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UTILITY

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835466

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Marta Weinstock Rosin, Michael Chorev and Zeev Tashma

For: PHENYL CARBAMATES

Enclosed are:

☐ sheets of drawing

☐ Associate power of attorney

Fee Calculation:

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	Total Claims	13	20	0	x	$12	=	0
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Respectfully submitted,

Dated: March 3, 1986

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Robin J. Moffat

Robin J. Moffat

PHENYL CARBAMATES

By

Prof. Marta Weinstock Rosin
Michael Chorev
Zeev Tashmac

Priority Claimed:

Priority Country:	Israel
Application No.:	74497
Filing date:	March 5, 1985

Case 118-6848

501
PHENYL CARBAMATES

The present invention relates to novel phenyl carbamates which are useful as pharmaceutical compositions. The invention further relates to pharmaceutical compositions having anticholinesterase activity.

Acetylcholine is a major neurotransmitter which is found in all parts of the body. Any reduction in its activity, either as a result of neuronal damage, degeneration etc. or as induced by drugs or toxins, causes marked changes in the function of the organism. Acetylcholine itself has an extremely short half life, since it is rapidly hydrolysed at its site of action and in plasma by specific cholinesterase enzymes. Drugs that inhibit acetylcholinesterase, markedly increase and prolong the action of acetylcholine, thereby enhancing cholinergic transmission. Three such agents are used clinically, i.e., physostigmine, a naturally occurring alkaloid, and two synthetic analogues, neostigmine and pyridostigmine. The latter two agents are strongly ionised at physiological pH and therefore are only poorly absorbed from the gastro-intestinal tract, and do not penetrate the central nervous system to any significant extent. Physostigmine is absorbed after

oral administration and readily enters the brain. As a therapeutic agent it has several disadvantages. It is chemically unstable and must be prepared in solution with an antioxidant, and protected from light. It has a relatively short half-life (20-40 mins) thereby necessitating frequent administration. The latter is of particular importance when the drug is to be administered chronically. It has a low therapeutic ratio, a value of 3-5 being reported in the majority of studies in laboratory animals, and a small therapeutic window, i.e. small range of dose in which it can be given without the accompaniment of side effects. Although physostigmine is absorbed from the gastro-intestinal tract, this is reported to be irregular and unpredictable, and therefore it is usually preferred to administer the drug parenterally. This is a serious drawback if it is to be used chronically on an outpatient basis.

There are a number of clinical and pathological conditions which are associated with cholinergic under-activity which can be improved by the administration of an anticholinesterase agent. These include reduction in cholinergic transmission induced by a variety of exogenous substances acting in the peripheral, or central nervous system. Peripherally acting agents are gallamine, d-tubocurarine and pancuronium, which are used as muscle relaxants. Their action can readily be overcome by an anticholinesterase drug. Drugs which interfere with central cholinergic transmission are numerous, anticholinergic, atropine-like drugs including antiparkinson drugs, tricyclic antidepressants, neuroleptics, opiate analgesics, benzodiazepines and some types of general anaesthetics. So far the only agent that has proved to be of any value in reversing the effects of the latter group of drugs is physostigmine. In all reported cases of drug overdose or lack of recovery when the agent was used peri-operatively, physo-

stigmine is usually administered parenterally, and administration is repeated every 20-30 minutes as required.

Chronic treatment with neuroleptics often results in tardive dyskinesias. The widespread use of agents having anticholinesterase activity for the treatment of schizophrenia makes this side effect an ever increasing possibility. Physostigmine injected intravenously produces a significant but short lived improvement in a proportion of patients.

A number of pathological and degenerative diseases has also been shown to be associated with a reduction or loss of cholinergic transmission. This includes myasthenia gravis and Eaton Lambert syndrome in which there is an interference with neuromuscular transmission.

A selective loss of choline acetyltransferase (the enzyme that synthesises acetylcholine) has been found in specific brain regions of patients with pre-senile dementia of the Alzheimer type. These include the frontal and temporal cortex, hippocampus, amygdala, caudate nucleus, substantia innominata. Degeneration of cholinergic neurons in some of these areas appears to be associated with the aphasia, apraxia, agnosia and loss of short term memory that occurs in Alzheimer's disease. A similar type of dementia is also found in patients with Down's syndrome that survive to the age of 40 years and show similar cholinergic deficits. There is also a loss of cholinergic transmission in the caudate nucleus and putamen of patients with Huntingdon's chorea. Physostigmine injections have also been of some benefit in this condition. Treatment with a centrally acting anticholinesterase should also prove to be beneficial in Friedrich's ataxia.

There are two major classes of potent inhibitors of the enzyme cholinesterase. The first group was modelled primarily on the natural alkaloids physostigmine (a carbamate) and an inhibitor of cholinesterase, and d-tubocurarine, an antagonist of acetylcholine. The second group consists of various organophosphorus compounds, such as diisopropylfluorophosphonate, paraxon etc. The vast majority of the compounds of both these series were designed primarily as insecticides. In the first group of carbamate derivatives, almost all of the potent insecticides are monomethyl carbamates lacking a charged nitrogen function. This enables the molecule to penetrate rapidly the insect cuticle and fatty nerve sheath. The dimethyl derivatives are slightly less potent but are particularly toxic to houseflies and aphids. The monomethyl derivatives tend to be unstable in solution and hydrolyse readily at physiological pH. This greatly limits their biological action in mammals and makes them less suitable as pharmaceutical or therapeutic agents.

The organo-phosphorus group of compounds causes irreversible inhibition of cholinesterase and other serine containing enzymes, which, together with their high relative toxicity, virtually precludes their use in pharmaceutical preparations. The only exception is echothiopate, a quaternary ammonium organophosphorus compound, employed in eye drops for the treatment of glaucoma.

The synthetic anticholinesterase agents currently employed as pharmaceuticals all contain a charged nitrogen function and can be broadly classified into 3 groups.

1) Reversible inhibitors which contain a charged nitrogen function attached to an aromatic ring, e.g. edrophonium.

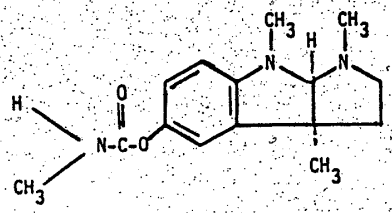
2) Dimethyl carbamates with an aromatic or heterocyclic ring containing a charged nitrogen, neostigmine, pyridostigmine.

3) Bisquaternary structures, e.g. Demacarium, Ambenonium. These agents tend to be more selective inhibitors of acetylcholinesterase than butyrylcholinesterase, compared with the monoquaternary molecules.

The pharmaceutical application of the quaternary anticholinesterase agents is limited because of their poor penetration through cell membranes. They are therefore used for actions outside the central nervous system, and are usually given parenterally, since they are not reliably absorbed from the gastrointestinal tract. Edrophonium, neostigmine and pyridostigmine and the bisquaternary analogues are used in anaesthetic practice for the reversal of the action of muscle relaxants. They are also used for the treatment of myasthenia gravis, and paralytic ileus.

Physostigmine is the only potent anti-cholinesterase agent which has been used clinically to treat conditions in which an elevation of brain acetylcholine activity is desired. These include, Alzheimer's disease, tardive dyskinesia, Down's syndrome and Huntingdon's chorea. Physostigmine is also used to reverse the effects of overdose of anticholinergic agents, anti-Parkinson drugs, benzodiazepines and opiate analgesics.

Physostigmine is a natural alkaloid extracted from calabar beans and the seeds of the vine Physostigma venenosum and has the formula



There is a need to provide new carbamate derivatives which show greater chemical stability than physostigmine.

Furthermore there is a need to provide new compounds which inhibit acetylcholinesterase in the brain for periods exceeding 3 hours but not more than 12 hours after a single administration.

There is also a need to provide new compounds which will be completely and reliably absorbed after oral administration.

There is also a need to provide new compounds which will be relatively less toxic than physostigmine. This means that the therapeutic ratio, defined as

$$\frac{\text{dose to produce therapeutic effect}}{\text{dose to produce mortality in 50 \% of animals}}$$

should be significantly higher than those of physostigmine and that the incidence and severity of side effects should be less than those of physostigmine at therapeutic doses.

There is also a need to provide new compounds which can be given orally or parenterally to treat chronic conditions in which it is

desired to raise cholinergic activity in the central nervous system. These include, Alzheimer's disease, Down's syndrome, Huntingdon's chorea, Friedrich's ataxia.

There is also a need to provide compounds that can be given parenterally at the end of operations, and anaesthetic procedures, to restore wakefulness, respiration and cardiovascular parameters to normal, after the use of anticholinergic, opiates, benzodiazepines, neuroleptics and general anaesthetics, thereby shortening the stay of patients in the recovery room.

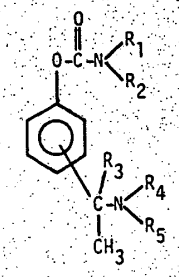
There is also a need to provide compounds that can be given together with narcotic analgesics to patients suffering from severe pain, e.g. traumatic, post-operative, or due to carcinomatosis etc. in order to reduce the side effects (respiratory depression, somnolence, constipation and urinary retention) commonly encountered with narcotics, without impairing their analgesic potency.

There is also a need to provide compounds that can be given to patients receiving antipsychotic drugs, which have developed tardive dyskinesias, in order to diminish or abolish the latter syndrome, without exascerbating the psychosis.

According to the present invention it has now been surprisingly found that certain novel and known phenyl carbamates also inhibit acetylcholinesterase in the mammalian brain after administration to provide systemic activity, e.g. oral or parenteral administration.

Thus according to the present invention there is now provided a pharmaceutical composition adapted to produce anticholinesterase

activity in the central nervous system of mammals comprising a compound of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

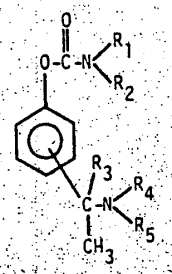
R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor. Hereinafter these compounds are called compounds of the invention.

Especially preferred are pharmaceutical compositions having anticholinesterase activity in the central nervous system of mammals, wherein the dialkylaminoalkyl group is in the meta position, and R_4 and R_5 are both methyl.

Certain compounds falling within the above formula have previously been described i.e. the m disubstituted compound in which R_1 and R_3 = H and R_2, R_4 and R_5 = methyl which is known as Miotine(R) was claimed to be an insecticide and a myopic agent for use in eye drops. The m disubstituted compound in which R_1 and R_2 are methyl, R_3 is H and R_4 and R_5 are methyl has been described as an insecticide. The p and o disubstituted derivatives in which R_1 and R_3 = H and R_2, R_4 and R_5 = CH_3 have been shown to inhibit a preparation of liver cholinesterase. The m disubstituted derivative in which R_1 = H and R_2, R_3, R_4 and R_5 = CH_3 has also been shown to inhibit liver cholinesterase.

The remaining compounds are believed to be novel and thus the present invention also provides novel phenyl carbamate derivatives of the general formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

Preferred compounds of the above formula are N-ethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate, N-allyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N-butyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-methyl, N-propyl-3[1-(dimethylamino)ethyl]phenyl carbamate and N-ethyl, N-methyl-3[1-(dimethylamino)isopropyl]phenyl carbamate.

As indicated, the invention also includes the pharmacologically acceptable salts of these compounds such as the acetate, salicylate, fumarate, phosphate, sulphate, maleate, succinate, citrate, tartrate, propionate and butyrate salts thereof.

The compounds of formula I can be prepared by amidating a compound of formula II

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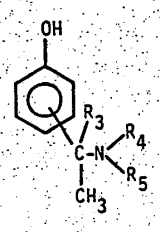
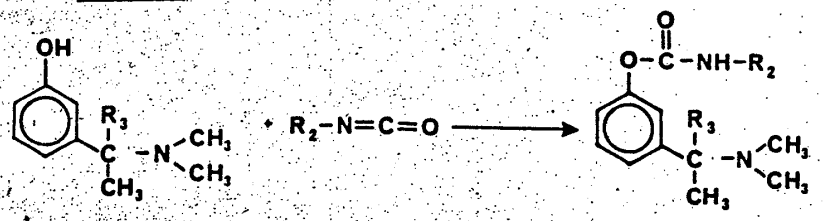


II

wherein R_3, R_4 and R_5 are as defined above.

The process can be effected in conventional manner, e.g. by reacting the compound of formula II with an appropriate isocyanate if a compound wherein R_1 is hydrogen is desired, or with an appropriate carbamoyl halogenide, e.g. as described below in processes A and B.

PROCESS A:



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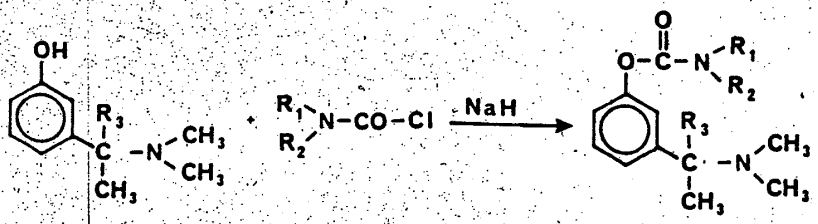
PROCESS B:



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PROCESS A:

A stirred suspension of α-m-Hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in benzene (0.2 - 0.3 g/ml) is treated with 2.5 - 3 fold molar excess of the isocyanate. After stirring for 15 - 24 hours at ambient temperature the reaction mixture is connected to a rotovaporator (20 mm Hg). The residue obtained is dissolved in dry ether (25 ml) and the solution, which is ice cooled, is saturated with dry HCl (g). The formed precipitate (the anticipated carbamate) is filtered off, washed with dry ether (25 ml) and dried to constant weight in a dessicator over KOH pellets under high vacuum (0.1 mm Hg).

PROCESS B:

A solution of α-m-hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in dry acetonitrile (0.1 - 0.5 M) is reacted with 50 - 70 % molar excess of the corresponding carbamoyl chloride in the presence of 200 % molar excess of NaH dispersion (50 - 80 % in mineral oil). The reaction mixture is left to stir at ambient temperature for 15 - 24 hours. Removal of the acetonitrile under reduced pressure (20 mm Hg) is followed by the addition of water (10 - 25 ml). The pH of the aqueous solution is adjusted to pH = 11 by the addition of the appropriate amount of NaOH 0.1 N followed by extraction with ether (3 x 25 ml). The combined organic phases are washed with brine (25 ml) dried over $MgSO_4$ anhydride which is then filtered off. The ice cooled etheral filtrate is saturated with a stream of HCl (g) resulting in the formation of a heavy precipitate (the anticipated carbamate) which is collected by filtration, washed with dry ether (20 ml) and dried to constant weight in a desiccator under high vacuum (0.1 mm Hg) over KOH pellets.

The compounds of the invention e.g. in free form or salt form can be utilized by formulating one or more of them in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. A compound or mixture of compounds of formula (I) or physiologically acceptable salt(s) thereof is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as mangnesium stearate; a sweetening agent such as sucrose, lactose or saccarin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other mterials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavour.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice by dissolving or suspending the active substance in a vehicle such as water for injection.

Buffers, preservatives, antioxidants and the like can be incorporated as required.

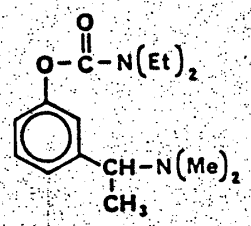
Preferred antioxidants for use with the compounds of the present invention include sodium metabisulphite and ascorbic acid.

While the invention will now be described in connection with certain preferred embodiments in the following examples, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars described are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the invention.

EXAMPLE 1

0.5 g (3.03 mmole) of α-m-hydroxyphenylethyldimethylamine are dissolved in 15 ml of dry acetonitrile and 0.70 g (5.2 mmole) of diethylcarbamylchloride are added to the mixture with stirring. This is followed by NaH 150 mg (50 %) of dispersion. The reaction mixture is stirred overnight at 25 - 30 ° C. Removal of acetonitrile under reduced pressure is followed by addition of water (10 ml) and adjustment of the pH to 11. The product is extracted in ether, which is washed by brine, dried over $MgSO_4$ and filtered. Upon addition of HCl (g) precipitation occurs immediately, the product is filtered off, washed by dry ether and dried in a desiccator under high vacuum over KOH pellets.

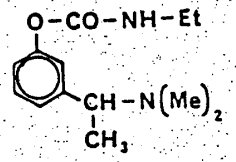
The carbamate is obtained as a white powder 640 mg (80 %) mp. 137 - 138 ° and identified as N,N-diethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, having the formula



EXAMPLE 2

0.75 g (4.55 mmol) of α-m-hydroxyphenylethyldimethylamine are suspended in benzene (3 ml) and 0.898 g of ethylisocyanate are added to the mixture with stirring. After stirring 12 hours at room temperature the solvent is removed under reduced pressure.

The residue obtained was dissolved in dry ether. Introduction of dry HCl gas into the reaction mixture causes a heavy precipitation. The product is filtered off, washed with ether and dried in a desiccator over KOH pellets. The carbamate is obtained as a white powder 800 mg (75 %) mp. 177 - 179 ° C and identified as N-ethyl-3[1-(dimethylamino)ethyl]phenyl carbamate having the formula



The compounds of the present invention are useful as pharmaceuticals. In particular they show the following activities in vitro and in vivo in the tests specified below.

The values are correct when taken in comparison with the standard drug physostigmine.

IN VITRO EXPERIMENTS:

Tests for anticholinesterase activity

A solubilized preparation of acetylcholinesterase was prepared from mouse whole brain (minus cerebellum). The brain was homogenized with (100 mg/ml) phosphate buffer; pH 8.0, centrifuged, the supernatant discarded, and the pellet mixed with a similar volume as above of buffer pH 8.0 plus 1 % Triton; mixed, centrifuged and the supernatant which contained most of the solubilized enzyme, was used for the subsequent determinations of anticholinesterase activity.

The activity of the enzyme (rate of hydrolysis of substrate, acetylthiocholine) was measured using at least 4 different concentrations of substrate, and at least 3 different concentrations of each inhibitor. The enzyme was incubated with inhibitor for periods ranging for 2 - 180 mins. at 37 ° C, substrate was then added, and its rate of hydrolysis measured by the spectrophotometric method of Ellman et al. (1961).

The molar concentration of each agent that inhibited the activity of the enzyme by 50 % (IC_{50}) at the peak time of activity (15 - 60 min) was calculated from this data and recorded in Table 1 hereinafter. The compounds in general produce a significant inhibition from about 10^{-5} to about 10^{-8} molar.

IN VIVO EXPERIMENTS:

a) Assessment of acetylcholinesterase inhibition

The effect of each compound on brain acetylcholinesterase in vivo was measured, after subcutaneous or oral administration to mice. Animals were sacrificed, at different times ranging from 0.25 - 8 hours after drug administration. The brain was rapidly removed, and the enzyme acetylcholinesterase extracted and solubilized with 0.1 % Triton, and its ability to hydrolyse acetylthiocholine assessed as described above (in vitro experiments), in comparison with the enzyme removed from mice injected with normal saline. The compounds have in general a potency of from about 2% to about 90% that of physostigmine.

b) Assessment of acute toxicity

Mice were given one of at least three different doses of each compound, orally or subcutaneously, a minimum of 10 mice allotted to each dose. The number of animals which died at

each dose within 3 hours was determined. From these data, the LD_{50} (dose in mg/kg which was lethal to 50 % of the mice) was computed.

This experiment was repeated after the animals had been pretreated with atropine sulphate, which blocks both peripheral and central muscarinic receptors. The data from these experiments enabled the assessment of the relative degrees of toxicity of the carbamates which result from excessive activation of muscarinic receptors, and from respiratory muscle paralysis, which is insensitive to this blocking agent.

The incidence and degree of side effects was noted for each dose of drug, starting with the lowest that caused any significant (> 20 %) inhibition of whole brain acetylcholinesterase.

c) Antagonism of the somnolent and respiratory depressant effects of opiates

Different doses of the carbamate compounds were injected intravenously with morphine in rabbits. Respiration rate, arterial blood gas tensions and pH were monitored continuously before and after drug administration for 4 - 5 hours. In another series of experiments the effect of the anticholinesterase drugs was assessed on the analgesic effect of opiates in rabbits after application of a nociceptive stimulus, i.e. electrical stimulation of the sciatic nerve.

All specific examples of formula I' mentioned hereinbefore, e.g. on specification page 10, and after especially Tables 1 to 3, are prepared in analagous manner to Example 1 when R_1 and R_2 are each other than hydrogen and Example 2 when one of R_1 and R_2 are hydrogen. They are thus obtained as hydrochloride salts (except where otherwise specified). The specific compounds have metal substitutions.

Table 1

In vitro activity on solubilized mouse brain enzyme

Compound (R_4=R_5=CH_3)	R_1	R_2	R_3	IC_{50}(M)	Time of peak activity (mins)
Physiostigmine (Salicylate)	H	CH_3	H	$1.1x10^{-8}$	30
Miotine HCl	H	CH_3	H	$1.3x10^{-8}$	30
RA_6 HCl	H	C_2H_5	H	$4.0x10^{-7}$	120
RA_{15} HCl	H	C_3H_7 n-propyl	H	$1.1x10^{-7}$	120
RA_{14} HCl	H	C_3H_5 allyl	H	$4.3x10^{-7}$	120
RA_{13} HCl	H	C_3H_7 isopropyl	H	$1.2x10^{-5}$	120
RA_5 HCl	H	C_4H_9 n-butyl	H	$7.6x10^{-8}$	120
RA_{12}	H	cyclohexyl	H	$9.3x10^{-8}$	120
RA_{10} HCl	CH_3	CH_3	H	$2.7x10^{-8}$	120
RA_7 HCl	CH_3	C_2H_5	H	$1.3x10^{-6}$	90
RA_8 HCl	C_2H_5	C_2H_5	H	$3.5x10^{-5}$	30
RA_{11} HCl	morpholino		H	$>2x10^{-5}$	30
RA_4 HCl	CH_3	propyl	H	$1.7x10^{-6}$	60

Melting points of compounds (all in the hydrochloride form except for RA_{12} which is in the free base form as it precipitated from the reaction mixture before addition of hydrogen chloride) are in degrees Centigrade: RA_6 167-170; RA_{15} 141-143; RA_{14} 147-152; RA_{13} 146-148; RA_5 158-162; RA_{12} 75-77; RA_{10} 145; RA_7 135-136; RA_8 137-138; RA_{11} amorphous; RA_4 148-149.

Compound RA_{11} has an RF value of 0.59 in a system of 95 parts of ethyl acetate and 5 parts of 33% (w/w) dimethylamine in ethanol.

Table 2

Anticholinesterase activity of compounds in mouse brain compared to that of physostigmine

TU210X

Compound	Relative potency to physostigmine after subcut. (s.c.) administration	Relative potency to physostigmine after oral administration	% cholinesterase inhibition 3 hours after s.c. administration
Physo-stigmine	100	100	0
Miotine	100	300	5
RA_6	11	19	35
RA_{15}	33	32	37
RA_{14}	15	22	35
RA_{13}	2	5	-
RA_5	36	29	30
RA_{12}	13	17	37
RA_{10}	81	92	7
RA_7	25	57	41
RA_8	2	5	32
RA_4	13	29	25

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Table 3

Acute toxicity of carbamates in mice

T0220X

Compound	LD_{50} µmoles/kg s.c.	Degree of* protection afforded by pretreatment with atropine	Therapeutic ratio LD_{50}/ED_{50} s.c.	LD_{50} oral / LD_{50} s.c.
Physostigmine	3.0	3.0	3.3	4.1
Miotine	4.5	2.4	4.9	1.2
RA_6	96	2.6	11.9	2.1
RA_{15}	31	4.1	11.1	4.5
RA_{14}	69	8.0	11.5	4.4
RA_{13}	65	4.5	1.6	1.1
RA_5	19	5.8	7.6	5.0
RA_{12}	42	3.8	5.8	3.6
RA_{10}	14	5.0	12.7	9.7
RA_7	46	10.4	12.4	1.2
RA_8	> 568	-	> 10.0	-
RA_4	72	4.9	10.0	1.7

*Ratio of LD_{50} after pretreatment with atropine sulphate 5 mg/kg to LD_{50} of drug alone.

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The data in Tables 1 and 2 demonstrate that somewhat larger quantities are required of all the drugs of the RA series than of physostigmine to inhibit the enzyme acetylcholinesterase. However, a comparison of the data in Table 1 with that in Table 2, shows that compounds RA_5, RA_6, RA_{15}, RA_{14}, RA_{10}, RA_7 and RA_8 are all relatively more active in vivo compared to physostigmine than one would expect from the in vitro data. This greater in vivo potency is particularly marked when the drugs are administered orally. This relatively greater in vivo activity may be due to:

a) greater chemical stability

b) a slower metabolic degradation or/and excretion

c) a higher lipid solubility, enabling a greater proportion of the drug to gain access to the enzyme in the central nervous system

d) more efficient absorption from gastro-intestinal tract.

For the purposes of their therapeutic application it is of little importance if one needs to give the drug (to human subjects) at a dose of 1 - 2 mg (physostigmine) or 2 - 50 mg that may be required of the compounds of the RA series. What is important is the safety of the drugs and the presence and severity of side effects that may occur at therapeutic doses. A commonly-used measure of drug safety is the therapeutic index - or LD_{50}/ED_{50}

$$\frac{\text{Dose to kill 50 \% of animals}}{\text{Dose to cause the desired therapeutic effect}}$$

It is assumed that the therapeutic effect of these anticholinesterase agents results from an elevation of brain cholinergic activity. This in turn, should be related to the degree of inhibition of acetylcholinesterase. For the purpose of the computation of the denominator of the therapeutic ratio, there is used the dose of drug that inhibits the activity of acetylcholinesterase by 50 %. This is based on the observation by Thal et al. (Ann. Neurology 13: 491, 1983) that the maximum improvement in short term memory obtained in a series of patients with Alzheimer's disease was achieved with a dose of physostigmine which blocked the acetylcholinesterase in the cerebro-spinal fluid by 50 %. The numerator is the dose found to kill 50 % of the animals within 4 hours of a subcutaneous injection.

The therapeutic ratios of compounds RA4, 5, 6, 7, 8, 10, 14 and 15 are all significantly higher than of physostigmine (see Table 3). This indicates that all these compounds have a wider margin of safety than that of physostigmine. Moreover, these RA compounds do not produce any significant undesirable side effects such as defaecation, lachrymation, fasciculations or tremor at the doses which inhibit the brain enzyme by 50 %, while the former 3 side effects are clearly evident when physostigmine is given at the appropriate dose (ED_{50}).

The data in Table 3 show that atropine can afford considerably greater protection against the lethality of the derivatives RA4, 5, 7, 10, 13 and 14. This is particularly important in the treatment of drug overdose since the respiratory muscle paralysis which is not affected by atropine and which is the cause of death induced by excess drug administration in the presence of atropine cannot be satisfactorily reversed by specific antidotes.

The duration of significant brain enzyme inhibition (> 30 %) induced by physostigmine (ED_{50} dose) is less than 2 hours. Compounds RA_4, 5, 6, 7, 8, 12, 14, 15 all act for more than 3 hours at their respective ED_{50} doses and RA_6 and RA_7 still causes significant inhibition (36 %) after 7 hours. Since none of these drugs caused noticeable side effects at the ED_{50} doses, an even longer duration of action may be achieved by giving between 50 and 100 % larger doses. The longer duration of action is a distinct advantage, particularly if the drugs are to be administered chronically to subjects suffering from neurological and behavioural conditions associated with a deficit in cholinergic transmission in the central nervous system, e.g. Alzheimer's disease, tardive dyskinesias, Huntingdon's chorea, Down's syndrome and Friedrich's ataxia.

The better the absorption of the drug after oral administration the more closely the LD_{50} given by this route resembles that after subcutaneous injection. Table 3 shows that RA_6, 13, 7 and 4 are more efficiently absorbed from the gastro-intestinal tract than is physostigmine. The ED_{50} of RA_8 after oral administration is the same as that after S.C. injection, indicating a much better oral bioavailability than that of physostigmine. The higher oral bioavailability of these compounds may be a considerable advantage for their clinical use.

RA_{10}, RA_6, RA_{14} and RA_{15} produce significant antagonism of the respiratory depressant effects of morphine in rabbits for periods lasting between 3 - 5 hours depending on the drug and the dose administered. The analgesic activity of morphine is not reduced by the RA compounds. Muscle fasciculations are not evident at the doses of drugs administered. Physostigmine (0.1 - 0.2 mg/kg) antagonizes the respiratory depressant effect of morphine for

30 - 60 mins only and fasciculations are marked at the higher dose.

These findings show that the RA compounds may be given together with morphine to obtain adequate analgesia without significant degrees of respiratory depression.

The most preferred compounds of the RA series are RA_4, RA_5, RA_6, RA_{15}, RA_{14}, RA_7 and RA_8, all of which produce inhibition of brain acetylcholinesterase after parenteral administration of significantly longer duration than that induced by physostigmine or miotine. These compounds also have a greater safety margin (therapeutic ratio) than physostigmine. $RA_{4, 6, 7}$ and $_8$ also show better bioavailability after oral administration than physostigmine. In addition, the acute toxicity (lethality) induced by RA_7 can be decreased more than 10-fold and that of RA_{14} more than 8-fold by the antidote atropine, compared to only a 3-fold decrease for physostigmine and miotine.

The compounds of the invention are therefore useful for the treatment of senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Down's syndrome and Friedrich's ataxia.

For these indications, the exact dosage will of course vary depending upon the compound employed, mode of administration and treatment desired. The compounds may be administered by any conventional route, non-oral or preferably orally.

In general, satisfactory results are obtained when administered at a daily dosage of from about 0.05 to 10 mg/kg animal body weight. For the larger mammals, an indicated total daily dosage

is in the range from about 0.5 to about 25 mg of the compound, conveniently administered in divided doses 2 to 4 times a day in unit dosage form containing for example from about 0.1 to about 12 mg of the compound or in sustained release form.

The compounds may be administered in similar manner to known standards for use in these utilities. The suitable daily dosage for a particular compound will depend on a number of factors such as its relative potency of activity.

The compounds according to the invention may be administered in free base form or as a pharmaceutically acceptable acid addition salt. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free forms.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative embodiments and examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is, therefore, desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come with the meaning and range of equivalency of the claims are, therefore, intended to be embraced therein.

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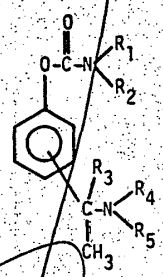
WHAT IS CLAIMED IS:

1. A pharmaceutical composition adapted to produce anticholinesterase activity in the central nervous system comprising a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

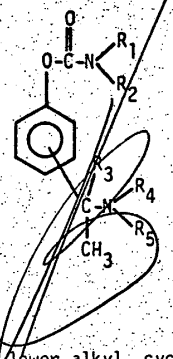
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor.

2. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

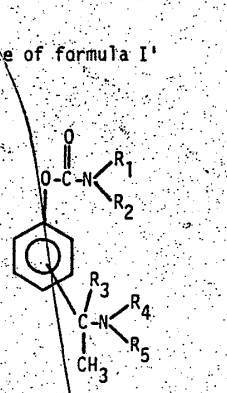
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof.

3. A phenylcarbamate of formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

4. A compound of claim 3 wherein the dialkylaminoalkyl group is in meta position and R_4 and R_5 are both methyl.

5. A compound of claim 3 which is N-ethyl-3-[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

6. A compound of claim 3 which is N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

7. A compound of claim 3 which is N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

8. A compound of claim 3 which is N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

9. A compound of claim 3 which is N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

10. A compound of claim 3 which is N-allyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

11. A compound of claim 3 which is N-butyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

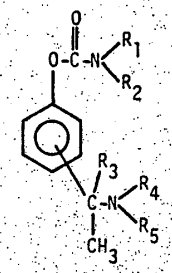
12. A compound of claim 3 which is N-methyl, N-propyl-3[1-dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

13. A compound of claim 3 which is N-methyl, N-ethyl-3[1-dimethylamino)isopropyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

PHENYL CARBAMATES

Abstract of the disclosure

Phenyl carbamates of the general formula



I

wherein R_1 to R_5 are as defined in the claims, are useful as pharmaceuticals.

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-1-

Attorney's Case No. 118-6848

DECLARATION AND POWER OF ATTORNEY
ORIGINAL APPLICATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled PHENYL CARBAMATES the specification of which is

☒ is attached hereto

☐ was filed on _____ as Application Serial No. _____ and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
74497 (Number)	Israel (Country)	March 5, 1985 (Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed to the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to

disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint Ronald G. Goebel (Registration No. 26,895), Bruce M. Collins (Registration No. 20,066) and William C. Long (Registration No. 18,545) to prosecute this application and transact all business in the Patent and Trademark Office connected threwith.

SEND CORRESPONDENCE TO:

601
602
701
702
704
Ronald G. Goebel, Esq.
MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS P.A.
22 Park Place, P.O. Box 112-M
Morristown, New Jersey 07960

DIRECT TELEPHONE CALLS TO:

Ronald G. Goebel, Esq.
(201) 267-3444

Full name of first inventor: 45100 Marta Weinstock Rosin

Inventors Signature _____

Date: _____

Residence: Jerusalem, Israel ILX

Citizenship: Israel

Post Office Address: 9 Herzog Str., Jerusalem, Israel

Full name of second joint inventor: Michael Chorev 40200

Inventors Signature _____

Date: _____

Residence: Jerusalem, Israel IL

Citizenship: British

Post Office Address: 135/4 Feinstein Str., Jerusalem, Israel

Full name of third joint inventor: Zeev Tashma 40300

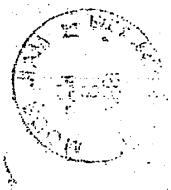
Inventors Signature _____

Date: _____

Residence: Jerusalem, Israel IL

Citizenship: Israel

Post Office Address: 2 Shahal Str., Jerusalem, Israel



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APR 04 1986

APPLICATION DIVISION
PATENT & TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Ronald G. Goebel
MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS
22 Park Place, P.O. Box 11
Morristown, NJ 07960

Applicant(s): MARTA W. ROSIN, ET AL
Serial Number: 835466
Filing Date: 03/03/86
Title: PHENYL CARBAMATES

Notice to File Missing Parts of Application-Filing Date Granted

If all missing parts are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is $ 110.00.

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity, ☐ small entity, must submit $ _____ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.

2. ☐ Additional claim fees of $ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.

3. ☐ The oath or declaration is:
   ☐ missing.
   ☐ does not cover items omitted at the time of execution.
   An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63 identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

5. ☒ The signature to the oath or declaration is: ☒ missing; ☐ a reproduction; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Serial Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration: _____. Applicant(s) should provide, if possible, an oath or declaration signed by the omitted inventor(s), identifying this application by the above Serial Number and Filing Date. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of $26.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE IS REQUIRED FOR THIS ITEM.

8. ☒ Other: 110.00 SURCHARGE IS DUE.

A Serial Number and Filing Date have been assigned to this application. However, to avoid abandonment under 37 CFR 1.53(d), the missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE OF $110.00 for large entities or $55.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- 40 -

Direct the response to, and any questions about, this notice to the undersigned, Attention, Application Branch, and include the above Serial Number and Filing Date.

F. W. Acevedo /by f

For: Manager, Application Branch
(703) 557-3264

Form PTO-1533 (6-xx)

#3

110 - 105-A/N

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Marta Winstock Rosin et al Examiner:

Serial No.: 835,466 Group Art Unit:

Filed: March 3, 1986

For: PHENYL CARBAMATES

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

ATTN: APPLICATION BRANCH
F. Morris

RESPONSE

SIR:

In response to Notice To File Missing Parts Of Application-Filing Date Granted mailed April 4, 1986 in the above-identified application applicant submits a combined Declaration and Power of Attorney fully executed by all inventors, which Declaration claims priority of a prior Israeli application filed March 5, 1985 bearing Application No. 74497.

Counsel's check in the amount of $110.00 in payment of the surcharge (large entity) as set forth in 37 CFR 1.16(e) is also enclosed. In the event the fee tendered is inadequate authority is hereby given to charge any such deficiency or credit any overpayment to Deposit Account No. 13-2160.

Respectfully submitted,

Ronald G. Goebel
Attorney for Applicants

Dated: May 15, 1986

MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS, P.A.
P.O. Box 112-M, 22 Park Place
Morristown, New Jersey 07960
Telephone: (201) 267-3444

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed To: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on May 15, 1986
Robert Moffat

040 05/21/86 835566
1 105 110.00 CK

#3

Attorney's Case No. 118-6848

DECLARATION AND POWER OF ATTORNEY
ORIGINAL APPLICATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled PHENYL CARBAMATES the specification of which is

☐ is attached hereto

☒ was filed on March 3, 1986 as Application Serial No. 835 466 and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

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(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

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disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint Ronald G. Goebel (Registration No. 26,895), Bruce M. Collins (Registration No. 20,066) and William C. Long (Registration No. ~~18,545~~) to prosecute this application and transact all business in the Patent and Trademark Office connected threwith.

SEND CORRESPONDENCE TO:

Ronald G. Goebel, Esq.
MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS P.A.
22 Park Place, P.O. Box 112-M
Morristown, New Jersey 07960

DIRECT TELEPHONE CALLS TO:

Ronald G. Goebel, Esq.
(201) 267-3444

Full name of first inventor: Marta Weinstock Rosin

Inventors Signature Marta Weinstock Rosin

Date: May 8th, 1986.

Residence: Jerusalem, Israel

Citizenship: Israel

Post Office Address: 9 Herzog Str., ~~Jerusalem~~, Israel

40200

Full name of second joint inventor: Michael Chorev

Inventors Signature Michael Chorev

Date: May 8th, 1986

Residence: Jerusalem, Israel IL X

Citizenship: Israel

Post Office Address: 135/4 Feinstein Str., Jerusalem, Israel

40300

Full name of third joint inventor: Zeev Tashma

Inventors Signature Zeev Tashma

Date: May 8th. 1986.

Residence: Jerusalem, Israel IL X

Citizenship: Israel

Post Office Address: 2 Shahal Str., Jerusalem, Israel

#3 56.00 - 115 - A/N

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Marta Weinstock Rosin et al Examiner:

Serial No.: 835,466 Group Art Unit:

Filed: March 3, 1986

For: PHENYL CARBAMATES

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

PETITION FOR EXTENSION OF TIME

SIR:

Pursuant to 37 CFR §1.136(a), request is hereby made for an extension of one month to render timely the attached response to the Notice To File Missing Parts of Application-Filing Date Granted dated April 4, 1986 which Notice established a period of response set to expire on May 4, 1986.

Counsel's check in the amount of $56.00 in payment of the extension fee is submitted herewith. In the event the fee tendered is inadequate for an extension sufficient to render the accompanying response timely, authority is hereby given to charge any such deficiency to Deposit Account No. 13-2160.

Respectfully submitted,

Ronald G. Goebel
Reg. No. 26,895

MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS, P.A.
P.O. Box 112-M, 22 Park Place
Morristown, New Jersey 07960
(201) 267-3444

Dated: May 15, 1986

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed To Commissioner of Patents and Trademarks, Washington, D.C. 20231, on May 15, 1986.

Robert Moffat

040 05/21/86 835466 1 115 56.00 CK

8351466

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
06/835,466	03/03/86	ROSIN	M	118-6848

RONALD G. GOEBEL
MATHEWS, WOODBRIDGE, GOEBEL,
PUGH & COLLINS
22 PARK PLACE, P.O. BOX 112-M
MORRISTOWN, NJ 07960

EXAMINER	
SHIFFEN, M	
ART UNIT	PAPER NUMBER
126	40

DATE MAILED: 01/23/87

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-13 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-13 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.
8. ☐ Allowable subject matter having been indicated, formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. These drawings are ☐ acceptable; ☐ not acceptable (see explanation).
10. ☐ The ☐ proposed drawing correction and/or the ☐ proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved. ☐ disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
12. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

PTOL-326 (Rev. 7-82) EXAMINER'S ACTION

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless-
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1 rejected under 35 U.S.C. 102(b) as being anticipated by Meltzer, ~~Canyé~~ Lange and Berry .

The claimed composition ~~uses as~~ reads on the prior art compositions and/or reaction mixtures regardless of intended use.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1,3-8 and 11-13 rejected under 35 U.S.C. 103 as being unpatentable over Aeschlimann (USP1,905,990), meltzer, Lange or Berry .

The Aeschlimann reference generically teaches the claimed compunds rendering them obvious. The references discloses homologous and/or isomeric compounds that are so structurally similar that are would expect them to possess a community of properties in common.

Claim 2 rejected under 35 U.S.C. 103 as being unpatentable over Berry and Aeschlimann (USP 1,905,990) opinally in view of Aeschlimann (UPS 2,493,710.

Berry and Aeschlimann (USP 1,905,990) teach that it is known that the compounds possessing anticholinesterase activity, Applicants admit that it is known to use anticholinesterase agents in the treatment of the recited disorders, see lines 12-16 of pages 1; lines 16-18 of page 2; and lines 16-22 of page 5 of the specification. Accordingly, it is considered obvious to use these known anticholinesterase agents for the treatment of the recited disorders. Aeschlimann (USP 2,493,710) is cited to show that the various R_1 and R_2 of the instant claims are recognized to be functionally equivalent in analogous compounds rendering such a modification of the primary reference compounds obvious.

The remaining reference are cited as of interest.

Shipper:vld
01/06/87
A/C 703 557-6930

Michael L. Shippen
MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP—APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

NOTICE OF REFERENCES CITED

SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
835,466	126	4

APPLICANT(S): Rosini, et al

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A	1905990	4-1933	Aeschlimann	560	32	
	B	2208485	7-1940	Aeschlimann	560	136	
	C	2493710	1-1950	Aeschlimann	560	136	
	D	2362508	11-1944	Stevens	—	—	
	E						
	F						
	G						
	H						
	I						
	J			1 of 2			
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
DEX	L	1037753	7-1956	Germany	Fitch	560	136		
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R Stedman, Biochemical Journal, 20 p 719-34 (1926) N

S Wassermann, Proc. Natl. Acad. Sci. USA, 79 p 4810-4 (1982) N

T Weiden, J. Agr. Food Chem., 13 p200-4 (1965) N

U Berry, Biochem. Pharmacol., 20 p3236-8 (1971) N

EXAMINER	DATE
M. Shippen	12/19/86

*A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP—APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED	835,466	126	4
	APPLICANT(S) Rosin, et al		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G			2 of 2			
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

	R	Lange, Haemostasis, 10 p 315-47 (1981) N
	S	Meltzer, Entomol. Exp. Appl. 12 p 169-82 (1969) N
	T	
	U	

EXAMINER	DATE
M. Shippen	12/19/86

*A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

390.00-111

16

AMENDMENT TRANSMITTAL FORM

"PATENT" 45

In re application of: M. Rosin, et al. Before the Examiner M. Shippen
Serial No: 835,466)
Filed: 03/03/86)
For: PHENYL CARBAMATES) Group Art Unit 126

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

[x] The undersigned hereby certifies having information and a reasonable basis for belief that this correspondence will be deposited as first-class mail with the United States Postal Service in an envelope addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 7/17/87.

Transmitted herewith is an amendment/response in the above-identified application.

Petition for extension of time pursuant to 37 CFR 1.136 and 1.137 is hereby made if, and to the extent, required. The fee for this extension of time is calculated to be 390.00 to extend the time for filing this response until 7/23/87.

The fee for any changes in number of claims has been calculated as shown below.

CLAIMS AS AMENDED							
	(1)	(2) Claims Remaining After Amendment	(3)	(4) Highest No. Previously Paid for	(5) Present Extra	(6) Rate	(7)
	Total Claims	*	Minus	**		x12.00	
	Indep. Claims	*	Minus	***		x34.00	
	MULTIPLE DEPENDENT CLAIM FEE					$110.00	
						FEE FOR CLAIM CHANGES	

* If the entry in Column 2 is less than the entry is Column 4, write "0" in Column 5.
** If the "Higher Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

The total fee for this amendment, including claim changes and any extension of time is calculated to be $ 390.00.

[x] A check in the amount of $ 390.00 is attached.

[ ] Charge $_____ to Deposit Account No.

[X] The Commissioner is hereby authorized to charge any additional fees under 37 CFR 1.16 and 1.17 which may be required by this paper, or credit any overpayment, to Deposit

120 07/27/87 835466 1 117 390.00 CK

July 17, 1987
Date of Signature

Attorney or Agent of Record

Richard T. Laughlin
Registration No. 17,264

Post Office Address (to which correspondence is to be sent):
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 Headquarters Plaza
Morristown, New Jersey 07901

8-3-87

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: M. W. Rosin, et al

SERIAL NUMBER: 835,466 GROUP ART UNIT: 126

FILED : 03/03/86 EXAMINER: M. SHIPPEN

FOR : PHENYL CARBAMATES

AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is in response to the Official Action of January 23, 1987.

Please amend the above identified application as follows:

In the Claims:

Cancel claim 1 in its entirety.

Amend the claims as follows:

Claim 2, last line, after "thereof" and before the period, insert the following

, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither

A.1 cont'd

hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamine group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl--

REMARKS

The claims in the application are claims 2 to 13. Claim 2 was amended so that it is directed to the same group of compounds as claim 3.

For the information of the Examiner, some of the compounds claimed in this application were disclosed after the priority date at the 3rd OHOLO Biol. conference in Eilat, Israel held in November of 1985, a copy of the publication is attached hereto as Exhibit "A". Subsequent to this, workers at Warner-Lambert synthesized some of the compounds, a copy of the publication is attached hereto and marked as Exhibit "B".

It is noted that claims 9 and 10 were not rejected on any basis.

Reconsideration is requested of the rejection of claims 3-8 and 11-13 under 35 USC 103 as being unpatentable over Aeschlimann 1,905,990; Meltzer, Lange or Berry in that the

references disclose homologous and/or isomeric compounds that are so structurally similar that it would be expected they would possess a community of properties in common.

The Meltzer article discloses two compounds with the code numbers KD 1207 and 1261 which fall under formula I, but not under formula I', and provides results as to their insecticidal activity. There is also a general statement mentioning that the anticholinesterase activity of alkylphenyl N-methylcarbamates can be improved by introducing a p-dimethylaminomethyl group. There is however no alkyl group in addition to the dialkylaminoalkyl group in the compounds disclosed. Meltzer concerns a different art, that of killing insects whereas the present invention is for treating patients to save their lives.

The Lange article discloses one compound (table II, No. 37) which is Miotine, falling under formula I, as an inhibitor of coagulation factors. Lange in page 338, 3rd paragraph indicates that other compounds are preferred. There is no motivation to modify compound 37 to a compound such as now embraced by the claims.

The Berry article discloses the acetylcholinesterase

activity of 6 carbamates including one of formula I, which is Miotine. Berry is an academic work and does not come to any conclusion and does not suggest structural changes for any purpose.

Aeschlimann 1,905,990 generically covers N-disubstituted carbamates of formula I, assuming the alkyl chain in the dialkylaminoalkyl group may be branched. However no compound having a dialkylaminoalkyl group is disclosed and there is no mention of any advantage of the compounds over physostigmine. In fact, Aeschlimann having prepared no compound with a dialkylaminoalkyl substituent could, of course, not have noticed the advantages bond to the alkyl bridge.

Applicants have now combined the two features of the alkyl bridge and tertiary nitrogen, and discovered further, unexpected advantages over physostigmine, comprising CNS selectivity, little side effects and low toxicity. These advantages are essential for the use of the compounds in senile mental decline.

Reconsideration is also requested of the rejection of claim 2 under 35 USC 103 as being unpatentable over Berry and Aeschlimann 1,905,990 or in view of Aeschlimann

2,493,710. The claim has been amended to limit it to the compounds of claim 3. The rejection is based on the allegation that the references teach the compounds of the references possess anticholinesterase activity and that applicants admit it is known to use such agents in the treatment of the recited disorders. As indicated above the basic references do not disclose the subject compounds. They also do not suggest that the claimed compounds have the recited activity. Accordingly, it is submitted that the recited method is novel and is not suggested. As to Aeschlimann 2,493,710, it discloses carbamic acid esters including compounds presenting the alkyl bridge, but wherein the nitrogen in the alkylamine radical is either primary or secondary, but not tertiary like in the compounds of the present invention. Thus he noticed some advantages over physostigmine like a good p.o. bioavailability and a certain specificity but no details are given. However, there was no incentive to improve the activity by preparing tertiary amine homologues, and this was not done until the present invention.

The other references have been considered but since they are only recited as of interest a detailed discussion

appears unnecessary. The references however do not disclose the claimed compounds.

For the reasons given hereinabove reconsideration of the rejection of the claims is respectfully requested.

Respectfully submitted,

M.W. Rosin, et al

By: _____
Richard T. Laughlin
Attorney for Applicant

Laughlin, Markensohn,
Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey
07960
(201) 539-0080

Dated: 7/17/87

1st Israel Conf. 3rd OHOLO
1. Conference Eilat
1985

PHARMACOLOGICAL ACTIVITY OF NOVEL ANTICHOLINESTERASE AGENTS OF POTENTIAL USE IN TREATMENT OF ALZHEIMER'S DISEASE

M. Weinstock, M. Razin and M. Chorev. Depts. of Pharmacology and Pharmaceutical Chemistry. Hebrew Univ. Medicine & Pharmacy Jerusalem, Israel

Alzheimer's disease has been associated with degeneration of cholinergic neurones in the hippocampus and cerebral cortex. Physostigmine has some beneficial effect in this condition, but its short half life, low therapeutic ratio and irregular intestinal absorption limit its usefulness to hospitalized patients. We have prepared and tested 10 novel anticholinesterase agents in a variety of preparations in vitro and in vivo. Several of these compounds were found to have obvious advantages over physostigmine. In vitro activity was assessed on a solubilized preparation of mouse brain acetylcholinesterase (AChE). In vivo activity and acute toxicity were assessed at various times after s.c. or oral administration to mice or rats. Five of the compounds, RA6,7,12,14 & 15 produced 50% AChE inhibition in mouse brain lasting 3-17 hours, while that of physostigmine lasted only 2 hours. The therapeutic ratio LD50/ED50 for physostigmine in mice was 5.6 after s.c. and <3.0 after oral administration, while those of five RA compounds ranged from 6.6-12. The maximum inhibition in AChE after oral or s.c. physostigmine in rats was only 31% in the cortex but reached 46% in the medulla, at doses which caused obvious fasciculations. In contrast, RA6,7,14 & 15 inhibited AChE in cortex by more than 50%, but that in the medulla by 25-45% at doses which did not cause signs of peripheral cholinergic overactivity. These drugs are currently being compared with physostigmine for their ability to restore memory in rats with lesions of the nucleus basalis. In view of their lower relative toxicity, longer duration of action, better oral bioavailability, and relatively greater inhibition of AChE in cortex than in medulla, these compounds may be superior to physostigmine in the treatment of Alzheimer's disease.

EXHIBIT "A"

145.8 CENTRAL CHOLINERGIC PHARMACOLOGY OF A SERIES OF CHOLINESTERASE INHIBITORS. R.E. Davis, L.L. Coughenour*, J.G. Marriott, W.H. Moos*, R.D. Schwarz, J.P. Symons, and A.J. Thomas*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

The short duration of action and poor therapeutic ratio of the anticholinesterase, physostigmine, has limited its utility in treating patients suffering from cholinergic deficiencies. Recently, a series of acetylcholinesterase inhibitors (ACHE-I) has been described by Weinstock-Rosen (RA series) with a potential for decreased toxicity and increased duration of action relative to physostigmine. We have examined the effects of this series in a variety of 'in vitro' biochemical and 'in vivo' behavioral tests which are designed to characterize central cholinergic function.

All compounds from the RA series exhibit higher affinity for muscarinic cholinergic agonist sites labeled by cis-methyldioxolane (CMD) than for sites labeled by the muscarinic antagonist, QNB. The IC-50's of these compounds for displacing ^{3}H-CMD is directly correlated to their potency for inhibiting cholinesterase activity (r^2 = 0.92). CMD displacement and cholinesterase activity of this series also are directly related to bulk parameters such as lipophilicity and molar refractivity. A similar relationship holds with respect to the ability of these compounds to decrease the K+-stimulated presynaptic release of acetylcholine from brain slices. However, these RA series compounds do not stimulate the turnover of phosphoinositides (PI). The order of potency for displacing CMD, inhibiting cholinesterase activity and decreasing ACH release is RA-2 > RA-10 > RA-6 > RA-7 ≥ RA-8.

A similar order of potency holds for the ability of these compounds to decrease spontaneous swimming and to reverse scopolamine-induced increases in swimming activity of rats. However, RA-6 does not reverse scopolamine-induced increases in swimming activity.

Taken together these data suggest that the ability of these compounds to displace CMD binding but not QNB binding may be related to their ability to inhibit cholinesterase activity. ACHE-I activity also is directly related to the ability of these compounds to decrease the presynaptic release of ACH from brain slices, decrease spontaneous swimming activity and reverse scopolamine-induced swimming activity. This pattern of effects also is seen with the known anticholinesterase, physostigmine.

EXHIBIT "B"

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

835,466

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/835,466	03/03/86	ROSIN M	118-6848

RONALD G. GOEBEL
MATHEWS, WOODBRIDGE, GOEBEL,
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MORRISTOWN, NJ 07960

EXAMINER	
SHIFFEN, M	
ART UNIT	PAPER NUMBER
126	7

DATE MAILED: 11/10/87

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 7/20/87 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 2-13 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 2-8 & 11-13 are rejected.
5. ☒ Claims 9 & 10 are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.
8. ☐ Allowable subject matter having been indicated, formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. These drawings are ☐ acceptable; ☐ not acceptable (see explanation).
10. ☐ The ☐ proposed drawing correction and/or the ☐ proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved. ☐ disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
12. ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

PTOL-326 (Rev. 7-82) EXAMINER'S ACTION

Claims 3-8 and 11-13 are rejected under 35 U.S.C. 103 as being unpatentable over Aeschlimann (USP 1,905,990), Meltzer, Lange or Berry for reasons of record.

Applicants state that Aeschlimann (USP 1,905,990) does not disclose a compound having a "dialkylaminoalkyl" group and there is no mention of any advantage over physostigmine. The fact is that the product of example 2 has a "dialkylaminoalkyl" group which is an adjacent homologue to the compounds instantly claimed, as to advantages of the prior art compounds, there is no requirement that the prior art disclose advantages over physostigmine. There is no evidence of the instant compounds possessing unexpected properties over the compounds of Aeschlimann (USP 1,905,990), note In re Hoch, 166 USPQ 406. The fact that Meltzer does not teach "treating patients" is of no moment, In re Hoch, supra. The fact that compound 37 of Lange may not be the most preferred agent disclosed is of no moment, see In re Mills, 176 USPQ 196. Applicants suggestion that Berry does not suggest structural changes is of no moment because one of ordinary skill in the art would recognize the obviousness of homologous or isomeric compounds without the reference suggesting such changes.

Claim 2 is rejected under 35 U.S.C. 103 as being unpatentable over Berry and Aeschlimann (USP 1,905,990) optionally in view of Aeschlimann (USP 2,493,710) for reasons of record.

Applicants arguments as to Berry and Aeschlimann (USP 1,905,990) that are the same as presented in response to the preceding rejection are not persuasive for the reasons given.

Claims 9 and 10 objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Applicant information about "some of the compounds claimed" being disclosed after the priority date is noted. It is pointed out to applicants from the information provided by applicants it is not possible to determine what compounds were actually disclosed making an evaluation of such information impossible. It is further pointed out to applicants that they are not entitled to their priority date unless it is actually prefected in accordance with 35 USC 119 (also see MPEP 201.15). If the disclosure raises issues under 35 USC 102 or 35 USC 103, such a determination cannot be made with the information provided, see 37 CFR 1.56.

THIS ACTION IS MADE FINAL.

Applicant is reminded of the extension of time policy set forth in 37 CFR 1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely first response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 CFR 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

MShippen/baf

A/C 703 557-3871

10/31/87

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
835,466	3/3/86	Rosin	

EXAMINER	
Shippen	
ART UNIT	PAPER NUMBER
126	8

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) M. Shippen (3) _____

(2) H. Kasper (4) _____

Date of interview 2/10/88

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☐ applicant's representative). 1 of 2

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: _____

Identification of prior art discussed: _____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

(1) Examiner maintained his position as to the rejections of record.

(2) Claims 9+10 would be allowable if placed in independent form. Method claims dependent upon

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
835,466			

EXAMINER	
ART UNIT	PAPER NUMBER
	8

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1)_____ (3)_____

(2)_____ (4)_____

Date of interview_____

Type: ☐ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative). 2 of 2

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description:_____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed:_____

Identification of prior art discussed:_____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

claims 9+10 would also be allowable,
(3) any comparison under 37 CFR 1.132 should compare the closest prior art compounds for applicants utility; however, a showing would ~~probably~~ probably not be considered after FINAL REJECTION in this application,

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., Items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

In re application of: M. Rosin, et al.)
Serial No: 835,466)
Filed: 3/3/86)
For: PHENYL CARBMATES) Group Art Unit 126

Before the Examiner M. Shippen

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

[X] The undersigned hereby certifies having information and a reasonable basis for belief that this correspondence will be deposited as first-class mail with the United States Postal Service in an envelope addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231, on FEB 10, 1988.

Transmitted herewith is an amendment/response in the above-identified application.

Petition for extension of time pursuant to 37 CFR 1.136 and 1.137 is hereby made if, and to the extent, required. The fee for this extension of time is calculated to be _____ to extend the time for filing this response until _____.

The fee for any changes in number of claims has been calculated as shown below.

CLAIMS AS AMENDED							
	(1)	(2) Claims Remaining After Amendment	(3)	(4) Highest No. Previously Paid for	(5) Present Extra	(6) Rate	(7)
	Total Claims	* 2	Minus	** 2		x12.00	0
	Indep. Claims	* 2	Minus	*** 2		x34.00	0
	MULTIPLE DEPENDENT CLAIM FEE					$110.00	0
					FEE FOR CLAIM CHANGES		0

* If the entry in Column 2 is less than the entry in Column 4, write "0" in Column 5.
** If the "Higher Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.

The total fee for this amendment, including claim changes and any extension of time is calculated to be 0.

[ ] A check in the amount of $ _____ is attached.

[ ] Charge $ _____ to Deposit Account No.

2/10/88
Date of Signature

Attorney or Agent of Record
Richard T. Lauchlin

Registration No. 17 264

#8
3-5-88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
ROSIN ET AL.	:	ART UNIT 126
Ser. No. 06/835,466	:	Michael L. Shippen Examiner
Filed: March 3, 1986	:	
For: Phenyl Carbamates	:	

POWER OF ATTORNEY

Honorable Commissioner of Patents and Trademarks

Washington, D. C. 20231

Dear Sir:

In the matter of the above identified application, the undersigned, the assignee of the application, hereby revokes all Powers of Attorney heretofore given and appoint as its attorney:

Richard T. Laughlin Reg. No. 17,264

Anthony Lagani, Jr. Reg. No. 24,126

of 129 Headquarters Plaza

Morristown, New Jersey 07960

Tel. No. 201-539-0080

with full power of substitution, association, and revocation, to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith.

Proterra AG

By _____
(Title)

Dr. Martin J. Lutz
Chairman of the Board of Directors

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 9

Shippen Art Unit 126
06/835466 03/03/86
Marta W. Rosin, et al

Ronald G. Goebel
Mathews, Woodbridge, Goebel
Puch & Collins
22 Park Place, P.O. Box 112-M
Morristown, NJ 07960

MAILED
MAR - 9 1988
GROUP 120

This is in response to the communication re the Power of Attorney filed 2-16-88.

1. ☑ The power of attorney to you in this application has been revoked by the ~~applicant~~ assignee.
2. ☐ In view of the notice in this application of the death of _____ his power of attorney is terminated.
3. ☑ The power of attorney to you in this application has been accepted by the Commissioner of Patents, & Trademarks.

_____
For Director, Operation

4. ☐ The assignee in this application has intervened and appointed an attorney of his own selection. Further correspondence will be held with said attorney. (Rule 36, Rules of Practice.)
5. ☐ The revocation of the power of attorney to _____ has been entered and said attorney has been notified. Further correspondence will be addressed to you.
6. ☐ On _____, the applicant (assignee) appointed _____ as additional attorney in this application. Further correspondence will continue to be addressed to you as specified in the new power of attorney.
7. ☐ On _____, the applicant (assignee) appointed _____ as additional attorney in this application. Further correspondence will be addressed to said attorney. MPEP 403.02
8. ☐ The associate power of attorney to you in this application has been revoked by the attorney of record.

Laughlin, Markensohn
Lajani & Pegg
129 Headquarters Plaza
Morristown, NJ 07960

Kathryn P. Perry
For Director, Operation

RETAIN THIS COPY IN THE APPLICATION FILE

Copy A

FORM PTOL-305 (REV.9/75)

GP. 126

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: M. W. Rosin, et al.

SERIAL NUMBER: 835,466 GROUP ART UNIT: 126

FILED: 3/3/86 EXAMINER: M. SHIPPEN

FOR: PHENYL CARBMATES

AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is in response to the Offical Action of November 10, 1987.

Please amend the application as follows:

Cancel claim 2 to 8 and 11 to 13.

Rewrite claims 9 and 10 as follows:

~~9.~~ (Rewritten) N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

~~10.~~ (Rewritten) N-allyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

REMARKS

The claims in the application are claims 9 and 10. All of the rejected claims have been cancelled and claims 9 and 10 have been rewritten as independent claims. These claims were indicated as allowable if the claims were written in independent form.

29

The case is now in condition for allowance and thereafter it is respectfully requested that it be passed to issue.

Respectfully submitted,

Richard T. Laughlin
Attorney for Applicant

Laughlin, Markensohn,
Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960

(201) 539-0080

DATED: FEB 10 1988

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
835,466	3/3/86	Rosin	

EXAMINER	
Shippen	
ART UNIT	PAPER NUMBER
126	11

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) M. Shippen (3)

(2) R. Laughlin (4)

Date of interview 3/21/88

Type: ☑ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description:

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed:

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

① Informed applicants that case was in condition for allowance

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1—7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☑ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

835,466

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER
	12

DATE MAILED:

NOTICE OF ALLOWABILITY

PART I.

1. [X] This communication is responsive to 2/16/88.
2. [X] All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. [X] The allowed claims are 9+10.
4. [ ] The drawings filed on _____ are acceptable.
5. [X] Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has [...] been received. [X] not been received. [...] been filed in parent application Serial No. _____, filed on _____
6. [ ] Note the attached Examiner's Amendment.
7. [ ] Note the attached Examiner Interview Summary Record, PTOL-413.
8. [ ] Note the attached Examiner's Statement of Reasons for Allowance.
9. [ ] Note the attached NOTICE OF REFERENCES CITED, PTO-892.
10. [ ] Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. [ ] Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. [ ] APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. [ ] Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. [ ] The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. [ ] Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. [ ] Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:

- _ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- _ Reasons for Allowance
- _ Notice of References Cited, PTO-892
- _ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

Michael L. Shippen
MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

PTOL-85 (Rev. 5-85)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

RICHARD T. LAGANI
129 HEADQUARTERS PLAZA
MORRISTOWN, N.J. 07960

All communications regarding this application should give the serial number, date of filing, name of applicant, and batch number.

Please direct all communications to the Attention of "OFFICE OF PUBLICATIONS" unless advised to the contrary.

The application identified below has been examined and found allowable for issuance of Letters Patent. PROSECUTION ON THE MERITS IS CLOSED.

SC/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
06/835,466	03/03/86	002	SHIPPEN, M 126	06/22/88

First Named Applicant: ROSIN, MARTA W.

TITLE OF INVENTION: PHENYL CARBAMATES

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
118-6848	560-115.000	H63	UTILITY	NO	$560.00	09/22/88

The amount of the issue fee is specified by 37 C.F.R. 1.18 as follows: for an original or reissue patent, except for a design or plant patent, $500; for a design patent, $175; and for a plant patent, $250. If the applicant qualifies for and has filed a verified statement of small entity status in accordance with 37 C.F.R. 1.27, the issue fee is one-half the respective amount aforementioned. The issue fee due printed above reflects applicant's status as of the time of mailing this notice. A verified statement of small entity status may be filed prior to or with payment of the issue fee. However, in accordance with 37 C.F.R. 1.28, failure to establish status as a small entity prior to or with payment of the issue fee precludes payment of the issue fee in the amount so established for small entities and precludes a refund of any portion thereof paid prior to establishing status as a small entity.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE as indicated above. The application shall otherwise be regarded as ABANDONED. The issue fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office. Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of the notice of allowance, the issue fee is charged to the deposit account at the time of mailing of this notice in accordance with 37 C.F.R. 1.311. If the issue fee has been so charged, it is indicated above.

In order to minimize delays in the issuance of a patent based on this application, this Notice may have been mailed prior to completion of final processing. The nature and/or extent of the remaining revision or processing requirements may cause slight delays of the patent. In addition, if prosecution is to be reopened, this Notice of Allowance will be vacated and the appropriate Office action will follow in due course. If the issue fee has already been paid and prosecution is reopened, the applicant may request a refund or request that the fee be credited to a Deposit Account. However, applicant may wait until the application is either found allowable or held abandoned. If allowed, upon receipt of a new Notice of Allowance, applicant may request that the previously submitted issue fee be applied. If abandoned, applicant may request refund or credit to a Deposit Account.

In the case of each patent issuing without an assignment, the complete post office address of the inventor(s) will be printed in the patent heading and in the Official Gazette. If the inventor's address is now different from the address which appears in the application, please fill in the information in the spaces provided on PTOL-85b enclosed. If there are address changes for more than two inventors, enter the additional addresses on the reverse side of the PTOL-85b.

The appropriate spaces in the ASSIGNMENT DATA section of PTOL-85b must be completed in all cases. If it is desired to have the patent issue to an assignee, an assignment must have been previously submitted to the Patent and Trademark Office or must be submitted not later than the date of payment of the issue fee as required by 37 C.F.R. 1.334. Where there is an assignment, the assignee's name and address must be provided on the PTOL-85b to ensure its inclusion in the printed patent.

Advance orders for 10 or more printed copies of the prospective patent can be made by completing the information in Section 4 of PTOL-85b and submitting payment therewith. If use of a Deposit Account is being authorized for payment, PTOL-85c should also be forwarded. The order must be for at least 10 copies and must accompany the issue fee. The copies ordered will be sent only to the address specified in section 1 or 1A of PTOL-85b.

☑ Note attached communication from Examiner.

☐ This notice is issued in view of applicant's communication filed _____

PATENT AND TRADEMARK OFFICE COPY

IMPORTANT REMINDER

Patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. See 37 CFR 1.20 (e)–(j).

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
06/835,466	03/03/86	ROSIN	M	118-6848

RICHARD T. LAGANI
129 HEADQUARTERS PLAZA
MORRISTOWN, N.J. 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	13

DATE MAILED: 08/12/88

NOTICE OF ABANDONMENT

This application is abandoned in view of:

1. ☐ Applicant's failure to respond to the Office letter, mailed _____.
2. ☐ Applicant's letter of express abandonment which is in compliance with 37 C.F.R. 1.138.
3. ☐ Applicant's failure to timely file the response received _____ within the period set in the Office letter.
4. ☑ Applicant's failure to pay the required issue fee within the statutory period of 3 months from the mailing date of 5-22-88 of the Notice of Allowance.

   ☐ The issue fee was received on _____.

   ☐ The issue fee has not been received in Allowed Files Branch as of _____.

   In accordance with 35 U.S.C. 151, and under the provisions of 37 C.F.R. 1.316(b), applicant(s) may petition the Commissioner to accept the delayed payment of the issue fee if the delay in payment was unavoidable. The petition must be accompanied by the issue fee, unless it has been previously submitted, in the amount specified by 37 C.F.R. 1.17 (l), and a verified showing as to the causes of the delay.

   If applicant(s) never received the Notice of Allowance, a petition for a new Notice of Allowance and withdrawal of the holding of abandonment may be appropriate in view of Delgar Inc. v. Schuyler, 172 U.S.P.Q. 513.

5. ☐ Applicant's failure to timely correct the drawings and/or submit new or substitute formal drawings by _____ as required in the last Office action.
   ☐ The corrected and/or substitute drawings were received on _____.
6. ☐ The reason(s) below.

DIRECT ANY INQUIRIES TO :
NAOMI SORRELL

OR
MARCIA CAMPBELL
PUBLISHING DIVISION
(703) 557-6403

PTO-1432 (REV. 5-83)

720 8/16/88 463

RECEIVED
SEP 21 1988
GROUP 120

14
9/24/88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
ROSIN ET AL.	:	ART UNIT 126
Ser. No. 06/835,466	:	Michael L. Shippen Examiner
Filed: March 3, 1986	:	
For: Phenyl Carbamates	:	

Honorable Commissioner of Patents and Trademarks

Washington, D. C. 20231

Dear Sir:

This is in response to the Notice of Abandonment in the above special case for failure to pay the issue fee.

This application was formally abandoned when the continuing application Serial No. 07/185,451 was filed on 04/25/88.

Would you please correct your records to show this fact.

Respectively submitted,

Dated September 14, 1988

Richard T. Laughlin
Attorney for Applicants
Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Place, North Tower
Morristown, New Jersey 07960
201-539-0080

PTO/SB/68 (11-96)
Approved for use through 10/31/99. OMB 0651-0031
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

RECEIVED
JUN 1 4 2001
File Information Unit

In re Application of	
Application Number: 08/835466	Filed: 3-3-86
Group Art Unit:	Examiner:

Paper No. 15

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

✓ (A) referred to in United States Patent Number 4948807 column _____

___ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. _____ filed _____ on page _____ of paper number _____

___ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. _____ filed _____ or

___ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

_____

_____

_____

Henry Owens
Signature

6-14-01
Date

HENRY OWENS
Typed or printed name

FOR PTO USE ONLY
Approved by: _____ (Initials)
Unit: _____

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Washington, DC 20231.

PTO/SB/68 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

RECEIVED
MAR 2 0 2003
File Information Unit

In re Application of	
Application Number 06/835,766	Filed 3/03/86
Art Unit	Examiner

Paper No. #16

Assistant Commissioner for Patents
Washington, DC 20231

1. ☐ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☐ (A) referred to in:

United States Patent Application Publication No. 4948807, page _____, line_____,

United States Patent Number_____, column _____, line _____, or

an International Application which was filed on or after November 29, 2000 and which

designates the United States, WIPO Pub. No. _____, page _____, line_ ____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No._____, paper No. _____, page _____, line _____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

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REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(a)

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In re Application of	
Application Number: 06/835,466	Filed: Mar. 3, 1986
Art Unit	Examiner

Paper No. 17

Assistant Commissioner for Patents
Washington, DC 20231

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☒ (A) referred to in:

United States Patent Application Publication No. _____, page _____, line _____,
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an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. _____, page _____, line _____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No._____, paper No: _____, page _____, line _____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

B. Rhodes
Signature

05-29-03
Date

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REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number	Filed
06/835466	3/3/86

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Paper No. #18

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United States Patent Application Publication No. _____, page, _____ line _____.

United States Patent Number 4948807, column _____, line, _____ or

WIPO Pub. No._____, page _____, line _____.

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Steven J. Diley
Signature

12/19/03
Date

Steven Diley
Typed or printed name

Registration Number, if applicable

703-416-0366
Telephone Number

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REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

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In re Application of: Bosi et al

Application Number	Filed
06/835,466	3/3/86

Paper No. 17

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. _____, page, _____ lins _____,

United States Patent Number 4,948,807, column _____, line, _____ or

WIPO Pub. No._____, page _____, line _____.

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Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

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BA Harris
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1-16-04
Date

BA Harris
Typed or printed name

Registration Number, if applicable

703-415-0006
Telephone Number

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REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

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In re Application of: ROSIN ET AL

Application Number	Filed
06/835,466	3-3-86

Paper No. 10

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United States Patent Number 4948807, column _____, line, _____ or

WIPO Pub. No._____, page_____, line _____.

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C. W.
Signature

2-19-04
Date

CHRISTOPHER WALKER
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REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of: Marta W. Rosin

Application Number: 06/835466 Filed: 3/03/86

Paper No. #21

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United States Patent Application Publication No. 4948807, page, _____ line _____,

United States Patent Number _____, column _____, line, _____ or

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Date

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In re Application of: Rosin et al

Application Number	Filed
06/835,466	Mar 3, 1986

Paper No. 22

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United States Patent Number 4948807, column _____, line, _____ or

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(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
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Bill Mueller
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June 18, 2004
Date

Bill Mueller
Typed or printed name

Registration Number, if applicable

332-8355
Telephone Number

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In re Application of

Application Number	Filed
06/835,466	03-03-86

Paper No. #23

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

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United States Patent Number 4948807, column _____, line, _____ or

WIPO Pub. No._____, page _____, line _____.

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Shoaib Chaykoz
Signature

7-6-04
Date

Shoaib Chaykoz
Typed or printed name

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United States Patent [19]

Rosin et al.

[11] **Patent Number: 4,948,807**

[45] **Date of Patent: Aug. 14, 1990**

[54] **PHENYL CARBAMATES**

[75] Inventors: **Marta W. Rosin; Michael Chorev; Zeev Tashma, all of Jerusalem, Israel**

[73] Assignee: **Proterra AG, Zug, Switzerland**

[21] Appl. No.: **320,700**

[22] Filed: **Mar. 8, 1989**

Related U.S. Application Data

[63] Continuation of Ser. No. 185,451, Apr. 25, 1988, abandoned, which is a continuation of Ser. No. 835,466, Mar. 3, 1986, abandoned.

[30] **Foreign Application Priority Data**

Mar. 5, 1985 [IL] Israel 74497

[51] Int. Cl.[5] C07C 125/067; A61K 31/27

[52] U.S. Cl. 514/484; 514/330; 514/487; 514/490; 514/237.5; 544/162; 546/226; 560/32; 560/115; 560/136

[58] Field of Search 560/115, 163, 136; 514/484, 490, 487

[56] **References Cited**

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FOREIGN PATENT DOCUMENTS

1037753	7/1956	Fed. Rep. of Germany	560/136

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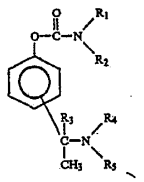
Primary Examiner—Michael L. Shippen
Attorney, Agent, or Firm—Ribis, Graham, Verdon & Curtin

[57] **ABSTRACT**

Phenyl carbamates of the general formula



I

wherein R_1 to R_5 are as defined in the claims, are useful as pharmaceuticals.

4 Claims, No Drawings

ENT NUMBER

APPLICATION SERIAL NUMBER
835,466

APPLICANT'S NAME (PLEASE PRINT)
Rosin, et al

IF REISSUE, ORIGINAL PATENT NUMBER

INTERNATIONAL CLASSIFICATION (INT. CL. 4)	
C07C	125/067
A61K	31/22

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
560	115

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
514	234	330	484	487
514	490			
544	162			
546	226			
560	32	163		

GROUP ART UNIT: 126

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)
Michael L. Shippen

PTO 270 (10-84)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

FORM PTO-875 (REV. 1-86)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	FILING DATE
PATENT APPLICATION FEE DETERMINATION RECORD		835 466	3-3-86
		APPLICANT (FIRST NAMED)	Roben, et al

CLAIMS AS FILED - PART I

FOR:	NO. FILED	NO. EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
BASIC FEE				$170	OR		$340
TOTAL CLAIMS	13 -20=	*	X8=	$	OR	X12=	$
INDEP. CLAIMS	3 -3=	*	X17=	$	OR	X34=	$
☐ MULTIPLE DEPENDENT CLAIM PRESENT			X55=	$	OR	X110=	$
			TOTAL	$	OR	TOTAL	$340

* If the difference in col. 1 is less than zero, enter "0" in col. 2

CLAIMS AS AMENDED - PART II

	(1)		(2)	(3)	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	X5=	$	OR	X10=	$
INDEP.	*	MINUS	***	=	X15=	$		X30=	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	X5=	$	OR	X10=	$
INDEP.	*	MINUS	***	=	X15=	$		X30=	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	X5=	$	OR	X10=	$
INDEP.	*	MINUS	***	=	X15=	$		X30=	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$		+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest No. Previously Paid For" (Total or Indep.) is the highest number found in the appropriate box in Col. 1.

PTO-1130 (Rev. 3-83) 835466 **PALM III APPLICATION FILE DATA CODING SHEET** U.S. DEPARTMENT OF COMMERCE–PATENT & TM OFFICE | PREPARED BY NB | DATE 5/27/86

FORMAT NO. 2 Serial No.	TYPE APPL	FILING DATE Month	Day	Year	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWINGS	ASGT	TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE RECEIVED	SECURITY CASE?	FOREIGN LICENSE?
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FORMAT NO. 3 ATTORNEY DOCKET NUMBER (12 spaces)	Atty.'s Reg. Nos.	FORMAT NO. 4 Applicant's Name & Address	FORMAT NO. 5 Title of Invention	FORMAT NO. 6&7 Correspondence Address
118-6848	☑ APPL. PAPERS ☐ CODING SHEET	☐ APPL. PAPERS ☐ CODING SHEET	☐ APPL. PAPERS ☐ CODING SHEET	☐ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 8

		CONTINUITY CODE	PARENT APPLICATION SERIAL NUMBER	PARENT FILING DATE Month Day Year	STATUS CODE	PARENT PATENT NUMBER
RECORD 801			0			
RECORD 802			0			
RECORD 803			0			
RECORD 804			0			
RECORD 805			0			
RECORD 806			0			
RECORD 807			0			
RECORD 808			0			
RECORD 809			0			
☐ MORE ON SUPPLEMENTAL CODING SHEET RECORD 810			0			

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RECORD 902				
RECORD 903				
RECORD 904				
RECORD 905				
RECORD 906				
☐ APPLICATION PAPERS RECORD 907				
RECORD 908				
RECORD 909				
☐ MORE ON SUPPLEMENTAL CODING SHEET RECORD 910				

SEARCH NOTES

	Date	Exr
CAS ONLINE		

SEARCHED

Class	Sub	Date	Exr
560	~~100~~	Pulled	
	163		
	32		
	115		
544	162		
546	226		
514	234		
	330		
	487		
	484		
	490		
	Searched	9/87	
	Updated	3/88	

INTERFERENCE SEARCHED

Class	Sub	Date	Exr
560	115	3/88	
	163		

PRINT CLAIM(S): 1 & 2

INDEX OF CLAIMS

Claim Final	Claim Original	Date 3/88	Claim Final	Claim Original	Date
	1	✓		26	
	2	✓		27	
	3	✓		28	
	4	✓		29	
	5	✓		30	
	6	✓		31	
	7	✓		32	
	8	✓		33	
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	12	✓		37	
	13	✓		38	
	14			39	
	15			40	
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	25			50	

SYMBOLS	STATUS
✓	Rejected
=	Allowed
– (Through numeral)	Canceled
+	Restriction requirement
N	Nonelected invention or species
I	Interference
A	Appeal
O	Objected

835466

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Briefed In Gr. 120

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CONTENTS

1. Applications papers.
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3. Dead Surcharge + Extension received 5/19/86 JUN 13 1986
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15. Request for Access 6-14-01
16. Request for Access 3/20/03
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APPLICANTS

MARTA W. ROSIN, JERUSALEM, ISRAEL; MICHAEL CHOREV, JERUSALEM, ISRAEL; ZEEV TASHMA, JERUSALEM, ISRAEL.

CONTINUING DATA********
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TITLE
PHENYL CARBAMATES

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE	CLAIMS ALLOWED	
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PRIMARY EXAMINER
ART UNIT 126
Primary Examiner

ISSUE CLASSIFICATION		ISSUE BATCH NUMBER
Class	Subclass	
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185451

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

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PTO-1556
(5/87)

Case 118-6848

501

PHENYL CARBAMATES

The present invention relates to novel phenyl carbamates which are useful as pharmaceutical compositions. The invention further relates to pharmaceutical compositions having anticholinesterase activity.

Acetylcholine is a major neurotransmitter which is found in all parts of the body. Any reduction in its activity, either as a result of neuronal damage, degeneration etc. or as induced by drugs or toxins, causes marked changes in the function of the organism. Acetylcholine itself has an extremely short half life, since it is rapidly hydrolysed at its site of action and in plasma by specific cholinesterase enzymes. Drugs that inhibit acetylcholinesterase, markedly increase and prolong the action of acetylcholine, thereby enhancing cholinergic transmission. Three such agents are used clinically, i.e., physostigmine, a naturally occurring alkaloid, and two synthetic analogues, neostigmine and pyridostigmine. The latter two agents are strongly ionised at physiological pH and therefore are only poorly absorbed from the gastro-intestinal tract, and do not penetrate the central nervous system to any significant extent. Physostigmine is absorbed after

oral administration and readily enters the brain. As a therapeutic agent it has several disadvantages. It is chemically unstable and must be prepared in solution with an antioxidant, and protected from light. It has a relatively short half-life (20-40 mins) thereby necessitating frequent administration. The latter is of particular importance when the drug is to be administered chronically. It has a low therapeutic ratio, a value of 3-5 being reported in the majority of studies in laboratory animals, and a small therapeutic window, i.e. small range of dose in which it can be given without the accompaniment of side effects. Although physostigmine is absorbed from the gastro-intestinal tract, this is reported to be irregular and unpredictable, and therefore it is usually preferred to administer the drug parenterally. This is a serious drawback if it is to be used chronically on an outpatient basis.

There are a number of clinical and pathological conditions which are associated with cholinergic under-activity which can be improved by the administration of an anticholinesterase agent. These include reduction in cholinergic transmission induced by a variety of exogenous substances acting in the peripheral, or central nervous system. Peripherally acting agents are gallamine, d-tubocurarine and pancuronium, which are used as muscle relaxants. Their action can readily be overcome by an anticholinesterase drug. Drugs which interfere with central cholinergic transmission are numerous, anticholinergic, atropine-like drugs including antiparkinson drugs, tricyclic antidepressants, neuroleptics, opiate analgesics, benzodiazepines and some types of general anaesthetics. So far the only agent that has proved to be of any value in reversing the effects of the latter group of drugs is physostigmine. In all reported cases of drug overdose or lack of recovery when the agent was used peri-operatively, physo-

stigmine is usually administered parenterally, and administration is repeated every 20-30 minutes as required.

Chronic treatment with neuroleptics often results in tardive dyskinesias. The widespread use of agents having anticholinesterase activity for the treatment of schizophrenia makes this side effect an ever increasing possibility. Physostigmine injected intravenously produces a significant but short lived improvement in a proportion of patients.

A number of pathological and degenerative diseases has also been shown to be associated with a reduction or loss of cholinergic transmission. This includes myasthenia gravis and Eaton Lambert syndrome in which there is an interference with neuromuscular transmission.

A selective loss of choline acetyltransferase (the enzyme that synthesises acetylcholine) has been found in specific brain regions of patients with pre-senile dementia of the Alzheimer type. These include the frontal and temporal cortex, hippocampus, amygdala, caudate nucleus, substantia innominata. Degeneration of cholinergic neurons in some of these areas appears to be associated with the aphasia, apraxia, agnosia and loss of short term memory that occurs in Alzheimer's disease. A similar type of dementia is also found in patients with Down's syndrome that survive to the age of 40 years and show similar cholinergic deficits. There is also a loss of cholinergic transmission in the caudate nucleus and putamen of patients with Huntingdon's chorea. Physostigmine injections have also been of some benefit in this condition. Treatment with a centrally acting anticholinesterase should also prove to be beneficial in Friedrich's ataxia.

There are two major classes of potent inhibitors of the enzyme cholinesterase. The first group was modelled primarily on the natural alkaloids physostigmine (a carbamate) and an inhibitor of cholinesterase, and d-tubocurarine, an antagonist of acetylcholine. The second group consists of various organophosphorus compounds, such as diisopropylfluorophosphonate, paraxon etc. The vast majority of the compounds of both these series were designed primarily as insecticides. In the first group of carbamate derivatives, almost all of the potent insecticides are monomethyl carbamates lacking a charged nitrogen function. This enables the molecule to penetrate rapidly the insect cuticle and fatty nerve sheath. The dimethyl derivatives are slightly less potent but are particularly toxic to houseflies and aphids. The monomethyl derivatives tend to be unstable in solution and hydrolyse readily at physiological pH. This greatly limits their biological action in mammals and makes them less suitable as pharmaceutical or therapeutic agents.

The organo-phosphorus group of compounds causes irreversible inhibition of cholinesterase and other serine containing enzymes, which, together with their high relative toxicity, virtually precludes their use in pharmaceutical preparations. The only exception is echothiopate, a quaternary ammonium organophosphorus compound, employed in eye drops for the treatment of glaucoma.

The synthetic anticholinesterase agents currently employed as pharmaceuticals all contain a charged nitrogen function and can be broadly classified into 3 groups.

1) Reversible inhibitors which contain a charged nitrogen function attached to an aromatic ring, e.g. edrophonium.

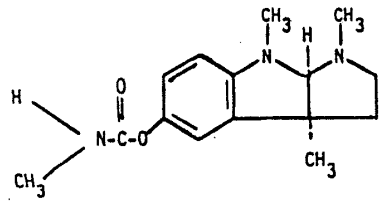
2) Dimethyl carbamates with an aromatic or heterocyclic ring containing a charged nitrogen, neostigmine, pyridostigmine.

3) Bisquaternary structures, e.g. Demacarium, Ambenonium. These agents tend to be more selective inhibitors of acetylcholinesterase than butyrylcholinesterase, compared with the monoquaternary molecules.

The pharmaceutical application of the quaternary anticholinesterase agents is limited because of their poor penetration through cell membranes. They are therefore used for actions outside the central nervous system, and are usually given parenterally, since they are not reliably absorbed from the gastrointestinal tract. Edrophonium, neostigmine and pyridostigmine and the bisquaternary analogues are used in anaesthetic practice for the reversal of the action of muscle relaxants. They are also used for the treatment of myasthenia gravis, and paralytic ileus.

Physostigmine is the only potent anti-cholinesterase agent which has been used clinically to treat conditions in which an elevation of brain acetylcholine activity is desired. These include, Alzheimer's disease, tardive dyskinesia, Down's syndrome and Huntingdon's chorea. Physostigmine is also used to reverse the effects of overdose of anticholinergic agents, anti-Parkinson drugs, benzodiazepines and opiate analgesics.

Physostigmine is a natural alkaloid extracted from calabar beans and the seeds of the vine Physostigma venenosum and has the formula



There is a need to provide new carbamate derivatives which show greater chemical stability than physostigmine.

Furthermore there is a need to provide new compounds which inhibit acetylcholinesterase in the brain for periods exceeding 3 hours but not more than 12 hours after a single administration.

There is also a need to provide new compounds which will be completely and reliably absorbed after oral administration.

There is also a need to provide new compounds which will be relatively less toxic than physostigmine. This means that the therapeutic ratio, defined as

$$\frac{\text{dose to produce therapeutic effect}}{\text{dose to produce mortality in 50 \% of animals}}$$

should be significantly higher than those of physostigmine and that the incidence and severity of side effects should be less than those of physostigmine at therapeutic doses.

There is also a need to provide new compounds which can be given orally or parenterally to treat chronic conditions in which it is

desired to raise cholinergic activity in the central nervous system. These include, Alzheimer's disease, Down's syndrome, Huntingdon's chorea, Friedrich's ataxia.

There is also a need to provide compounds that can be given parenterally at the end of operations, and anaesthetic procedures, to restore wakefulness, respiration and cardiovascular parameters to normal, after the use of anticholinergic, opiates, benzodiazepines, neuroleptics and general anaesthetics, thereby shortening the stay of patients in the recovery room.

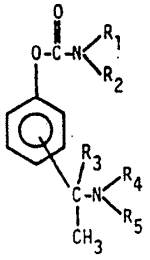
There is also a need to provide compounds that can be given together with narcotic analgesics to patients suffering from severe pain, e.g. traumatic, post-operative, or due to carcinomatosis etc. in order to reduce the side effects (respiratory depression, somnolence, constipation and urinary retention) commonly encountered with narcotics, without impairing their analgesic potency.

There is also a need to provide compounds that can be given to patients receiving antipsychotic drugs, which have developed tardive dyskinesias, in order to diminish or abolish the latter syndrome, without exascerbating the psychosis.

According to the present invention it has now been surprisingly found that certain novel and known phenyl carbamates also inhibit acetylcholinesterase in the mammalian brain after administration to provide systemic activity, e.g. oral or parenteral administration.

Thus according to the present invention there is now provided a pharmaceutical composition adapted to produce anticholinesterase

activity in the central nervous system of mammals comprising a compound of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

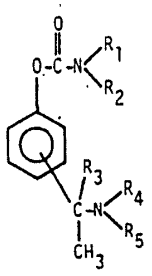
R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor. Hereinafter these compounds are called compounds of the invention.

Especially preferred are pharmaceutical compositions having anticholinesterase activity in the central nervous system of mammals, wherein the dialkylaminoalkyl group is in the meta position, and R_4 and R_5 are both methyl.

Certain compounds falling within the above formula have previously been described i.e. the m disubstituted compound in which R_1 and R_3 = H and R_2, R_4 and R_5 = methyl which is known as Miotine(R) was claimed to be an insecticide and a myopic agent for use in eye drops. The m disubstituted compound in which R_1 and R_2 are methyl, R_3 is H and R_4 and R_5 are methyl has been described as an insecticide. The p and o disubstituted derivatives in which R_1 and R_3 = H and R_2, R_4 and R_5 = CH_3 have been shown to inhibit a preparation of liver cholinesterase. The m disubstituted derivative in which R_1 = H and R_2, R_3, R_4 and R_5 = CH_3 has also been shown to inhibit liver cholinesterase.

The remaining compounds are believed to be novel and thus the present invention also provides novel phenyl carbamate derivatives of the general formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

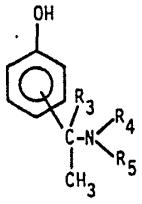
R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

Preferred compounds of the above formula are N-ethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate, N-allyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N-butyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-methyl, N-propyl-3[1-(dimethylamino)ethyl]phenyl carbamate and N-ethyl, N-methyl-3[1-(dimethylamino)isopropyl]phenyl carbamate.

As indicated, the invention also includes the pharmacologically acceptable salts of these compounds such as the acetate, salicylate, fumarate, phosphate, sulphate, maleate, succinate, citrate, tartrate, propionate and butyrate salts thereof.

The compounds of formula I can be prepared by amidating a compound of formula II

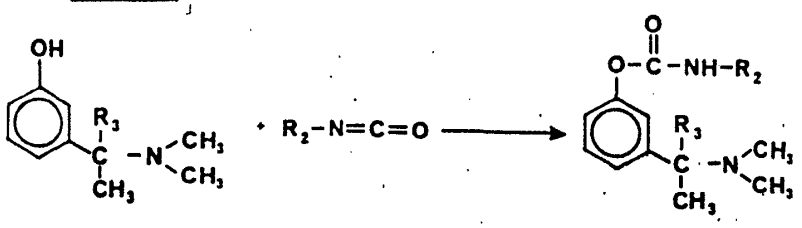


II

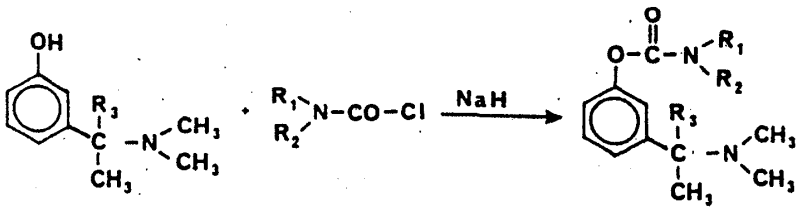
wherein R_3, R_4 and R_5 are as defined above.

The process can be effected in conventional manner, e.g. by reacting the compound of formula II with an appropriate isocyanate if a compound wherein R_1 is hydrogen is desired, or with an appropriate carbamoyl halogenide, e.g. as described below in processes A and B.

PROCESS A:



PROCESS B:



PROCESS A:

A stirred suspension of α-m-Hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in benzene (0.2 - 0.3 g/ml) is treated with 2.5 - 3 fold molar excess of the isocyanate. After stirring for 15 - 24 hours at ambient temperature the reaction mixture is connected to a rotovaporator (20 mm Hg). The residue obtained is dissolved in dry ether (25 ml) and the solution, which is ice cooled, is saturated with dry HCl (g). The formed precipitate (the anticipated carbamate) is filtered off, washed with dry ether (25 ml) and dried to constant weight in a dessicator over KOH pellets under high vacuum (0.1 mm Hg).

PROCESS B:

A solution of α-m-hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in dry acetonitrile (0.1 - 0.5 M) is reacted with 50 - 70 % molar excess of the corresponding carbamoyl chloride in the presence of 200 % molar excess of NaH dispersion (50 - 80 % in mineral oil). The reaction mixture is left to stir at ambient temperature for 15 - 24 hours. Removal of the acetonitrile under reduced pressure (20 mm Hg) is followed by the addition of water (10 - 25 ml). The pH of the aqueous solution is adjusted to pH = 11 by the addition of the appropriate amount of NaOH 0.1 N followed by extraction with ether (3 x 25 ml). The combined organic phases are washed with brine (25 ml) dried over $MgSO_4$ anhydride which is then filtered off. The ice cooled etheral filtrate is saturated with a stream of HCl (g) resulting in the formation of a heavy precipitate (the anticipated carbamate) which is collected by filtration, washed with dry ether (20 ml) and dried to constant weight in a desiccator under high vacuum (0.1 mm Hg) over KOH pellets.

The compounds of the invention e.g. in free form or salt form can be utilized by formulating one or more of them in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. A compound or mixture of compounds of formula (I) or physiologically acceptable salt(s) thereof is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as mangnesium stearate; a sweetening agent such as sucrose, lactose or saccarin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other mterials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavour.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice by dissolving or suspending the active substance in a vehicle such as water for injection.

Buffers, preservatives, antioxidants and the like can be incorporated as required.

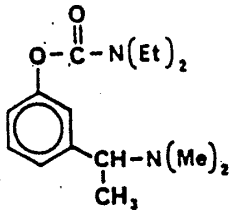
Preferred antioxidants for use with the compounds of the present invention include sodium metabisulphite and ascorbic acid.

While the invention will now be described in connection with certain preferred embodiments in the following examples, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars described are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the invention.

EXAMPLE 1

0.5 g (3.03 mmole) of α-m-hydroxyphenylethyldimethylamine are dissolved in 15 ml of dry acetonitrile and 0.70 g (5.2 mmole) of diethylcarbamylchloride are added to the mixture with stirring. This is followed by NaH 150 mg (50 %) of dispersion. The reaction mixture is stirred overnight at 25 - 30 ° C. Removal of acetonitrile under reduced pressure is followed by addition of water (10 ml) and adjustment of the pH to 11. The product is extracted in ether, which is washed by brine, dried over $MgSO_4$ and filtered. Upon addition of HCl (g) precipitation occurs immediately, the product is filtered off, washed by dry ether and dried in a desiccator under high vacuum over KOH pellets.

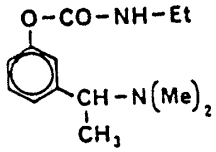
The carbamate is obtained as a white powder 640 mg (80 %) mp. 137 - 138 ° and identified as N,N-diethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, having the formula



EXAMPLE 2

0.75 g (4.55 mmol) of α-m-hydroxyphenylethyldimethylamine are suspended in benzene (3 ml) and 0.898 g of ethylisocyanate are added to the mixture with stirring. After stirring 12 hours at room temperature the solvent is removed under reduced pressure.

The residue obtained was dissolved in dry ether. Introduction of dry HCl gas into the reaction mixture causes a heavy precipitation. The product is filtered off, washed with ether and dried in a desiccator over KOH pellets. The carbamate is obtained as a white powder 800 mg (75 %) mp. 177 - 179 ° C and identified as N-ethyl-3[1-(dimethylamino)ethyl]phenyl carbamate having the formula



The compounds of the present invention are useful as pharmaceuticals. In particular they show the following activities in vitro and in vivo in the tests specified below.

The values are correct when taken in comparison with the standard drug physostigmine.

IN VITRO EXPERIMENTS:

Tests for anticholinesterase activity

A solubilized preparation of acetylcholinesterase was prepared from mouse whole brain (minus cerebellum). The brain was homogenized with (100 mg/ml) phosphate buffer; pH 8.0, centrifuged, the supernatant discarded, and the pellet mixed with a similar volume as above of buffer pH 8.0 plus 1 % Triton; mixed, centrifuged and the supernatant which contained most of the solubilized enzyme, was used for the subsequent determinations of anticholinesterase activity.

The activity of the enzyme (rate of hydrolysis of substrate, acetylthiocholine) was measured using at least 4 different concentrations of substrate, and at least 3 different concentrations of each inhibitor. The enzyme was incubated with inhibitor for periods ranging for 2 - 180 mins. at 37 ° C, substrate was then added, and its rate of hydrolysis measured by the spectrophotometric method of Ellman et al. (1961).

The molar concentration of each agent that inhibited the activity of the enzyme by 50 % (IC_{50}) at the peak time of activity (15 - 60 min) was calculated from this data and recorded in Table 1 hereinafter. The compounds in general produce a significant inhibition from about 10^{-5} to about 10^{-8} molar.

IN VIVO EXPERIMENTS:

a) Assessment of acetylcholinesterase inhibition

The effect of each compound on brain acetylcholinesterase in vivo was measured, after subcutaneous or oral administration to mice. Animals were sacrificed, at different times ranging from 0.25 - 8 hours after drug administration. The brain was rapidly removed, and the enzyme acetylcholinesterase extracted and solubilized with 0.1 % Triton, and its ability to hydrolyse acetylthiocholine assessed as described above (in vitro experiments), in comparison with the enzyme removed from mice injected with normal saline. The compounds have in general a potency of from about 2% to about 90% that of physostigmine.

b) Assessment of acute toxicity

Mice were given one of at least three different doses of each compound, orally or subcutaneously, a minimum of 10 mice allotted to each dose. The number of animals which died at

each dose within 3 hours was determined. From these data, the LD_{50} (dose in mg/kg which was lethal to 50 % of the mice) was computed.

This experiment was repeated after the animals had been pretreated with atropine sulphate, which blocks both peripheral and central muscarinic receptors. The data from these experiments enabled the assessment of the relative degrees of toxicity of the carbamates which result from excessive activation of muscarinic receptors, and from respiratory muscle paralysis, which is insensitive to this blocking agent.

The incidence and degree of side effects was noted for each dose of drug, starting with the lowest that caused any significant (> 20 %) inhibition of whole brain acetylcholinesterase.

c) Antagonism of the somnolent and respiratory depressant effects of opiates

Different doses of the carbamate compounds were injected intravenously with morphine in rabbits. Respiration rate, arterial blood gas tensions and pH were monitored continuously before and after drug administration for 4 - 5 hours. In another series of experiments the effect of the anticholinesterase drugs was assessed on the analgesic effect of opiates in rabbits after application of a nociceptive stimulus, i.e. electrical stimulation of the sciatic nerve.

All specific examples of formula I' mentioned hereinbefore, e.g. on specification page 10, and after especially Tables 1 to 3, are prepared in analagous manner to Example 1 when R_1 and R_2 are each other than hydrogen and Example 2 when one of R_1 and R_2 are hydrogen. They are thus obtained as hydrochloride salts (except where otherwise specified). The specific compounds have metal substitutions.

Table 1

In vitro activity on solubilized mouse brain enzyme

Compound (R_4=R_5=CH_3)	R_1	R_2	R_3	IC_{50}(M)	Time of peak activity (mins)
Physiostigmine (Salicylate)	H	CH_3	H	$1.1x10^{-8}$	30
Miotine HCl	H	CH_3	H	$1.3x10^{-8}$	30
RA_6 HCl	H	C_2H_5	H	$4.0x10^{-7}$	120
RA_{15} HCl	H	C_3H_7 n-propyl	H	$1.1x10^{-7}$	120
RA_{14} HCl	H	C_3H_5 allyl	H	$4.3x10^{-7}$	120
RA_{13} HCl	H	C_3H_7 isopropyl	H	$1.2x10^{-5}$	120
RA_5 HCl	H	C_4H_9 n-butyl	H	$7.6x10^{-8}$	120
RA_{12}	H	cyclohexyl	H	$9.3x10^{-8}$	120
RA_{10} HCl	CH_3	CH_3	H	$2.7x10^{-8}$	120
RA_7 HCl	CH_3	C_2H_5	H	$1.3x10^{-6}$	90
RA_8 HCl	C_2H_5	C_2H_5	H	$3.5x10^{-5}$	30
RA_{11} HCl	morpholino		H	$>2x10^{-5}$	30
RA_4 HCl	CH_3	propyl	H	$1.7x10^{-6}$	60

Melting points of compounds (all in the hydrochloride form except for RA_{12} which is in the free base form as it precipitated from the reaction mixture before addition of hydrogen chloride) are in degrees Centigrade: RA_6 167-170; RA_{15} 141-143; RA_{14} 147-152; RA_{13} 146-148; RA_5 158-162; RA_{12} 75-77; RA_{10} 145; RA_7 135-136; RA_8 137-138; RA_{11} amorphous; RA_4 148-149.

Compound RA_{11} has an RF value of 0.59 in a system of 95 parts of ethyl acetate and 5 parts of 33% (w/w) dimethylamine in ethanol.

T220X

Table 2

Anticholinesterase activity of compounds in mouse brain compared to that of physostigmine

Compound	Relative potency to physostigmine after subcut. (s.c.) administration	Relative potency to physostigmine after oral administration	% cholinesterase inhibition 3 hours after s.c. administration
Physo-stigmine	100	100	0
Miotine	100	300	5
RA_6	11	19	35
RA_{15}	33	32	37
RA_{14}	15	22	35
RA_{13}	2	5	-
RA_5	36	29	30
RA_{12}	13	17	37
RA_{10}	81	92	7
RA_7	25	57	41
RA_8	2	5	32
RA_4	13	29	25

22X

Table 3

Acute toxicity of carbamates in mice

Compound	LD_{50} µmoles/kg s.c.	Degree of* protection afforded by pretreatment with atropine	Therapeutic ratio LD_{50}/ED_{50} s.c.	$\frac{LD_{50}\ oral}{LD_{50}\ s.c.}$
Physostigmine	3.0	3.0	3.3	4.1
Miotine	4.5	2.4	4.9	1.2
RA_6	96	2.6	11.9	2.1
RA_{15}	31	4.1	11.1	4.5
RA_{14}	69	8.0	11.5	4.4
RA_{13}	65	4.5	1.6	1.1
RA_5	19	5.8	7.6	5.0
RA_{12}	42	3.8	5.8	3.6
RA_{10}	14	5.0	12.7	9.7
RA_7	46	10.4	12.4	1.2
RA_8	> 568	-	> 10.0	-
RA_4	72	4.9	10.0	1.7

*Ratio of LD_{50} after pretreatment with atropine sulphate 5 mg/kg to LD_{50} of drug alone.

The data in Tables 1 and 2 demonstrate that somewhat larger quantities are required of all the drugs of the RA series than of physostigmine to inhibit the enzyme acetylcholinesterase. However, a comparison of the data in Table 1 with that in Table 2, shows that compounds RA_5, RA_6, RA_{15}, RA_{14}, RA_{10}, RA_7 and RA_8 are all relatively more active in vivo compared to physostigmine than one would expect from the in vitro data. This greater in vivo potency is particularly marked when the drugs are administered orally. This relatively greater in vivo activity may be due to:

a) greater chemical stability

b) a slower metabolic degradation or/and excretion

c) a higher lipid solubility, enabling a greater proportion of the drug to gain access to the enzyme in the central nervous system

d) more efficient absorption from gastro-intestinal tract.

For the purposes of their therapeutic application it is of little importance if one needs to give the drug (to human subjects) at a dose of 1 - 2 mg (physostigmine) or 2 - 50 mg that may be required of the compounds of the RA series. What is important is the safety of the drugs and the presence and severity of side effects that may occur at therapeutic doses. A commonly-used measure of drug safety is the therapeutic index - or LD_{50}/ED_{50}

$$\frac{\text{Dose to kill 50 \% of animals}}{\text{Dose to cause the desired therapeutic effect}}$$

24

It is assumed that the therapeutic effect of these anticholinesterase agents results from an elevation of brain cholinergic activity. This in turn, should be related to the degree of inhibition of acetylcholinesterase. For the purpose of the computation of the denominator of the therapeutic ratio, there is used the dose of drug that inhibits the activity of acetylcholinesterase by 50 %. This is based on the observation by Thal et al. (Ann. Neurology 13: 491, 1983) that the maximum improvement in short term memory obtained in a series of patients with Alzheimer's disease was achieved with a dose of physostigmine which blocked the acetylcholinesterase in the cerebro-spinal fluid by 50 %. The numerator is the dose found to kill 50 % of the animals within 4 hours of a subcutaneous injection.

The therapeutic ratios of compounds RA[4], 5, 6, 7, 8, 10, 14 and 15 are all significantly higher than of physostigmine (see Table 3). This indicates that all these compounds have a wider margin of safety than that of physostigmine. Moreover, these RA compounds do not produce any significant undesirable side effects such as defaecation, lachrymation, fasciculations or tremor at the doses which inhibit the brain enzyme by 50 %, while the former 3 side effects are clearly evident when physostigmine is given at the appropriate dose (ED_{50}).

The data in Table 3 show that atropine can afford considerably greater protection against the lethality of the derivatives RA[4], 5, 7, 10, 13 and 14. This is particularly important in the treatment of drug overdose since the respiratory muscle paralysis which is not affected by atropine and which is the cause of death induced by excess drug administration in the presence of atropine cannot be satisfactorily reversed by specific antidotes.

The duration of significant brain enzyme inhibition (> 30 %) induced by physostigmine (ED_{50} dose) is less than 2 hours. Compounds RA_4, 5, 6, 7, 8, 12, 14, 15 all act for more than 3 hours at their respective ED_{50} doses and RA_6 and RA_7 still causes significant inhibition (36 %) after 7 hours. Since none of these drugs caused noticeable side effects at the ED_{50} doses, an even longer duration of action may be achieved by giving between 50 and 100 % larger doses. The longer duration of action is a distinct advantage, particularly if the drugs are to be administered chronically to subjects suffering from neurological and behavioural conditions associated with a deficit in cholinergic transmission in the central nervous system, e.g. Alzheimer's disease, tardive dyskinesias, Huntingdon's chorea, Down's syndrome and Friedrich's ataxia.

The better the absorption of the drug after oral administration the more closely the LD_{50} given by this route resembles that after subcutaneous injection. Table 3 shows that RA_6, 13, 7 and 4 are more efficiently absorbed from the gastro-intestinal tract than is physostigmine. The ED_{50} of RA_8 after oral administration is the same as that after S.C. injection, indicating a much better oral bioavailability than that of physostigmine. The higher oral bioavailability of these compounds may be a considerable advantage for their clinical use.

RA_{10}, RA_6, RA_{14} and RA_{15} produce significant antagonism of the respiratory depressant effects of morphine in rabbits for periods lasting between 3 - 5 hours depending on the drug and the dose administered. The analgesic activity of morphine is not reduced by the RA compounds. Muscle fasciculations are not evident at the doses of drugs administered. Physostigmine (0.1 - 0.2 mg/kg) antagonizes the respiratory depressant effect of morphine for

30 - 60 mins only and fasciculations are marked at the higher dose.

These findings show that the RA compounds may be given together with morphine to obtain adequate analgesia without significant degrees of respiratory depression.

The most preferred compounds of the RA series are RA_4, RA_5, RA_6, RA_{15}, RA_{14}, RA_7 and RA_8, all of which produce inhibition of brain acetylcholinesterase after parenteral administration of significantly longer duration than that induced by physostigmine or miotine. These compounds also have a greater safety margin (therapeutic ratio) than physostigmine. $RA_{4, 6, 7}$ and $_8$ also show better bioavailability after oral administration than physostigmine. In addition, the acute toxicity (lethality) induced by RA_7 can be decreased more than 10-fold and that of RA_{14} more than 8-fold by the antidote atropine, compared to only a 3-fold decrease for physostigmine and miotine.

The compounds of the invention are therefore useful for the treatment of senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Down's syndrome and Friedrich's ataxia.

For these indications, the exact dosage will of course vary depending upon the compound employed, mode of administration and treatment desired. The compounds may be administered by any conventional route, non-oral or preferably orally.

In general, satisfactory results are obtained when administered at a daily dosage of from about 0.05 to 10 mg/kg animal body weight. For the larger mammals, an indicated total daily dosage

is in the range from about 0.5 to about 25 mg of the compound, conveniently administered in divided doses 2 to 4 times a day in unit dosage form containing for example from about 0.1 to about 12 mg of the compound or in sustained release form.

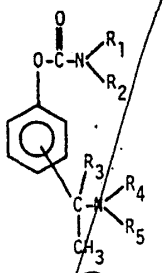
The compounds may be administered in similar manner to known standards for use in these utilities. The suitable daily dosage for a particular compound will depend on a number of factors such as its relative potency of activity.

The compounds according to the invention may be administered in free base form or as a pharmaceutically acceptable acid addition salt. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free forms.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative embodiments and examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is, therefore, desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come with the meaning and range of equivalency of the claims are, therefore, intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition adapted to produce anticholinesterase activity in the central nervous system comprising a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

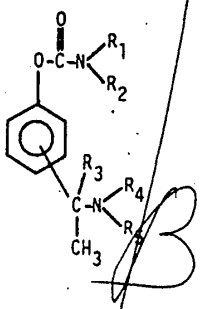
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor.

2. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

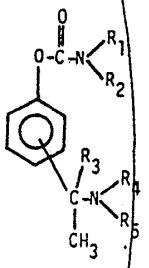
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof.

3. A phenylcarbamate of formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morphonino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

4. A compound of claim 3 wherein the dialkylaminoalkyl group is in meta position and R_4 and R_5 are both methyl.

5. A compound of claim 3 which is N-ethyl-3-[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

6. A compound of claim 3 which is N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

7. A compound of claim 3 which is N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

8. A compound of claim 3 which is N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

9. A compound of claim 3 which is N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

10. A compound of claim 3 which is N-allyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

11. A compound of claim 3 which is N-butyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

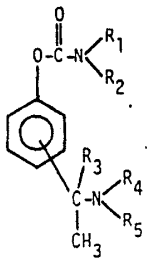
12. A compound of claim 3 which is N-methyl, N-propyl-3[1-dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

13. A compound of claim 3 which is N-methyl, N-ethyl-3[1-dimethylamino)isopropyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

PHENYL CARBAMATES

Abstract of the disclosure

Phenyl carbamates of the general formula



I

wherein R_1 to R_5 are as defined in the claims, are useful as pharmaceuticals.

3700/WY/ER

Attorney's Case No. 118-6848

DECLARATION AND POWER OF ATTORNEY
ORIGINAL APPLICATION

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled PHENYL CARBAMATES the specification of which is

☐ is attached hereto

☒ was filed on March 3, 1986 as Application Serial No. 835 466 and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
74497 (Number)	Israel (Country)	March 5, 1985 (Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed to the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to

disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (Patent, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint Ronald G. Goebel (Registration No. 26,895), Bruce M. Collins (Registration No. 20,066) and William C. Long (Registration No. 18,545) to prosecute this application and transact all business in the Patent and Trademark Office connected threwith.

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4010V

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Inventors Signature Zeev Tashma

Date: May 8th. 1986.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. 469-102•/

Anticipated Classification of this application:

Class 560 Subclass 136

Prior Application: 06/835,466

Examiner: Michael C. Shippen

Art Unit: 126

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

Sir: This is a request for filing a

[X] Continuation
[ ] Divisional

application under 37 CFR 1.60, of pending prior application serial no._____, filed on _____ of for

1. [X] Enclosed is a copy of the prior application, including the oath or declaration as originally filed and an affidavit or declaration verifying it as a true copy. (See 8 and 8a for drawing requirements.)

2. [ ] Prepare a copy of the prior application.

3. [X] The filing fee is calculated below:

CLAIMS AS FILED IN THE PRIOR APPLICATION, LESS ANY CLAIMS CANCELLED BY AMENDMENT BELOW

For	Number filed		Number extra		Rate		Basic Fee $340.00
Total Claims	10-20	=	0	x	$10.00	=	0
Independent Claims	4-3	=	1	x	$34.00	=	34.00
Total filing fee						=	$374.00

4. [ ] The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Account No. . A duplicate copy of this sheet is enclosed.

5. [X] A check in the amount of $374.00 is enclosed.

6. [ ] Cancel in this application original claims

of the prior art application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

7. [X] Amend the specification by inserting before the first line the sentence: - This is a [X] continuation, [ ] division, of application serial no. 835,466, filed March 3, 1986, now abandoned

8. [ ] Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by 1.138 and before payment of base issue fee.)

8a. [ ] New formal drawings are enclosed.

8b. [X] Priority of application serial no. 74497 filed on March 5, 1985 in Irael is claimed under 35 U.S.C. 119.

[ ] The certified copy has been filed in prior application serial no. , filed

9. [ ] The prior application is assigned of record to and the assignment is recorded in the U.S. Patent and Trademark Office at reel , Frame[s] .

10. [X] The power of attorney in the prior application is to:

Richard T. Laughlin, Reg. No. 17,264

a. [X] The power appears in the original papers in the prior application

b. [ ] Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. [X] Address all future communications to:

Richard T. Laughlin, Esq.
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 Headquarters Plaza
Morristown, New Jersey 07960

11. [X] A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.

12. [ ] I hereby verify that the attached papers are a true copy of the prior application serial no.
as originally filed on

The undersigned declares further that all statements made herein of his or her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent pending thereon.

Dated: April 21, 1994

Attorney of Record
Reg. No. 17,264
Telephone (201) 539-0080

3/3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: M. W. Rosin, et al.

SERIAL NUMBER: 185,451 GROUP ART UNIT: 126

FILED: EXAMINER: M. Shippen

FOR: PHENYL CARBAMATES

AMENDMENT

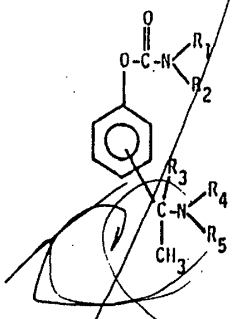
Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows:

Cancel all of the claims and substitute the following claims:

14. A phenylcarbamate of formula



wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

15. A compound of claim 14 wherein the dialkylaminoalkyl group is in meta position and R_4 and R_5 are both methyl.

16. A compound of claim 14 which is N-ethyl-3-[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

17. A compound of claim 14 which is N-propyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

18. A compound of claim 14 which is N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

19. A compound of claim 14 which is N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

20. A compound of claim 14 which is N-butyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharacologically acceptable salt thereof.

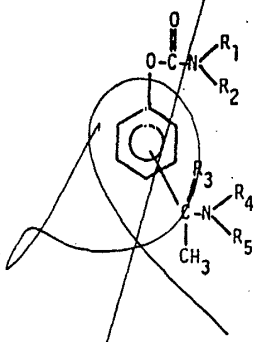
21. A compound of claim 3 which is N-methyl, N-propyl-3[1-dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

22. A compound of claim 3 which is N-methyl, N-ethyl-3[1-dimethylamino)isopropyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

23. ~~(Rewritten)~~ N-cyclohexyl-3[1-(dimethylamino)ethyl] phenyl carbamate and pharmacologically acceptable salts thereof.

24. ~~(Rewritten)~~ N-allyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

25. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering a therapeutically effective amount of a compound of formula



wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

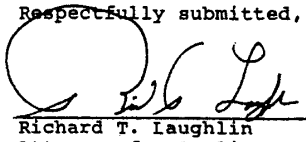
R_4 and R_5 are the same or different, and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof.

REMARKS

New claims 14 to 25 are presented. Claims 23 and 24 are identical to claims 9 and 10 which were allowed in the parent application.

Respectfully submitted,



Richard T. Laughlin
Attorney for Applicant

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129 Headquarters Plaza
Morristown, New Jersey 07960
(201) 539-0080

CERTIFICATION

This is to certify that the attached copy is a true copy of United States Patent Application 06/835,466 entitled PHENYL CARBAMATES and Declaration and Power of Attorney has originally been filed in the United States Patent and Trademark Office on March 3, 1986.

Michelle Lopa

Michelle Iopa
Notary Public Of New Jersey
My Commission expires February 9, 1990

MICHELLE LOPA
A Notary Public of New Jersey
My Commission Expires Feb. 9, 1990

Dated: April 20, 1988

185,451

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/185,451	04/25/88	ROSIN M	469-102-1

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	4

DATE MAILED: 10/11/88

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 4/25/88 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 14-25 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☒ Claims 23+24 are allowed.
4. ☒ Claims 14-22+25 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings which are acceptable for examination purposes until such time as allowable subject matter is indicated.
8. ☐ Allowable subject matter having been indicated, formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. These drawings are ☐ acceptable; ☐ not acceptable (see explanation).
10. ☐ The ☐ proposed drawing correction and/or the ☐ proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved. ☐ disapproved (see explanation). However, the Patent and Trademark Office no longer makes drawing changes. It is now applicant's responsibility to ensure that the drawings are corrected. Corrections MUST be effected in accordance with the instructions set forth on the attached letter "INFORMATION ON HOW TO EFFECT DRAWING CHANGES", PTO-1474.
12. ☒ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☒ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 14 -22 are rejected under 35 U.S.C. 103 as being unpatentable over Aeschlimann (USP 1,905,990), Meltzer, Lange or Berry.

The Aeschlimann reference generically teaches the claimed compounds rendering them obvious. The references discloses homologous and/or isomeric compounds that are so structurally similar that ~~are~~ one would expect them to possess a community of properties in common. Note the product of example 2 of Aeschlimann has a "dialkylaminoalkyl" group which is an adjacent homologue to the compounds instantly claimed. As to advantages of the prior art compounds, there is not evidence of the instant compounds possessing unexpected properties over the compounds of Aeschlimann (USP 1,905,990), note In re Hoch, 166 USPQ 406. The fact that Meltzer does not teach "treating patients is of no moment, In re Hoch, supra.

The fact that compound 37 of Lange may not be the most preferred agent disclosed is of no moment, ~~see In re moment~~, see In re Mills, 176 USPQ 196. Assertions that the prior art does not suggest structural changes is of no moment because one of ordinary skill in the art would recognize the obviousness of homologous or isomeric compounds without the reference suggesting such changes.

Claim 25 is rejected under 35 U.S.C. 103 as being unpatentable over Berry and Aeschlimann (USP 1,905,990) optionally in view of Aeschlimann (USP 2,493,710).

Berry and Aeschlimann (USP 1,905,990) teach that it is known that the compounds possessing anticholinesterase activity. Applicants admit that it is known to use anticholinesterase agents in the treatment of the recited disorders, see lines 12-16 of page 1; lines 16-18 of page 2; and lines 16-22 of page 5 of the specification. Accordingly, it is considered obvious to use these known anticholinesterase agents for the treatment of the recited disorders. Aeschlimann (USP 2,493,170) is cited to show that the various R_1 and R_2 of the instant claims are recognized to be functionally equivalent in analogous compounds rendering such a modification of the primary reference compounds obvious.

Claims 23 and 24 are allowed.

It unclear what is meant by "(Rewritten)" in claims 23 and 24. It is suggested that such be deleted from the claims.

The remaining references are cited as of interest.

This is a continuation of applicant's earlier application S.N. 835,466. All rejected claims are drawn to the same invention claimed in the earlier application and could have been finally rejected on the grounds or art of record in the next Office action if they had been entered in the earlier application. Accordingly, THIS ACTION IS MADE FINAL even though it is a first action in this case. See MPEP 706.07(b).

Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely first response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 CFR 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Shippen whose telephone number is (703) 557-0805.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 557-3920.

SHIPPEN:cij
10/04/88

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP–APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER	
NOTICE OF REFERENCES CITED	185,451	126		4
	APPLICANT(S) Rosin, et al			

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

X	R	Lange, Hormostasis, 10 p 315-47 (1961)
X	S	Meltzer, Enzymol. Exp. App. 12 p 169-82 (1969)
	T	
	U	

EXAMINER	DATE
M. Shuppan	9/29/88

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP–APART AND REMOVE CARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
NOTICE OF REFERENCES CITED	185,457	126	4
	APPLICANT(S) Rosin, et al		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
X	A	1905990	4-1933	Aeschlimann	560	32	
X	B	2208485	7-1940	Aeschlimann	560	136	
X	C	2493710	1-1950	Aeschlimann	560	136	
X	D	2362508	11-1944	Stevens	560	136	
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
X	L	1037753	7-1956	Germany	Fitch	560	136		
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

X	R	Stedman, Biochemical Journal, 20 p 719-34 (1926)
X	S	Wassermann, Proc. Natl. Acad. Sci., USA, 79 p 4810-4 (1982)
X	T	Weiden, J. Agr. Food Chem., 13 p 200-4 (1965)
X	U	Berry, Biochem. Pharmacol., 20 p 3236-8 (1971)

EXAMINER	DATE
M. Shippen	9/29/88

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

MISSING PAGE(S) FROM THE

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Haemostasis *10:* 315–347 (1981)

Inhibition of Activated Factors II, VII, IX, and X by Synthetic Organic Compounds Directed against the Active-Site Seryl Residue[1]

J.A. v.d. Woerd-de Lange, M.C.E. van Dam-Mieras, H.C. Hemker

Department of Biochemistry, State University of Limburg, Maastricht, The Netherlands

Key Words. Thrombin · Factor VII_a · Factor IX_a · Factor X_a · Inhibition · Organic compounds

Abstract. A series of 53 organic chemicals belonging to the groups of organic phosphates, sulfonyl derivatives and carbamates were screened for their activity against the coagulation factors II_a (thrombin), VII_a, IX_a and X_a. Relatively specific inhibitors for the factors II_a, VII_a and X_a were found.

Introduction

In the past decade, much progress has been made in the characterization of the molecular processes underlying blood coagulation (table I). The activation reactions occurring in the coagulation pathways are highly specific, limited proteolysis reactions; the activated coagulation factors with the exception of factors V_a and $VIII_a$ belong to the class of the serine proteases. When comparing the amino acid sequences around the active-site serine residues of the coagulation factors with those of the pancreatic enzymes chymotrypsin, trypsin and elastase, it can be seen that a high degree of sequence homology exists among these proteins [4]. Furthermore, thrombin and factor X_a contain histidine and asparagin residues in positions corresponding with the active-site histidine and asparagin residues present in trypsin and chymotrypsin. In the case of thrombin and factor X_a,

[1] This work has been described in full detail in the PhD thesis of the first author (Leyden University, 1976).

Table I. A survey of the coagulation factors with proteolytic activity

Coagulation factor	Specifications of the zymogen molecule	Activation
XII	bovine factor XII: single-chain glycoprotein, molecular weight (MW) 74,000; preliminary amino acid sequence data by *Fujikawa* et al. [7, 9]; human factor XII: single-chain glycoprotein, MW 76,000 [10]	by plasma kallikrein by a single proteolytic arg-val cleavage, leading to a disulfide-linked two-chain enzyme; the in vivo initiation of the activation is not yet understood
XI	bovine factor XI: glycoprotein containing two similar disulfide-linked polypeptide chains, MW 124,000 [19]; human factor XI: glycoprotein, containing two identical disulfide-linked polypeptide chains; partial amino acid sequence known [2, 22]	by factor XII_a, in both chains a new N-terminal Ile residue is created; factor XI_a is a disulfide-linked four-chain molecule
IX	bovine factor IX: single-chain glycoprotein, MW 55,400; amino acid sequence nearing completion [39]; human factor IX: single-chain glycoprotein; MW 57,000; amino acid sequence nearly identical to that of bovine factor IX [35]	by factor XI_a ($+Ca^{++}$) in a two-step reaction; in the first step an arg-ala bond is cleaved, leading to a disulfide-linked two-chain molecule, next an activation peptide is removed from the heavy chain by arg-val cleavage
VII	bovine factor VII: single-chain glycoprotein, MW 45,500 [18, 33, 34]	by arg-ile cleavage; the in vivo initiation of the activation is not clear
X	bovine factor X: two-chain glycoprotein, MW 55,000; amino acid sequence completely known [6, 8, 15, 40]; human factor X: two-chain glycoprotein, MW 58,900 [35]	by tenase complex (IX_a, $VIII_a$, Ca^{++}, phospholipids) through arg-ile cleavage in the N-terminal region, releasing a 9,000-dalton fragment
II	bovine factor II: single-chain glycoprotein, MW 70,000; amino acid sequence completely known [24]; human factor II: single-chain glycoprotein, MW 70,000; amino acid sequence almost elucidated [3, 38]	by prothrombinase complex (X_a, V_a, Ca^{++}, phospholipids) in a two-step reaction; in the first step an arg-thr bond is cleaved; the second arg-ile cleavage yields the two-chain disulfide-linked thrombin molecule with MW 37,000

the amino acid sequence of the active-site-bearing chain shows about 40–45% sequence homology with trypsin. These data strongly suggest a close analogy between the coagulation factors and the pancreatic enzymes with respect to the activation of the zymogen and the catalytic mechanism of the active enzyme. Most likely, the substrate-binding pocket of the coagulation proteases will resemble that of trypsin because all coagulation proteases are specific towards basic amino acids (table I).

The main differences between the coagulation factors and the digestive enzymes are the presence of a second protein chain in the coagulation factors not involved in the catalytic reaction per se and, perhaps as a consequence of the occurrence of this additional protein part, the higher substrate specificity of the coagulation factors.

Of course, the tertiary structure of the coagulation factors can only be proven by crystallographic studies of these enzymes and their (natural) substrates, but valuable information can be obtained from work with model substrates. In these studies, small changes in the substrate structure can be introduced, and the effects on the enzyme-substrate interaction brought about by these small changes can give useful information about the substrate-binding site and the catalytic site of the enzymes. The same holds true for inhibitors.

The aim of the work presented here was to find specific inhibitors for the different coagulation factors. The availability of such specific inhibitors would be useful for the study of the mechanism of blood coagulation and for the chemical determination of the coagulation factors. The stereochemistry of the specific inhibitors could contribute to our knowledge of the structure of the active site of the coagulation factors, and finally, the specific inhibitors might possibly be used therapeutically. In the experiments described here, the inhibitory capacity towards the activated coagulation factors was checked for a series of organic compounds belonging to the organic phosphates, the sulfanyl derivatives and the carbamates. Other groups have used the same approach, but the families of inhibitors investigated here have, to our knowledge, not been screened before [13, 16, 30, 31].

Materials and Methods

Isolation and Purification of the Factors II_a, VII_a, IX_a and X_a [see also 40]

As starting material, a coagulation factor concentrate containing the factors II, VII, IX, and X was prepared according to *Swart* [37]. The coagulation factor concentrate was activated with thromboplastin and Ca^{2+}; the activated coagulation factors were separated

amino acid sequence completely known [24]; human factor II: single-chain glycoprotein, MW 70,000; amino acid sequence almost elucidated [3, 38]

a two-step reaction; in the first step an arg-thr bond is cleaved; the second arg-ile cleavage yields the two-chain disulfide-linked thrombin molecule with MW 37,000

by chromatography on a Whatman DE 32 column. (For the factors II_a and VII_a the best results were obtained when the isolation was carried out at 4 °C; for factor X_a the best results were obtained when the isolation occurred at room temperature.) It was not possible to obtain factor IX_a by this procedure, presumably because the starting material did not contain a sufficient amount of this factor. However, when the coagulation factor concentrate was activated with contact product, a factor IX_a preparation could be obtained. This preparation always contained factor XI_a (from the contact product) and was only used in preliminary experiments. It was also possible to isolate a factor $VII_a - IX_a - X_a$ concentrate from serum and to isolate the factors VII_a and X_a from this concentrate by chromatography on Sephadex G-100.

No attempt was made to obtain preparations that contained clotting factors that could be considered pure by the usual physicochemical criteria. Preparations that contained one coagulation factor in excess and no or trace activities of the others were considered sufficiently pure for our purposes. This is because the inhibitors are added in large molar excess anyhow, and the tests employed are sufficiently specific not to be influenced by trace amounts of inhibited or uninhibited coagulation factors other than the one under investigation.

Coagulation Factor Reagents

These reagents were prepared according to *Koller* et al. [20] and *Loeliger and Koller* [23] (factor II); according to *Borchgrevink* et al. [1] (factor V); according to *Hemker* et al. [12] (factors VII and X); according to *Denson* [5] (factor VII/X reagent). The factor VIII and factor IX reagents were obtained from a patient with a severe deficiency of the respective coagulation factor (< 1 % activity) (440 ml blood collected in a siliconized glass vessel containing 60 ml ACD solution; storage at −20 °C). Factor XI reagent was obtained from congenital factor XI deficient plasma.

Thromboplastin was prepared from human brain according to *Owren and Aas* [32]. Contact product was prepared according to *Niewiarowski* et al. [29].

Determination of Coagulation Factors

For the estimation of the factors II, V, VII and X, one-stage estimations were carried out as described by *van der Meer* et al. [26]; the factors VIII and IX were estimated according to *Veltkamp* et al. [42]; factor XI was estimated according to *Horowitz* et al. [14].

Determination of the Activated Coagulation Factors

Factor II_a was determined according to *Hemker* et al. [11]; factor VII_a in the same system as factor VII [26] but with phospholipids instead of thromboplastin; the factors $VIII_a$ and IX_a in the same systems as used for factors VIII and IX [42] except that no kaolin was added to the test system and the incubation time of sample and reagent prior to recalcification was 1 min instead of 30 min; factor X_a was determined in a one-stage test with a factor VII and X deficient reagent and phospholipid [26].

Inhibitors were the same as described by *Meyers* [27] and *Meyers* et al. [28].

Inhibition Experiments

The coagulation factor (concentration about 10^{-6}–10^{-7} mol/l) was incubated with the inhibitor (concentration about 10^{-2}–10^{-4} mol/l) at pH 7.4, 37 °C, in siliconized glass or in plastic during 30 min (the concentrations of the coagulation factors were: thrombin,

12.5 NIH U/ml; factor VII_a, 0.4–0.6 U/ml; factor IX_a, 0.15–0.25 U/ml; factor X_a, 0.5–0.75 U/ml; the final inhibitor concentration is given in tables II–V. If there was no or only a slight inhibition after 30 min, the enzymatic activity was tested again after 1, 2, 3 or, sometimes, 18 h.

All chemicals were p.a. grade from Merck.

Protein concentrations were determined by measuring $E_{280\,nm}$ in a Zeiss PM Q 11 spectrophotometer. It was assumed that for prothrombin and thrombin, 1 extinction unit at 280 nm corresponds with 1.6 mg protein/ml [17, 37] and for all other protein mixtures with 1 mg protein/ml [36].

For a number of compounds the nuclear magnetic resonance (NMR) spectra were recorded in a Jeol PS 100 NMR apparatus; sweep time 250 s, sweep width 1,080 Hz (×0.01 ppm). The organic compounds were dissolved in $CDCl_3$; with the compounds No. 1, 7, 13, and 30, a little deuterated aceton was added.

Results and Discussion

Within a series of related inhibitors that are all able to react with an active-site serine residue, the actual course of the reaction is determined by the stereochemistry of the enzyme around the active-site residue [21]. Therefore, it can be expected that the differences between the coagulation factors will be reflected in their susceptibility towards related inhibitors with varying chemical structure. As far as small substrates are considered, this is the same phenomenon that accounts for substrate specificity. For this reason, the interaction of series of serine esterase inhibitors with the factors II_a, VII_a, IX_a, and X_a has been investigated.

The evaluation of the kinetic constants of the inhibition process was based on the following general scheme for the reaction of serine proteases with irreversible inhibitors.

$$E + I \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E \cdot I \xrightarrow{k_{+2}} E_{inhibited} + P$$

For this situation the steady-state approximation yields

$$\frac{d[E \cdot I]}{dt} = k_{+1}([E][I] - K_i[E \cdot I]) = 0$$

with

$$K_i = \frac{k_{-1} + k_{+2}}{k_{+1}}$$

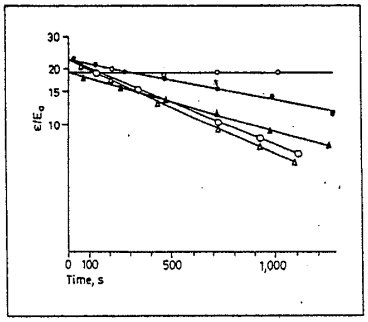


Fig. 1. Time course of the inhibition of thrombin by dinitrophenyl-benzyl-phosphate in the following concentrations: ○ = blank; ● = 0.08 mmol; ★ = 0.19 mmol; ◯ = 0.31 mmol; ☆ = 0.39 mmol.

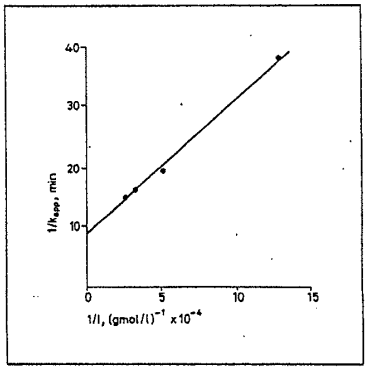


Fig. 2. The dependence of k_{app} on the concentration of dinitrophenyl-benzyl-phosphate.

If $[E_{akt}]$ stands for $[E] + [E \cdot I]$, the total amount of uninhibited enzyme, it can be deduced that the formation of the irreversibly inhibited enzyme proceeds with the velocity

$$v = \frac{-d[E_{akt}]}{dt} = k_{+2} \frac{[I]}{[I] + [K_i]} [E_{akt}].$$

Integration of this differential equation yields

$$[E_{akt}]_t = [E_{akt}]_0 \exp\left(-k_{+2} \frac{[I]}{[I] + K_i} t\right).$$

When the residual activity ($[E_{akt}]_t/[E_{akt}]_0$) after the incubation time t is plotted on semilogarithmic paper against the incubation time, a straight line is obtained. The slope of this line gives the apparent reaction constant (k_{app}) of the inactivation process. In formula:

$$k_{app} = -k_{+2} \frac{[I]}{[I] + K_i}.$$

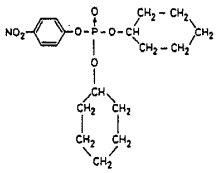
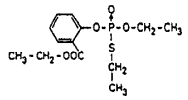
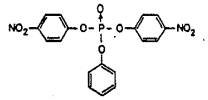
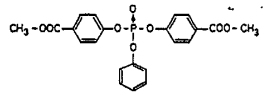
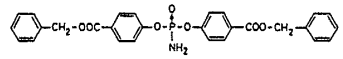
The values of k_{+2} and K_i can be obtained in the usual way by plotting $1/k_{app}$ against $1/[I]$. An example of this approach is given in figures 1 and 2.

The inhibitors used in this study were first described by *Myers* and co-workers [26, 27] who tested their inhibitory activity towards acetylcholine esterases; their chemical structure is given in table II. Table II summarizes the results of a first screening of the inhibitory activity of the tested compounds towards the factors II_a, VII_a, IX_a and X_a. The k_{30} values given in table II were calculated after a fixed constant time interval (30 min) without a control of the actual time course of the inactivation and, therefore, must be considered as a rough estimation of the inhibitory capacity of the compounds under consideration.

The compounds that seemed to be good and/or specific inhibitors during this first screening were tested again with the factors II_a, VII_a, IX_a, and X_a, but this time the inhibitor concentration was the same with all four coagulation factors. The purity of the compounds used for this second screening was also checked by NMR spectroscopy. All compounds except compounds No. 13, 16, and 35 had a purity of at least 97%; the compounds No. 13, 16, and 35 seemed to be partially degraded. The results of this second screening are shown in italics in table II.

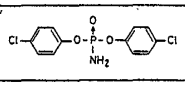
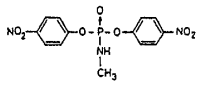
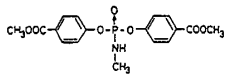
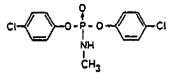
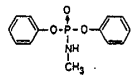
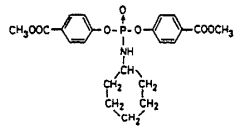
In order to facilitate the evaluation of the inhibitory capacity of the compounds tested, the k_{app} values for the inhibition of the coagulation factors with diisopropylfluorophosphate (DFP) are given in table III.

Table II

No.	Chemical structure of the inhibitor	II_a inhibition	
		[I]^a mmol/l	k_{30} 10^{-4}
1.		0.5 *1.0*	1 2
2.		0.03	
3.		3.1	
4.		0.1 *0.04*	> 1 > 1
5.		0.6	>
6.		0.02 *0.02*	

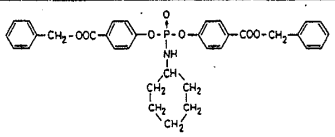
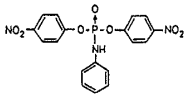
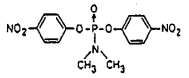
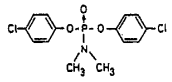
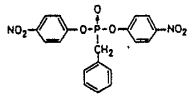
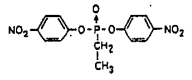
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}
0.5	15	1.0	0	1.0	9	0.9	58
1.0	*20*	*1.0*	*0*	*1.0*	*9.0*	*1.0*	*60*
0.03	3	0.03	0	0.03	0	0.03	0
3.1	0	1.7	16	3.1	0	3.1	20
0.1	>128	0.1	70	2.6	3	0.2	105
0.04	*>128*	*0.04*	*20*	*0.04*	*0*	*0.04*	*2.9*
0.6	>128	0.6	44	1.2	1	1.1	58
0.02	89	0.02	<6	4.04	1	0.04	24
0.02	*89.4*	*0.02*	*<5.9*	*0.02*	*0*	*0.02*	*15.9*

Table II (cont.)

No.	Chemical structure of the inhibitor	II_a inhibit [I]ª mmol/l	k l
7.		1.3 *0.02*	≥ ≥
8.		0.02 *0.02*	
9.		0.04	
10.		0.06 *0.1* *0.2*	
11.		0.6 *0.2*	
12.		0.5	

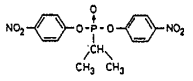
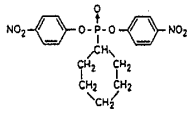
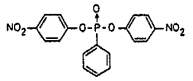
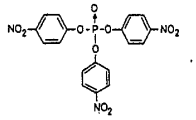
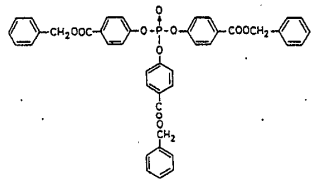
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}
1.3	>128	1.3	44	2.5	1	2.3	40
0.02	*>128*	*0.02*	*0*	*0.02*	*0*	*0.02*	*0*
0.02	44	0.02	0	0.04	0	0.04	12
0.02	*50.9*	*0.02*	*0*	*0.02*	*0*	*0.02*	*0*
0.04	20	0.04	16	0.08	6	0.07	16
0.06	12	0.6	<3	1.4	1	1.1	12
0.1	*0*	*0.1*	*0*	*0.1*	*0*	*0.1*	*0*
0.2	*9.0*	*0.2*	*0*	*0.2*	*0*	*0.2*	*0*
0.6	12	0.6	<3	1.3	0	1.3	0
0.2	*5.9*	*0.2*	*0*	*0.2*	*0*	*0.2*	*0*
0.5	6	0.5	20	0.8	<0.5	0.9	6

Table II (cont.)

No.	Chemical structure of the inhibitor	II_a inhibitio	
		[I]ª mmol/l	k_{30} 10⁻
13.		–	
		–	
14.		0.1	
		0.2	
15.		0.2	
		0.2	
16.		–	
		–	
17.		0.05	≥
		0.02	≥
		0.04	≥
18.		0.03	≥
		0.02	≥

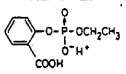
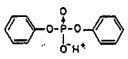
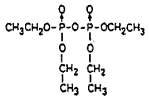
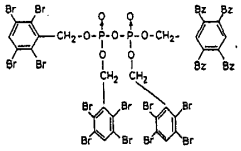
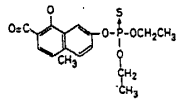
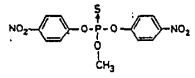
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}
–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–
0.1	12	0.9	0	0.8	0	0.7	6
0.2	*23,9*	*0.2*	*0*	*0.2*	*0*	*0.7*	*0*
0.2	20	0.2	0	0.2	0	0.4	6
0.2	*20*	*0.2*	*2.8*	*0.2*	*0*	*0.2*	*2.8*
–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–
0.05	> 128	0.05	60	1.1	0	0.1	105
0.02	*> 128*	*0.02*	*60*	*0.02*	*0*	*0.02*	*28.4*
0.04	*> 128*	*0.04*	*60*	*0.04*	*< 2.8*	*0.04*	*38.5*
0.03	> 128	0.03	20	0.06	0	0.6	58
0.02	*> 128*	*0.02*	*19.8*	*0.02*	*0*	*0.02*	*0*

Table II (cont.)

No.	Chemical structure of the inhibitor	II_a inhibitic	
		[I]^a mmol/l	k_{30} 10
19.		0.02 0.02	> >
20.		0.1 *0.05*	>
21.		0.04 *0.02*	> >
22.		0.08	
23.		0.1	

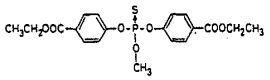
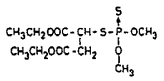
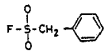
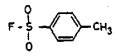
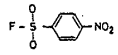
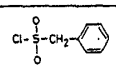
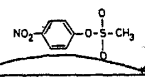
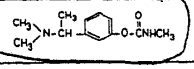
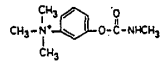
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}	[I]^a mmol/l	k_{30} 10^{-4} min^{-1}
0.02	> 128	0.04	10	0.04	0	0.04	33
0.02	> 128	0.02	5.9	0.02	0	0.02	< 5.9
0.1	> 128	0.4	0	0.4	0	0.3	28
0.05	*58.3*	*0.05*	*0*	*0.05*	*0*	*0.05*	*0*
0.04	> 128	0.04	128	0.8	12	0.06	77
0.02	*> 128*	*0.02*	*44.4*	*0.02*	*0*	*0.02*	*9.0*
0.08	20	0.08	16	0.1	0	0.1	0
0.1	6	0.1	0	0.1	0	0.1	0

Table II (cont.)

No.	Chemical structure of the inhibitor	I_s inhibit [I]^a mmol/l
24.		0.2 *0.2*
25.		0.1
26.		2.0
27.		0.003
28.		0.4
29.		0.6

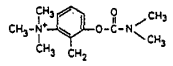
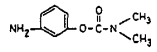
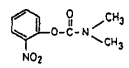
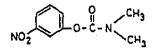
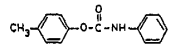
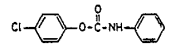
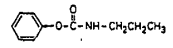
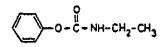
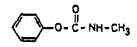
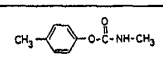
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}
0.2	16	0.2	33	0.4	0	0.4	20
0.2	*16.0*	*0.2*	*33.2*	*0.2*	*0*	*0.2*	*15*
0.1	20	7.3	0	7.5	5	7.5	40
2.0	> 128	2.0	40	4.0	50	4.0	> 128
0.003	< 3	0.003	6	0.2	6	0.006	12
0.4	16	0.7	0	0.7	0	0.7	6
0.6	0	0.6	3	0.6	0	1.1	13

Table II (cont.)

No.	Chemical structure of the inhibitor	I_{50} inhibit [I]ª mmol/l
30.		0.3 *0.3*
31.		0.2 *0.2*
32.	 Isoprop. a.d. 4%	0.8 2.0
33.		2.0
34.		2.5
35.		–
36.		0.2 *0.2*
37.		0.3 *0.2*
38.		0.01 *0.01*

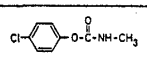
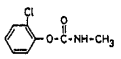
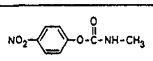
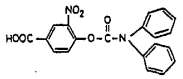
II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}
0.3	> 128	0.3	20	0.9	0	0.5	20
0.3	*> 128*	*0.3*	*19.8*	*0.3*	*0*	*0.3*	*15*
0.2	< 9	0.2	77	0.2	0	0.4	20
0.2	*9.0*	*0.2*	*77*	*0.2*	*0*	*0.2*	*5.9*
0.8	24	0.8	0	0.8	0	0.8	0
2.0	28	2.0	0	3.6	6	3.6	77
2.0	128	2.0	128	4.0	0	3.6	20
2.5	> 128	2.5	77	8.0	12	4.5	> 128
–	–	–	–	–	–	–	–
0.2	6	0.2	9	18.6	0	18.6	0
0.2	*5.9*	*0.2*	*9.0*	*0.2*	*0*	*0.2*	*0*
0.3	20	0.3	12	0.5	0	0.5	0
0.2	*2.9*	*0.2*	*0*	*0.2*	*0*	*0.2*	*0*
0.01	10	0.01	0	0.01	0	0.01	0
0.01	*10*	*0.01*	*0*	0.01	*0*	*0.01*	*0*

Table II (cont.)

No.	Chemical structure of the inhibitor	I_{50} inhib [I]* mmol/l
39.		0.01 *0.01*
40.		1.0 *2.0*
41.		0.3 *0.4*
42.		0.2 *0.4*
43.		0.4 *0.2* *0.4*
44.		0.4 *0.6*
45.		2.5 *0.5*
46.		3.2 *0.5*
47.		0.8 *0.2* *1.0*
48.		0.3 *0.4* *0.2*

II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}	[I]* mmol/l	k_{30} 10^{-4} min^{-1}
0.01	12	0.01	0	0.01	0	0.01	0
0.01	*12.4*	*0.01*	*0*	*0.01*	*0*	*0.01*	*0*
1.0	6	1.0	6	1.0	0	1.0	0
2.0	*23.9*	*2.0*	*0*	*2.0*	*0*	*2.0*	*0*
0.3	3	0.3	3	0.5	0	0.5	12
0.4	*0*	*0.4*	*0*	*0.4*	*0*	*0.4*	*0*
0.2	9	0.2	3	0.4	6	0.4	0
0.4	*16.0*	*0.4*	*0*	*0.4*	*2.9*	*0.4*	*3.9*
0.4	20	0.4	33	1.7	0	0.7	<6
0.2	*16.0*	*0.2*	*2.9*	*0.2*	*0*	*0.2*	*0*
0.4	*19.8*	*0.4*	*33.0*	*0.4*	*0*	*0.4*	*<2.9*
0.4	6	0.4	6	0.6	0	0.6	0
0.6	*19.8*	*0.6*	*9.0*	*0.6*	*0*	*0.6*	*<2.9*
2.5	9	2.5	44	5.1	3	4.6	20
0.5	*5.9*	*0.5*	*0*	*0.5*	*0*	*0.5*	*5.9*
3.2	0	1.6	44	3.2	0	2.8	20
0.5	*0*	*0.5*	*0*	*0.5*	*0*	*0.5*	*9.0*
0.8	0	0.4	44	0.8	0	1.4	6
0.2	*0*	*0.2*	*0*	*0.2*	*0*	*0.2*	*12*
1.0	*0*	*1.0*	*20*	*1.0*	*16*	*1.0*	*0*
0.3	3	0.3	3	0.6	12	0.6	12
0.4	*2.8*	*0.4*	*0*	*0.4*	*1.9*	*0.4*	*12*
0.2	*0*	*0.2*	*0*	*0.2*	*1.9*	*0.2*	*12*

Table II (cont.)

No.	Chemical structure of the inhibitor	I_{50} in [I]* mm
49.		0.2 *0.2*
50.		0.2 *0.2*
51.		0.2 *0.2*
52.	 Tris-HCl buffer	2.4 *0.2*
53.	 alc. Tris buffer 4%	0.5 *0.5*

* The in vitro inhibitory activity of the $(R)_3 - O - P \rightarrow S$ type compounds is mainly due to the $(R)_3 - S - P \rightarrow O$ isomers, which are present as an impurity in the preparations in equilibrium with the former compounds.

II_a inhibition		VII_a inhibition		IX_a inhibition		X_a inhibition	
[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}	[I]ª mmol/l	k_{30} 10^{-4} min^{-1}
0.2	9	0.2	0	0.2	0	0.2	6
0.2	*9.0*	*0.2*	*0*	*0.2*	*0*	*0.2*	*12*
0.2	< 3	0.2	< 3	0.2	0	0.2	10
0.2	*< 2.8*	*0.2*	*< 2.8*	*0.2*	*0*	*0.2*	*12*
0.2	< 20	0.2	< 6	0.2	0	0.6	< 6
0.2	*19.8*	*0.2*	*2.9*	*0.2*	*0*	*0.2*	*2.9*
2.4	> 128	2.4	105	4.3	6	4.3	128
0.2	*33.2*	*0.2*	*19.8*	*0.2*	*0*	*0.2*	*0*
0.5	22	0.5	12	0.8	20	0.8	77
0.5	*22.2*	*0.5*	*11.7*	*0.5*	*19.8*	*0.5*	*28*

nainly due to the (R)-
equilibrium with the

Table III. Inhibition of the coagulation factors II_a, VII_a, IX_a, and X_a by DFP

Coagulation factor	[DFP] mmol/l	k_{app} 10^{-4} min^{-1}
II_a	0.2	3,600
VII_a	6.9	60
IX_a	4.2	
X_a	50	300

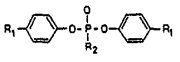
As judged from table II, there seem to occur rather specific inhibitors for the different coagulation factors among the organic compounds tested. The inhibitors that gave k_{30} values of 50×10^{-4} min^{-1} in these screenings were used further to determine the k_{+2} and K_i values of the inactivation processes (see below). As the data on the ineffective inhibitors gave information on the active site as well, we did not omit the negative results.

Tables IV–VI give the kinetic constants of the interaction of the factors II_a, VII_a, and X_a, respectively, with their more or less specific inhibitors. For the determination of the k_{+2} and K_i values given in these tables, samples were taken from the incubation mixture at different times and the residual activity was measured. k_{+2} and K_i values were calculated as described above. By sampling from the incubation mixture and dilution with the substrate, the velocity of the inhibition reaction decreases and competition between the inhibitor and natural substrate occurs. Because of this dilution, and because the clotting times are short compared to the incubation times, it can be assumed that the inactivation reaction does not proceed further during the clotting test. For the same reasons, the inhibition of thromboplastin and of the coagulation factors in the reagent by the inhibitor during the activity determination can be neglected.

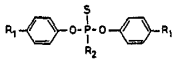
As can be deduced from table II, among the rather specific irreversible thrombin inhibitors, compounds No. 7 and 19 are the most promising. Furthermore, it can be seen in this table that compound No. 31 inhibits preferentially factor VII_a and compound No. 1 inhibits preferentially factor X_a. Compound No. 17 inhibits strongly all three factors, thrombin, VII_a and X_a. Of course, it should be kept in mind that table II shows the results of a rough first screening and gives no real kinetic constants.

When comparing the tables IV, V, and VI, it is striking that the 'specific' inhibitors all belong to the class of the organic phosphorus com-

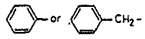
pounds. Moreover, all the irreversible thrombin inhibitors except compound No. 30 have the structure:



while compound No. 30 has the structure:



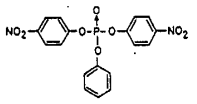
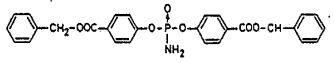
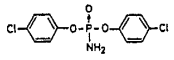
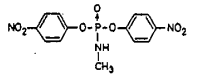
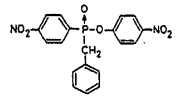
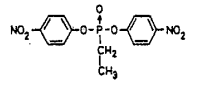
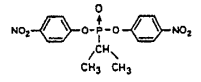
As can be seen in the general scheme for the reaction of serine proteases with irreversible inhibitors given above, the inhibitor first forms a reversible complex with the enzyme [E · I] which is further converted into an irreversible complex [$E_{inhibited}$]. K_i reflects the affinity of the enzyme for the substrate, and k_2 is the reaction velocity of the irreversible step. As can be seen in table IV, the compounds with a



group in the R_2 position give relatively large k_2 values; the smallest K_i values are found with the compounds which do not have aromatic groups in the R_2 portion.

From the results presented above it is clear that also small synthetic compounds can discriminate between the closely related compounds thrombin, factor VII_a and factor X_a. The same conclusion was reached by other groups. *Okamoto* et al. [30] studied a series of thrombin inhibitors belonging to N^a-naphtalenesulfonyl-*L*-argine derivates. The structure of these inhibitors is characterized by three entities: a positively charged group (the amino acid argine), an aromatic group (the dansyl group, attached to the α-NH_2 group of arginine), and a hydrophobic carbon chain (attached to the carboxyl group of arginine; optimal chain length 3 or 4 C-atoms). These authors reported I_{50} values for these compounds in the range from 0.03 to 2 µmol/l when fibrinogen (3 µmol/l) or N^a-benzoyl-*L*-phenylalanyl-*L*-valyl-*L*-arginine-*p*-nitroanilide (100 µmol/l) is used as a substrate. These values are of the same order of magnitude as the K_i values described in this article. *Kettner and Shaw* [16] reported inactivation experiments of thrombin by tetra- and tripeptide argine chloromethyl ketones. The peptide part of these substrates contains the amino acid sequence at the site cleaved by thrombin in its physiological substrates. The compounds Val-Pro-Arg-CH_2Cl (analog of the factor XIII cleavage site) and Ile-Pro-Arg-CH_2Cl (analog of the pro-

Table IV. Specific inhibition of factor II_a

No.	Chemical structure of the inhibitor	Inhibitor concentra mmol/l
4.		1.30 1.04 0.65 0.26
6.		0.20 0.16 0.10 0.04
7.		1.24 0.99 0.62 0.25
8.		0.47 0.28 0.14
17.		0.56 0.39 0.28
18.		0.33 0.33 0.20 0.10
19.		2.00 1.60 1.00 0.40

Inhibitor concentration mmol/l	Enzyme concentration 10^{-3} mmol/l	k_{app} 10^{-2} min^{-1}	k_2 10^{-2} min^{-1}	K_i 10^{-5} mol/l
1.30	1.6	2.60	3.1	20
1.04		2.62		
0.65		2.42		
0.26		1.77		
0.20	2.3	2.21	2.5	5.3
0.16		1.80		
0.10		1.65		
0.04		1.16		
1.24	1.7	2.36	2.8	34
0.99		1.97		
0.62		1.30		
0.25		1.25		
0.47	4	3.68	4.9	15
0.28		3.10		
0.14		2.39		
0.56	4	23.1	25	22
0.39		28.9		
0.28		11.6		
0.33	4	3.03	4	7.9
0.33		3.47		
0.20		1.62		
0.10		2.17		
2.00	1.7	2.41	3	100
1.60		1.40		
1.00		1.23		
0.40		1.04		

Table IV. (cont.)

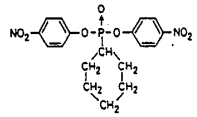
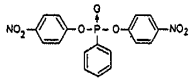
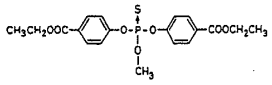
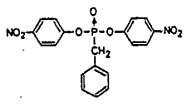
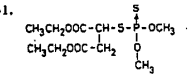
No.	Chemical structure of the inhibitor	Inhibitor concentration mmol/l
20.		1.63 1.31 0.82 0.33
21.		0.39 0.31 0.19 0.08
30.		0.44 0.35 0.22 0.09

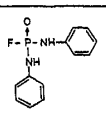
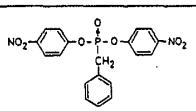
Table V. Specific inhibition of factor VII_a

No.	Chemical structure of the inhibitor	Inhibitor concentration mmol/l
17.		1.11 0.89 0.55 0.22
31.		22.8 18.2 18.2 11.4 4.6

Inhibitor concentration mmol/l	Enzyme Concentration 10^{-3} mmol/l	k_{app} 10^{-2} min^{-1}	k_2 10^{-2} min^{-1}	K_i 10^{-5} mol/l
1.63	2.3	2.29	2.5	8
1.31		2.13		
0.82		2.64		
0.33		2.00		
0.39	2.3	6.71	11	25
0.31		6.12		
0.19		5.20		
0.08		2.65		
0.44	2.3	2.05	2.5	10
0.35		1.96		
0.22		1.73		
0.09		1.16		

Inhibitor concentration mmol/l	Enzyme concentration 10^{-3} mmol/l	k_{app} 10^{-2} min^{-1}	k_2 10^{-2} min^{-1}	K_i 10^{-5} mol/l
1.11	3.6	3.30	calculation impossible	
0.89		3.15		
0.55		3.78		
0.22		9.04		
22.8	3.6	9.2	25	2,500
18.2		13.9		
18.2		16.0		
11.4		11.2		
4.6		3.1		

Table VI. Specific inhibition of factor X_a

No.	Chemical structure of the inhibitor	Inhibitor concentratio mmol/l
1.		0.97 0.78 0.49 0.19
17.		1.11 0.88 0.55 0.22

thrombin cleavage site) inactivate thrombin by 50% in less than 25 min at a concentration of 7.5×10^{-8} *M*. The compound Gly-Val-Arg-CH_2Cl [analog of the cleavage site in the fibrinogen A (α) chain] was effective at a concentration of 2×10^{-6} mol/l. The authors reported that the compounds with the proline residue in the P_2 composition were markedly more reactive with thrombin than with plasma kallikrein, plasmin, urokinase, and factor X_a. *Markwardt* [25] described irreversible thrombin inhibitors derived from the competitive inhibitors benzylamine and benzamidine by introduction of a reactive fluorosulfonyl moiety at the aromatic ring. These compounds, aminomethyl and amidinofluorosulfonylbenzenes, possessed an inhibitory effect towards thrombin that surpassed that of DFP and phenylmethyl sulfonylfluoride (PMSF).

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Inhibitor concentration mmol/l	Enzyme concentration 10^{-3} mmol/l	k_{app} 10^{-2} min^{-1}	k_2 10^{-2} min^{-1}	K_i 10^{-5} mol/l
0.97	0.25	5.7	20	200
0.78		4.8		
0.49		3.2		
0.19		2.0		
1.11	0.25	7.3	7.7	27.6
0.88		5.1		
0.55		4.9		
0.22		3.4		

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INSECTICIDAL ACTIVITY OF SUBSTITUTED PHENYL *N*-METHYLCARBAMATES

BY

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The insecticidal and the acaricidal activities of a number of substituted phenyl *N*-methylcarbamates have been determined on the housefly (*Musca domestica*), the black bean aphid (*Aphis fabae*), the Colorado potato beetle (*Leptinotarsa decemlineata*), the cabbage worm (*Pieris brassicae*) and the carmine spider mite (*Tetranychus cinnabarinus*).

It is demonstrated that the thesis of Kolbezen, Metcalf & Fukuto (1954), Metcalf, Fukuto & Winton (1962) and Kohn, Ospenson & Moore (1965), that the meta-isomers of alkylphenyl *N*-methylcarbamates are the most active, has to be restricted to some insect groups (e.g. flies and caterpillars). In the case of the Colorado potato beetle the *o*- and *m*-isomers were equally active; for aphids the *o*-isomer was the most toxic one.

This fact as well as the different responses of the test insects if the compounds are further alkylated indicate that various carbamates exhibit more or less selective activities. Most striking was the high level of activity in the new group of *p*-dimethylaminomethylphenyl *N*-methylcarbamates on most of the insects, in combination with a complete lack of toxicity to houseflies.

As previously pointed out by Kolbezen *et al.* (1954) and Metcalf *et al.* (1962), it was found that lengthening of the *N*-methyl group or *N,N*-dialkylation resulted in loss of insecticidal activity.

The most active dimethylaminophenyl compounds were those with a *p*-dimethylamino group in combination with alkyl substituents in the 2,5- and 3,5-positions. Several *p*-dimethylaminomethylphenyl *N*-methylcarbamates with two or three alkyl substituents (except the 2,6-combination) proved to be highly active, except on flies, to which they were virtually non-toxic. Greatest broad-spectrum activity was shown by 2,3-dimethyl-4-dimethylaminomethylphenyl *N*-methylcarbamate. It is demonstrated that by introducing a *p*-dimethylamino- or a *p*-dimethylaminomethyl group in alkylphenyl *N*-methylcarbamates a considerable gain in anticholinesterase and insecticidal activity is obtained.

1. *Introduction*

During the last 15 years a considerable number of carbamates have been investigated in respect of insecticidal activity. The compounds presented here include several members of a new class of carbamates, viz. the *p*-dimethylaminomethyl substituted phenyl-*N*-methyl carbamates. All these carbamates were synthesized by

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the second author, while he was connected with the Institute for Organic Chemistry T.N.O.

Although many papers on carbamates have been published, so far no comparison of the action on four insect orders and Acarina has been made. The compounds were examined by using houseflies (*Musca domestica* L.), black bean aphids (*Aphis fabae* Scop.), larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), caterpillars of the large cabbage white (*Pieris brassicae* (L.)) and females of the carmine spider mite (*Tetranychus cinnabarinus* Boisd.).

2. *Materials*

The synthesis and the physical data of most of the carbamates used in this study are described in the thesis of Welle (1964). Since then, several new 4-dimethylaminomethylphenyl *N*-methyl carbamates (Table I) have been synthesized by the method first published by him for the preparation of ring-alkylated 4-dimethylaminomethylphenyl *N*-methyl carbamates. The new *N,N*-dimethyl carbamate KD 1490 was prepared by condensing 2,3-dimethyl-4-dimethylaminomethyl phenol with dimethylcarbamoyl chloride in excess pyridine. The intermediate 2,6-dimethyl-4-dimethylaminomethyl phenol could also be prepared by refluxing for 5 hours equimolecular quantities of 2,6-dimethyl phenol, dimethylamine and formaldehyde in water.

The carbamates were recrystallized from light petroleum b.p. 60—80°, with the exception of KD 1446, which was recrystallized from a methanol-ethanol mixture, and of KD 1413 and 1435, which were used without purification.

All boiling points and melting points are uncorrected.

TABLE I

3. *Methods*

3.1. *Musca domestica*

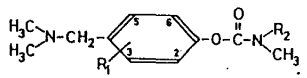
One ml of an acetonic solution of the test compound was poured into a Petri dish of 9 cm diameter. The dish was gently shaken, in order to distribute the substance evenly over the surface. The acetone was allowed to evaporate for exactly 10 minutes, after which the dish was closed. In the meantime a disc of filter paper of 2.5 cm diameter, soaked in water, was placed on the inner surface of the lid. Then the dishes were placed upside down, and provided with five male and five female flies. The experiments were carried out with at least three replicates. The dishes were kept at room temperature. Final mortality counts took place after 21 hours.

3.2. *Aphis fabae*

Potted seedlings of broad bean (*Vicia faba* L.) with two well-developed leaves were dipped in emulsions or solutions prepared by pouring an acetonic solution of the test compound into the appropriate amount of water. If necessary, an emulsifying agent was added. After the plants had been dried, they were placed in plexiglass

TABLE I

Physical data of substituted 4-dimethylaminomethylphenyl N-methyl and N,N-dimethyl carbamates



Code KD	R_1	R_2	B. p/m.p. of intermediate anisole	B. p/m.p. of intermediate phenol	M.p./b.p. of carbamate	Molecular formula	Analysis	Calc.	Found
1446	2-*i*-C_3H_7	H	82—84/0.6	118—120	195—197 (dec.) oxalate	$C_{14}H_{22}N_2O_2 \cdot C_2H_2O_4$	N	8.23	8.19
1434	2,3-di-CH_3	H	83/0.4	97— 98	134.5	$C_{13}H_{20}N_2O_2$	N	11.85	12.09
1490	2,3-di-CH_3	CH_3			122/0.06	$C_{14}H_{22}N_2O_2$	N	11.19	10.93
1447	2,3,5-tri-CH_3	H	82/0.15	91— 92	111—112	$C_{14}H_{22}N_2O_2$	N	11.19	11.21
1435	2,5-di-CH_3	H	80/0.5	110	229 hydrochloride	$C_{13}H_{20}N_2O_2 \cdot HCl$	Cl	13.03	12.85
1458	2,6-di-CH_3	H	oil	113—114	87—88	$C_{13}H_{20}N_2O_2$	N	11.85	12.11
1395	2-CH_3-5-*i*-C_3H_7	H	92/0.5	120—125	83—84	$C_{15}H_{24}N_2O_2$	N	10.60	10.91
1412	2-*i*-C_3H_7-5-CH_3	H	85/0.4	96	89—90	$C_{15}H_{24}N_2O_2$	N	10.60	10.97
1413	3-CH_3-5-C_2H_5	H	90—92/0.5	81	163—168 hydrochloride	$C_{14}H_{22}N_2O_2 \cdot HCl$	N	9.77	9.72

cylinders and infested with ten young adult aphids. Subsequently the cylinders were covered with lens paper and incubated in climatically conditioned racks at 24° C and 60—70% R.H. The racks were illuminated by tubular fluorescent lamps for 16 hours per day. Final mortality counts took place after 5 days.

3.3. *Leptinotarsa decemlineata*

Potato haulms were dipped in emulsions or solutions and placed in flasks filled with tap water. They were further treated like the broad-bean plants mentioned under 3.2, with the exception that the plants were infested with ten third-stage larvae of the Colorado potato beetle and that the cylinders were left uncovered.

3.4. *Pieris brassicae*

Potted cauliflower seedlings were treated in the same manner as for the broad beans described above. The plants were infested with 10 third-stage caterpillars, and further treated like the broadbean plants mentioned under 3.2. Final mortality counts took place after 5 days.

3.5. *Tetranychus cinnabarinus*

Potted French beans were dipped like the other plants referred to above. After the plants had been dried, the leaves were provided with plexiglass cages of 2.5 cm diameter, in which ten young female mites were placed. Then the plants were incubated in the climatically conditioned racks and treated like the others mentioned. Final mortality counts took place after 5 days.

3.6. Determination of anticholinesterase activity

Inhibition of fly head cholinesterase by the compounds involved was determined as follows. From frozen flies the heads were separated by sieving, and homogenized at 0° by means of an Ultraturrax homogenizer using 7.5 ml of 0.05 M tris-HCl buffer of pH 7.5 per gram of fly heads. The homogenate was sonicated during 3 × 10 minutes, using a cooling bath at —10°. The resulting homogenate was passed through cheese cloth and the filtrate was centrifuged at 25000 g and 4° for 60 minutes. The clear supernatant with an average cholinesterase activity of 4 μ moles of acetylthiocholine per ml per minute was used as the enzyme preparation. It was usually diluted 100-fold with cold 0.05 M tris HCl-buffer of pH 7.5.

Incubation with the inhibitors was effected by adding 0.3 ml of inhibitor solution in 0.05 M tris HCl, containing 5% ethanol, to 0.3 ml of the diluted cholinesterase preparation. After 30-minutes incubation at 25° the remaining cholinesterase activity was determined according to Ellman, Courtney, Andres & Featherbone (1961) by adding 0.3 ml of buffer solution, 0.3 ml of 5.5'-dithio-bis-(2-nitrobenzoate) reagent and 0.3 ml of acetylthiocholine solution, to a final concentration of 1 mmole/l and measuring the optical density at 412 nm during 6 minutes, using a Unicam SP 600 spectrofotometer. The increase in optical density with time followed a linear course with all the inhibitors tested at all relevant concentrations. The

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Code KD	R
1154	*i*-C_3H_7
1157	
1160	
1222	C_2H_5O
1208	
1224	
1105	$N(CH_3)_2$
1040	
98	
1148	$CH_2N(CE$
1149	
1174	

Concentration no:
ppm :
Mortality :

inhibitor concentration was taken to be the concentration in the pre-incubation solution.

4. *Discussion of results*

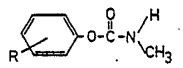
4.1. Influence of single-ring substituents on insecticidal activity

Some examples shown in Table II illustrate the selective activity of the carbamates. Acaricidal activity was generally weak. The compounds with the listed substituents in the para-position showed only weak activities. Both the *o*- and the *m*-substituted compounds, however, were active.

Kolbezen *et al.* (1954), Metcalf *et al.* (1962), and Kohn *et al.* (1965) reported that both insecticidal activity and cholinesterase inhibition are optimum in *m*-substituted alkylphenyl *N*-methylcarbamates. As can be seen from the table, however, we found a specific effect on aphids, where the *o*-isopropyl compound was the most active. In the case of the Colorado potato beetle the activities of the *o*- and *m*-isopropyl derivatives are equal.

TABLE II

Influence of the position of ring substituents on the insecticidal activity of phenyl N-methylcarbamates



Code KD	R		Musca 1 2 3 4 5	Aphis 1 2 3 4 5	Leptinot. 1 2 3 4 5	Pieris 1 2 3 4	Tetran. 1 2 3
1154	i-C_3H_7	o	++—	+++—	+++—	—	—
1157		m	+++±—	+±—	+++—	+——	—
1160		p	—	±	±——	—	—
1222	C_2H_5O	o	+++±±	++—	+++—	—	—
1208		m	+++±—	—	++—	—	—
1224		p	—	—	±	—	—
1105	$N(CH_3)_2$	o	+++—	+——	++—	++—	+——
1040		m	—	+±—	+±—	++—	—
98		p	±——	±——	+——	+±—	—
1148	$CH_2N(CH_3)_2$	o	+±—	+++—	±	±	—
1149		m	+±—	+++±—	+——	++±—	—
1174		p	—	—	—	—	—

Concentration no: 1 2 3 4 5
ppm : 1000 300 100 30 10
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

The alkoxy compounds, however, were most active on all insects examined when the alkoxy group was in *o*-position. This is in agreement with the results of Metcalf & Fukuto (1965).

With the dimethylaminophenyl *N*-methylcarbamates there was no significant difference between the activities of the *o*- and the *m*-isomers on aphids, beetles and caterpillars. In this case, however, the *o*-isomer was the most active one on flies and spider mites. With the dimethylaminomethylphenyl *N*-methylcarbamates there was no significant difference between the activities of the *o*- and the *m*-isomers on flies, aphids and beetles. In this case, however, the *m*-isomer was the most active one on caterpillars.

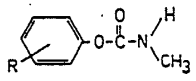
In general we can see from the results in Table II that the *o*- and *m*-isomers are superior to the *p*-isomer. The superiority of *o*- or *m*-position depends on the test insect, however.

4.2. Insecticidal activity of mono- and dialkyl substituted compounds

Table III illustrates the influence of further alkylation by starting from either *o*- or *m*-isopropylphenyl *N*-methylcarbamates. In the case of the *o*-isomer, introduction of a methyl group in position 5 annihilated insecticidal activity; introduction of another isopropyl group in the 5-position resulted in loss of activity on aphids and beetles, but not on flies.

TABLE III

Insecticidal activity of some alkylphenyl N-methylcarbamates



Code KD	R	Musca 1 2 3 4 5	Aphis 1 2 3 4 5	Leptin. 1 2 3 4 5	Pieris 1 2 3	Tetran. 1 2 3
1154	2-*i*-C_3H_7	++—	+++—	+++—	—	—
1035	2-*i*-C_3H_7-5-CH_3	—	—	±	—	—
1237	2,5-di-*i*-C_3H_7	++±	—	+——	—	—
1157	3-*i*-C_3H_7	+++±—	+±—	+++—	+——	—
1070	3-*i*-C_3H_7-5-CH_3	++++±	++—	++++±	+——	±
1238	3,5-di-*i*-C_3H_7	++++±	±	+++±—	—	—
1066	3-*i*-C_3H_7-6-CH_3	±	+——	+++±	—	±

Concentration no: 1 2 3 4 5
ppm : 1000 300 100 30 10
Mortality : + = 90—100%; ± = ⩾ 50 < 90%; — = < 50%.

Introduction of a 5-methyl group in the *m*-isopropylphenyl *N*-methylcarbamate enhanced insecticidal activity. A second isopropyl group in the 5-position caused an increase in toxicity to flies and beetles, whereas the activity on aphids and caterpillars was diminished.

Finally, introduction of a methyl group in the 6-position dramatically reduced the toxicity to flies. Also the activity on aphids and caterpillars was lessened, but there was no adverse effect on the activity on beetles and spider mites.

From Tables II and III it is apparent that the response of the various test insects to the substituents and their positions is different and therefore more or less selective activities might be expected within the group of carbamates.

4.3. Insecticidal activity of phenyl *N*-alkyl and *N,N*-dialkylcarbamates

Several derivatives are listed in Tables IV & V. It is apparent that *N*-methylation of the carbamic ester enhanced toxicity, but that lengthening of the *N*-alkyl group abolished toxicity. This is in agreement with the findings of Kolbezen *et al.* (1954). Furthermore the *N,N*-dimethyl compounds were less active than their corresponding *N*-methyl derivatives. In some cases this decrease in activity was found rather dramatic, as is illustrated in Table V. This is in accordance with analogous results of Metcalf *et al.* (1962).

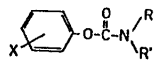
4.4. Insecticidal activity of dimethylaminophenyl *N*-methylcarbamates

In Table VI the results obtained with the dimethylamino group in respectively the *o*- and *m*-positions are listed. Of the ortho-compounds the 4- and 5-alkyl derivatives were the most active. The meta-derivatives showed less activity.

Far greater insecticidal activities were encountered with the compounds that have the dimethylamino group in *p*-position, as can be seen in Table VII. Introduction of all alkyl groups shown in the table resulted in a gain of insecticidal activity, if they were introduced into the 2-, 3-, or 5-position. It seems that *m*-substitution produces insecticides that are superior to the *o*-substituted ones. This is in agreement with Kaeding, Shulgin & Kenaga (1965). The high toxicity of most of the listed compounds to aphids and beetles is in sharp contrast to the weak activity on flies. In the case of spider mites, too, these compounds showed only little activity, except the 3-isopropyl-5-methyl derivative. The weak action on flies may be caused by a lack of contact activity, or it may be the result of the capability of flies to decompose the compounds rapidly. In the case of the spider mites decomposition seems more likely, for the compounds concerned possess systemic properties and, if

TABLE IV

Insecticidal activity of N-alkyl and N,N-dialkylphenylcarbamates

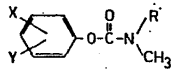


Code KD	X	R	R'	Musca 1 2 3 4 5	Aphis 1 2 3 4 5 6 7	Leptinot. 1 2 3 4 5 6 7	Pieris 1 2 3 4 5	Tetran. 1 2 3 4 5
1176	2-*i*-C_3H_7	H	H	—	+— —	+++—	—	—
1154		H	CH_3	++—	+++—	+++—	—	—
1177		CH_3	CH_3	+±—	—	—	—	—
1179		H	C_2H_5	±	—	+±—	—	—
1180		H	*i*-C_3H_7	—	—	+— —	—	—
1157	3-*i*-C_3H_7	H	CH_3	+++±—	+±—	+++—	+— —	—
1204		CH_3	CH_3	++—	+— —	+— —	±	—
1281	3-CH_3-5-*i*-C_3H_7-4-$N(CH_3)_2$	H	CH_3	+±—	+++++±—	+++++—	++++±	+++±—
1322		CH_3	CH_3	+++—	+++±—	+++±—	++—	++±—
1321		H	C_2H_5	—	++±—	++++—	+++	—

Concentration no: 1 2 3 4 5 6 7
ppm : 1000 300 100 30 10 3 1
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

TABLE V

Insecticidal activity of N-alkyl and N,N-dialkylphenylcarbamates

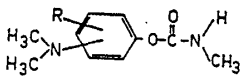


Code KD	X	Y	H	Musca 1 2 3	Aphis 1 2 3 4 5 6 7	Leptinot. 1 2 3 4 5 6 7	Pieris 1 2 3 4 5	Tetran. 1 2 3 4 5 6
1207	3-CH(CH$_3$)N(CH$_3$)$_2$	H	H	+ — —	+ + + + + —	+ + + + ± —	+ ± —	+ + + + —
1261			CH$_3$	+ ± —	+ + + + ± —	+ ± —	—	+ ± —
1434	4-CH$_2$N(CH$_3$)$_2$	2,3-di-CH$_3$	H	—	+ + + + ± —	+ + + + + + —	+ + + + —	+ + + + ± —
1490			CH$_3$	—	—	+ ± —	± — —	—
1312	4-CH$_2$N(CH$_3$)$_2$	3,5-di-CH$_3$	H	± — —	+ + + ± —	+ + + + + + —	+ + —	± — —
1347			CH$_3$	± — —	—	—	—	—

Concentration no: 1 2 3 4 5 6 7
ppm : 1000 300 100 30 10 3 1
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

TABLE VI

Insecticidal activity of o- *and* m-*dimethylaminophenyl* N-*methylcarbamates*

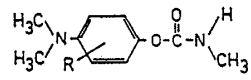


Code KD	Position $N(CH_3)_2$	R	Musca 1 2 3 4 5	Aphis 1 2 3 4 5	Leptinot. 1 2 3 4 5	Pieris 1 2 3 4 5	Tetra 1 2 3 4
1105	2	H	+++—	+——	++—	++—	+—
1218		3-*i*-C_3H_7	—	+——	+——	—	—
1194		4-CH_3	+±—	++±	++++—	—	—
1217		5-*i*-C_3H_7	++±	++±	++++—	—	—
1163		6-*i*-C_3H_7	+——	—	—	—	—
1040	3	H	—	+±—	+±—	++—	—
1166		4-CH_3	±	+±—	+++	—	—
1159		4-*i*-C_3H_7	—	±	++—	—	—
1167		6-CH_3	—	±——	++—	—	—

Concentration no: 1 2 3 4 5
ppm : 1000 300 100 30 10
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

TABLE VII

Insecticidal activity of P-*dimethylaminophenyl* N-*methylcarbamates*



Code KD	R	Musca 1 2 3	Aphis 1 2 3 4 5 6 7	Leptinot. 1 2 3 4 5 6 7	Pieris 1 2 3 4 5	Tetra 1 2 3
98	H	±——	±——	+——	+±—	—
1137	3-CH_3	+±—	+±—	++—	+±—	—
1158	3-*i*-C_3H_7	++±	+++++—	+++++—	+++—	+——
1117	2-CH_3-5-*i*-C_3H_7	+±—	+++++—	+++++—	±——	+——
1323	2-*i*-C_3H_7-5-CH_3	—	+++±—	++±—	+——	+±—
1336	3,5-di-CH_3	++±	+++++—	+++++—	+++++	±——
1281	3-*i*-C_3H_7-5-CH_3	+±—	+++++——	+++++——	+++++	+++±—

Concentration no: 1 2 3 4 5 6 7
ppm : 1000 300 100 30 10 3 1
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

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Code KD

1174	H
1446	2-*i*-C_3H_7
1434	2,3-di-CH
1447	2,3,5-tri-(
1435	2,5-di-CH
1458	2,6-di-CH
1395	2-CH_3-5-
1412	2-*i*-C_3H_7
1365	3-CH_3
1312	3,5-di-CH
1413	3-CH_3-5-

Concentration no:
ppm :
Mortality :

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compound was su
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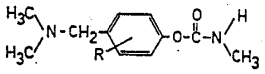
contact action is absent, both aphids and mites must take up the substances by feeding. As the aphids were killed at low concentrations, whereas the mites were not, the latter must possess an internal defence mechanism.

4.4. Insecticidal activity of dimethylaminomethylphenyl *N*-methylcarbamates

Table VIII records the insecticidal activities of the *p*-dimethylaminomethylphenyl *N*-methylcarbamates. The absence of activity of this whole group of compounds on the housefly was striking. This is in sharp contrast to the high anticholinesterase activity which these compounds possess, as appears from *in vitro* tests with fly-head cholinesterase (see Table IX).

TABLE VIII

Insecticidal activity of P-dimethylaminomethylphenyl N-methylcarbamates



Code KD	R	Musca 1 2 3	Aphis 3 4 5 6 7	Leptinot. 3 4 5 6 7	Pieris 3 4 5	Tetran. 3 4 5 6
1174	H	—	—	—	—	—
1446	2-*i*-C_3H_7	—	+++±—	++±—	+— —	+±—
1434	2,3-di-CH_3	—	+++±—	++++—	++—	++±—
1447	2,3,5-tri-CH_3	—	++—	+++±±	+— —	+±±
1435	2,5-di-CH_3	—	+±—	++++—	—	±— —
1458	2,6-di-CH_3	—	—	—	—	—
1395	2-CH_3-5-*i*-C_3H_7	—	+++±—	+++±—	±	—
1412	2-*i*-C_3H_7-5-CH_3	—	+++±—	++—	+±—	—
1365	3-CH_3	—	++—	—	+— —	—
1312	3,5-di-CH_3	±— —	+±—	+++±—	—	—
1413	3-CH_3-5-C_2H_5	—	+— —	+++±—	+±—	+±±

Concentration no: 1 2 3 4 5 6 7
ppm : 1000 300 100 30 10 3 1
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

Greatest broad-spectrum activity was shown by the 2,3-dimethyl derivative. In comparison with this substance the 2-isopropyl, the 2-methyl-5-isopropyl and the 2-isopropyl-5-methyl derivatives were equally toxic to aphids. On Colorado potato beetles the 2,3,5-trimethyl compound was even better; the 3,5-dimethyl and the 3-methyl-5-ethyl derivatives were equal in activity. On spider mites the 2,3-dimethyl compound was superior to all others, though the 2,3,5-trimethyl and the 3-methyl-5-ethyl derivatives also showed considerable acaricidal activity.

TABLE IX

Effect of the introduction of a p-dimethylamino and a p-dimethylaminomethyl group on the insecticidal and the anticholinesterase activities of some alkylphenyl N-methylcarbamates

Code KD	X	Y	Musca 1 2 3 4 5 6	Aphis 1 2 3 4 5 6 7	Leptinot. 1 2 3 4 5 6 7	Pieris 1 2 3 4 5	Tetran. 1 2 3 4 5 6	pI 50 Fly-head ChE
1503	2,3-di-CH_3	H	+ ± —	+ — —	+ — —	—	—	6.6
1434		$CH_2N(CH_3)_2$	—	+ + + + ± —	+ + + + + —	+ + + + —	+ + + + ± —	8.4
1066	2-CH_3-5-i-C_3H_7	H	±	+ — —	+ + + ±	—	±	6.5
1117		$N(CH_3)_2$	+ ± —	+ + + + —	+ + + + ± —	+ + —	+ ± —	7.3
1395		$CH_2N(CH_3)_2$	—	+ + + + ± —	+ + + + ± —	+ + ±	+ ± —	8.9
153	3,5-di-CH_3	H	+ ± —	—	—	—	—	6.4
1336		$N(CH_3)_2$	+ + ±	+ + + ± —	+ + + + ± —	+ + + —	± — —	7.6
1312		$CH_2N(CH_3)_2$	± — —	+ + + ± —	+ + + + + —	+ + —	+ — —	8.7
1070	3-i-C_3H_7-5-CH_3	H	+ + + + ± —	+ + —	+ + + ± —	+ — —	±	8.0
1281		$N(CH_3)_2$	+ ± —	+ + + + ± —	+ + + + ± —	+ + + + ±	+ + + ± —	8.4

Concentration no: 1 2 3 4 5 6 7
ppm : 1000 300 100 30 10 3 1
Mortality : + = 90—100%; ± = > 50 < 90%; — = < 50%.

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4.6. Effect of the introduction of a *p*-dimethylamino and a *p*-dimethylaminomethyl group on the insecticidal and the anticholinesterase activities of some alkylphenyl *N*-methylcarbamates

When comparing the *N*-methyl carbamic esters of the alkyl phenols with their corresponding *p*-dimethylamino and *p*-dimethylaminomethyl derivatives, we observe the important effect on toxicity obtained by the introduction of the latter groups.

All cases listed in Table IX show a considerable gain in insecticidal activity by the introduction of the *p*-dimethylamino or the *p*-dimethylaminomethyl groups into the corresponding alkyl phenols, except on flies. In Table IX are also listed the cholinesterase inhibition values expressed as the negative logarithms of the dilutions required for 50% inhibition of fly-head cholinesterase. These values show clearly that the anticholinesterase activities also are enhanced as a result of the introduction of a dimethylamino or a dimethylaminomethyl group at the *p*-position.

Acknowledgements

The authors are much indebted to Drs. W. H. Dekker, and Messrs. J. D. van Beek, and W. A. Bosboom for assistance in the preparation of the compounds; to Dr. L. C. Post for the determination of the anticholinesterase activities of the compounds; and to Messrs. K. Olivier, B. J. van der Kolk, and A. H. Adriaansen for the execution of the entomological evaluation tests.

RÉSUMÉ

ACTIVITÉ INSECTICIDE DE N-MÉTHYLCARBAMATES DE PHÉNYLE SUBSTITUÉS

L'activité insecticide et acaricide de plusieurs N-méthylcarbamates de phényle substitués a été examinée sur la mouche domestique (*Musca domestica*), le puceron noir (*Aphis fabae*), le doryphore (*Leptinotarsa decemlineata*), la chenille de la piéride du chou (*Pieris brassicae*) et l'araignée rouge des serres (*Tetranychus cinnabarinus*). Les N-méthylcarbamates de phényle monosubstitués ne présentent qu'une faible activité acaricide. Les substances avec des substitutions en position méta ou ortho montrent une meilleure activité insecticide que celles avec la substitution en position para. Pour les mouches domestiques l'alcoylation en position méta se trouve donner les composés les plus actifs; pour les pucerons l'alcoylation en position ortho était la plus efficace et pour les doryphores les combinaisons o- et m-étaient d'une activité égale. Les isomères ortho des dérivés alcoxylés étaient plus efficaces.

En cas de substitution par un groupe diméthylamino il n'y a pas de différence importante quant à l'activité insecticide entre les positions ortho ou méta. En général il en est de même pour les substitutions par diméthylaminométhyle, bien que le m-isomère soit le plus efficace sur les chenilles.

L'introduction d'un deuxième groupe alcoyle dans un N-méthylcarbamate de o- ou m-isopropylphényle change le spectre d'action. Un groupe 5-méthyle diminue beaucoup l'activité de l'o-isomère. Un groupe 5-isopropyle diminue l'activité sur les pucerons, mais pas sur les mouches. Quand un groupe 5-méthyle ou 5-isopropyle est introduit dans un N-méthylcarbamate de m-isopropyl-phényle l'activité est généralement augmentée. L'introduction d'un groupe

6-méthyle diminue toutefois l'activité sur les mouches, les pucerons et les chenilles, mais pas sur les Coléoptères et les araignées rouges.

Les N-méthylcarbamates de phényle s'avèrent posséder une meilleure activité que les combinaisons N,N-diméthylcarbamates correspondantes. Une prolongation du groupe N-alcoyle diminue également l'activité.

Les dérivés de N-méthylcarbamates de 2-diméthylamino-phényle ont une plus grande activité que les isomères méta correspondants. On trouve toutefois la meilleure activité dans les N-méthylcarbamates de 4-diméthylamino-phényle alcoylés. Tout groupe alcoyle, à condition d'être introduit en position 2-, 3-, ou 5-augmente l'activité insecticide. Les composés méta-substitués semblent encore un peu plus actifs que les dérivés d'o-alcoyle. La grande activité sur les pucerons contraste nettement avec la faible activité sur les mouches et les araignées rouges. Le seul dérivé présentant une bonne activité sur les araignées rouges est le N-méthylcarbamate de 3-isopropyl-4-diméthylamino-5-méthylphényle.

Les N-méthylcarbamates de p-diméthylaminométhyl-phényle alcoylés présentent également une forte action insecticide, sauf sur les mouches. Le N-méthylcarbamate de 2, 3-diméthyl-4-diméthylaminométhyl-phényle présente la plus grande activité et le plus large spectre d'action.

A partir des N-méthylcarbamates d'alcoylphényle l'introduction d'un groupe p-diméthylamino ou p-diméthylamino-méthyle augmente considérablement l'activité anticholinestérasique aussi bien que l'activité insecticide.

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Received for publication : 6 December 1968.

It is tentatively suggested that an effect of the carcinogen may be to cause a deletion of hormone receptor proteins with a subsequent loss of hormonal activity. If these receptor proteins are considered part of the protein synthesis control in the target tissue, then a role of carcinogens may be to cause specific protein deletion as suggested by Miller and Miller.[8]

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Acceleration by free carbamate of the spontaneous reactivation of carbamylated acetylcholinesterase

(*Received* 10 *March* 1971; *accepted* 13 *May* 1971)

SEVERAL workers have shown that inhibition of acetylcholinesterase (AChE; EC 3.1.1.7) by carbamates is adequately described by the mechanism[1–3]

$$E + I \underset{}{\overset{k_1}{\longrightarrow}} EI' \xrightarrow{k_r} E + \text{products} \qquad (1)$$

where E is the enzyme, I a carbamate and EI' a carbamyl enzyme. *In vitro* the velocity declines to a steady state at which rates of inhibition and spontaneous reactivation are equal. The forward bimolecular rate is first order because the concentration of inhibitor is greatly in excess of that of enzyme. Previous workers have estimated inhibition rates by a method which involves discarding some of the data of progress curves, viz. those beyond the range in which a semilogarithmic plot of velocity against time is sensibly linear. Reactivation rates have usually been estimated by greatly diluting enzyme–inhibitor mixtures and observing the rate of increase of velocity. Reiner and Simeon-Rudolf[2] have also estimated them by multiplying the inhibition rate constant by the "equilibrium constant" obtained by assuming that the steady state represents a true equilibrium. A different method, which uses all the data from progress curves, and which has not previously been used in studies of AChE, is based on the assumption that the approach to a steady state is kinetically equivalent to approach to a true equilibrium, a mathematical treatment for which has been presented.[4] Since both forward and reverse reactions are first order the approach to equilibrium is also first order, and is related to the initial velocity v_o, the equilibrium velocity v_e and the intermediate velocities v at times t thus:

$$k = \frac{2{\cdot}303}{t} \log \left(\frac{v_o - v_e}{v - v_e}\right). \qquad (2)$$

This rate is also the sum of the forward and reverse rates:

$$k = k_1(I) + k_r. \qquad (3)$$

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From each progress curve a value of k and its variance can be calculated by methods described by Cleland.[8] When several values of k for different values of (I) are obtained they are used to fit equation (3), using the inverse variances as weighting factors.[5] Thus estimates of k_1, k_r and their standard errors are found.

Rates of spontaneous reactivation of inhibited particulate AChE preparations were also measured by washing off the surplus inhibitor, analogous to the method of great dilution employed for studies on highly purified solubilized preparations. The estimates were found to differ significantly from those calculated by the first method, as will now be described.

Particulate AChE was prepared from diaphragm muscle by the "short method" of Berry and Rutland[6] and the activity was determined by automatic continuous titration, using a twin-syringe assembly to keep the substrate concentration constant. The saline medium was 0·15M KCl, pH 7·42, 38°, and the substrate was 5·5 mM acetylcholine. A typical progress curve of an inhibition experiment is illustrated. The normal velocity was first recorded (AB), the slope of AB giving a measure of v_s. A small volume of carbamate solution was added at B. The trace became curved as inhibition progressed, and it was ultimately recognized that a steady rate had been reached, the straight line CD providing a measure of v_e. Any intermediate velocity v_j is measured by the slope of the tangent at the point J. To avoid the difficulty of drawing tangents, each short portion of the trace IJK, defined by the points of intersection of the time marks on the chart paper, is regarded as an arc of a circle of large radius, the point J being, with sufficient accuracy, the midpoint of the arc IK. The tangent at J is parallel to the chord IK, the slope of which is the required measure of v_j. In control experiments in which no carbamate was added, the traces remained linear during the consumption of more titrants than was consumed during an inhibition run: no corrections for dilution were therefore required.

Direct measurement of spontaneous reactivation was made by incubating a portion of preparation for 30 min at room temperature with enough carbamate to give 95–100 per cent inhibition. The portion was then washed three times by centrifugation, each with 30–40 vol. of 0·15M KCl. Since in earlier experiments with intact red cells such washing had caused complete reactivation of physostigmine-inhibited AChE[7] it was assumed that washing had effectively removed free carbamate. In the present experiments washing caused only a little reactivation, which could be accounted for as the result of the time taken to wash. The washed inhibited preparation was then put up for assay with acetylcholine and the rate of recovery observed for 1·5–2 hr. Reactivation was assumed to be first order with respect to (v_e-v), using a washed uninhibited portion to estimate v_e.

In all cases the data were graphed to verify that the appropriate first-order equation was obeyed, before computing the results.

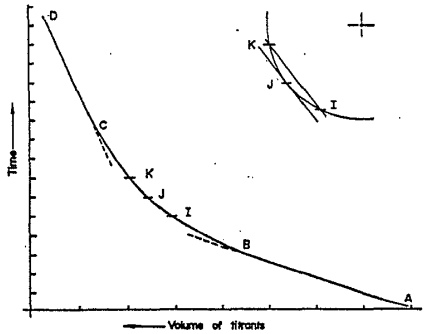

FIG. 1. Copy of trace of representative inhibition experiment. AB, normal velocity; BC diminishing velocity during approach to the steady "equilibrium" velocity CD. Inset, method of drawing tangent at J parallel to the chord IK. The curvature has been exaggerated to show clear separation of chord and tangent. See description in text.

TABLE 1. RATE CONSTANTS OF INHIBITION AND SPONTANEOUS REACTIVATION: CARBAMATES AND DIAPHRAGM PARTICULATE ACETYLCHOLINESTERASE IN THE PRESENCE OF 5·5mM ACETYLCHOLINE

Carbamate No.*	Inhibition l.mole^{-1}sec^{-1} × 10^{-2}	Spontaneous reactivation, sec^{-1} × 10^{3} With carbamate	Washed	Acceleration factor and P that it is unity
1 (Human)	16·6 ± 5·50 (5)	2·50 ± 0·37 (5)	0·59 ± 0·03 (28)	4·2 <0·001
1	8·91 ± 0·18 (5)	1·69 ± 0·08 (5)	0·24 ± 0·028 (17)	7·1 <0·001
2	8·66 ± 0·63 (5)	1·62 ± 0·11 (5)	0·38 ± 0·032 (34)	4·2 <0·001
3	18·2 ± 2·3 (5)	0·99 ± 0·26 (5)	0·90 ± 0·048 (38)	1·1 <0·05
4	85·9 ± 10·8 (4)	1·86 ± 0·26 (4)	0·82 ± 0·077 (35)	2·3 <0·001
5	0·70 ± 0·067 (5)	1·60 ± 0·13 (5)	0·33 ± 0·025 (23)	4·9 <0·001
6	8·54 ± 1·40 (5)	1·77 ± 0·43 (5)	0·225 ± 0·063 (45)	7·9 <0·001

Guinea-pig diaphragm, except where noted. S.E. of estimate. Figures in brackets—Cols. 2 and 3, No. of k, (I) pairs; Col. 4, total No. of points from two concordant experiments.

* Names of carbamates: 1. Physostigmine sulphate. 2. Miotine, 2-dimethylamino-2-(3′-methylcarbamoyloxyphenyl)ethane dihydrobromide. 3. 2-pyrrolidino-2-(3′-methylcarbamoyloxyphenyl)-ethane dihydrobromide. 4. 3-methylcarbamoyloxy-trimethylaminophenyl bromide hydrobromide. 5. Pyridostigmine, 3-dimethylcarbamoyloxy-*N*-methylpyridinium methylsulphate. 6. Benzpyrinium, 3-dimethylcarbamoyloxy-*N*-phenylmethylpyridinium bromide.

Table 1 shows that rates of reactivation measured on washed preparations were significantly smaller than rates calculated from experiments in which there was an excess of carbamate. Since all measurements were made in the presence of 5·5 mM acetylcholine it may be concluded that decarbamylation was accelerated by the carbamates themselves. An analogous phenomenon has been described by Brestkin and Brik:[8] the hydrolysis of high concentrations of butyrylcholine by a purified cholinesterase (EC 3.1.1.8) was more rapid than would have been predicted by the Michaelis–Menten equation from data at the lowest substrate concentrations. The postulated mechanism involved acceleration of deacylation by high concentrations of substrate.

Kitz, Braswell and Ginsburg[3] used the method of great dilution to measure decarbamylation rates, which is equivalent to the present washing method. They found that decarbamylation was accelerated by certain non-depolarizing neuromuscular blocking drugs, and postulated that these drugs induced an allosteric change in configuration. The acceleration factors, 1·8–4·3, were roughly similar to those reported here, 1·1–7·9. It is therefore tempting to associate the well-known pharmacological antagonism between carbamates and non-depolarizing neuromuscular blocking drugs with such a change in configuration which results in accelerated decarbamylation. However, the present results give grounds for supposing that the acceleration is not specific to nondepolarizing drugs, and is indeed produced by the carbamates themselves, possibly by a similar allosteric mechanism. It is thus very doubtful if the antagonism is associated with the decarbamylation of AChE. Another objection relates to concentration effects. *In vitro*, Kitz *et al.*[3] used 50 μM gallamine in many experiments, and showed that 1 μM had no effect. Since the paralysing dose of the drug, molecular weight 892, is about 1 mg/kg[9] or about 10^{-6} moles/kg assuming even distribution without loss, it is doubtful if a concentration of gallamine could be attained in vital organs *in vivo* sufficiently high to affect decarbamylation.

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Case 469-104

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Rosin	
Serial No.:	07/ 185,451	Art Unit 126
Filed:	4/25/88	Michael L. Shippen
Title:	Phenyl Carbamates	Examiner

AMENDMENT UNDER RULE 116

Hon. Commissioner of Patents and Trademarks
Washington, D. C. 20231

Dear Sir:

This is in reply to the final rejection of October 11, 1988. Please amend the application as follows:

IN THE CLAIMS

Claims 23 and 24 line 1 cancel -- (Rewritten) --

REMARKS

The claims in the application are claims 14 to 25. The allowance of claims 23 and 24 is appreciated.

Attached hereto is a copy of a portion of Volume 29 of Advances in Behavioral Biology pages 539 to 549. This article sets forth the unexpected advantages of the compounds covered by the claims.

It is true, as the Examiner pointed out, that Aeschlimann 1,905,990 has prepared a compound with a dialkylaminoalkyl substituent, but he disclosed no such compound wherein, as in all the compounds of the present application, the alkyl between the

Two

phenyl and the amino is branched. Although according to U. S. patent 2,493,710 he prepared at a later date a compound with such a branched alkyl, this was not a dialkylamino compound. Thus present inventor has combined the features of the branched alkyl bridge and the tertiary nitrogen.

Second, though it is true that miotine as well as the Meltzer compound (identified in the present application under the code number RA_{10}) both do present the branched alkyl bridge and the tertiary nitrogen, and thus are structurally closer to the new compounds than the Aeschlimann compounds, it is stressed that by introducing for the first time high alkyl substituents than methyl on the nitrogen of the carbamate moiety, the applicant has prepared high homologues of miotine (which bears a hydrogen and a methyl) and the Meltzer compound (which bears two methyls), which surprisingly exhibit very significant advantages not only over physostigmine and the Aeschlimann art, but also over miotine and the Meltzer compound. It was totally unexpected that the mere substitution of a methyl by a higher alkyl would significantly improve the pharmacological profile of these compounds.

It was our intention to file an affidavit under Rule 132 attesting to the superiority of the claimed compounds. It was just realized that all the data available for this showing are already disclosed in the patent or specifications and in a publication of the inventors, which appeared in the priority year. This data,

Three

summarized below, appears to be adequate for establishing superiority not only of RA_7 versus RA_{10} but also of all the higher alkyl homologues of the present invention versus RA_{10} or miotine.

As mentioned in the description or the publication, physostigmine has serious disadvantages such as a low therapeutic ratio and a short duration of action which necessitates frequent dosing. The known carbamates have similar disadvantages and thus have never been used as acetylcholinesterase inhibitors: miotine (used as miotic) has a low therapeutic ratio and a short duration of action.

The new carbamates with at least one alkyl higher than methyl on the N of the carbamate unexpectedly exhibit a higher therapeutic ratio than miotine and a longer duration of action than miotine and RA_{10}. This can be seen in Table 3 of the patent application specification and Table 4 of the publication as regards the therapeutic ratio and in Table 2 of the patent application specification and table 3 of the publication (% inhibited by ED_{10} after 3 hours) as regards the duration of action.

The longer duration of action seems to be an advantage shared by all the higher homologues of RA_{10} as defined above. This is very important and totally unexpected.

It is believed that based on this showing the compounds defined by the claims are patentable over the references. For the reasons given hereinabove reconsideration of the rejection of the claims is respectively requested.

Respectfully submitted,

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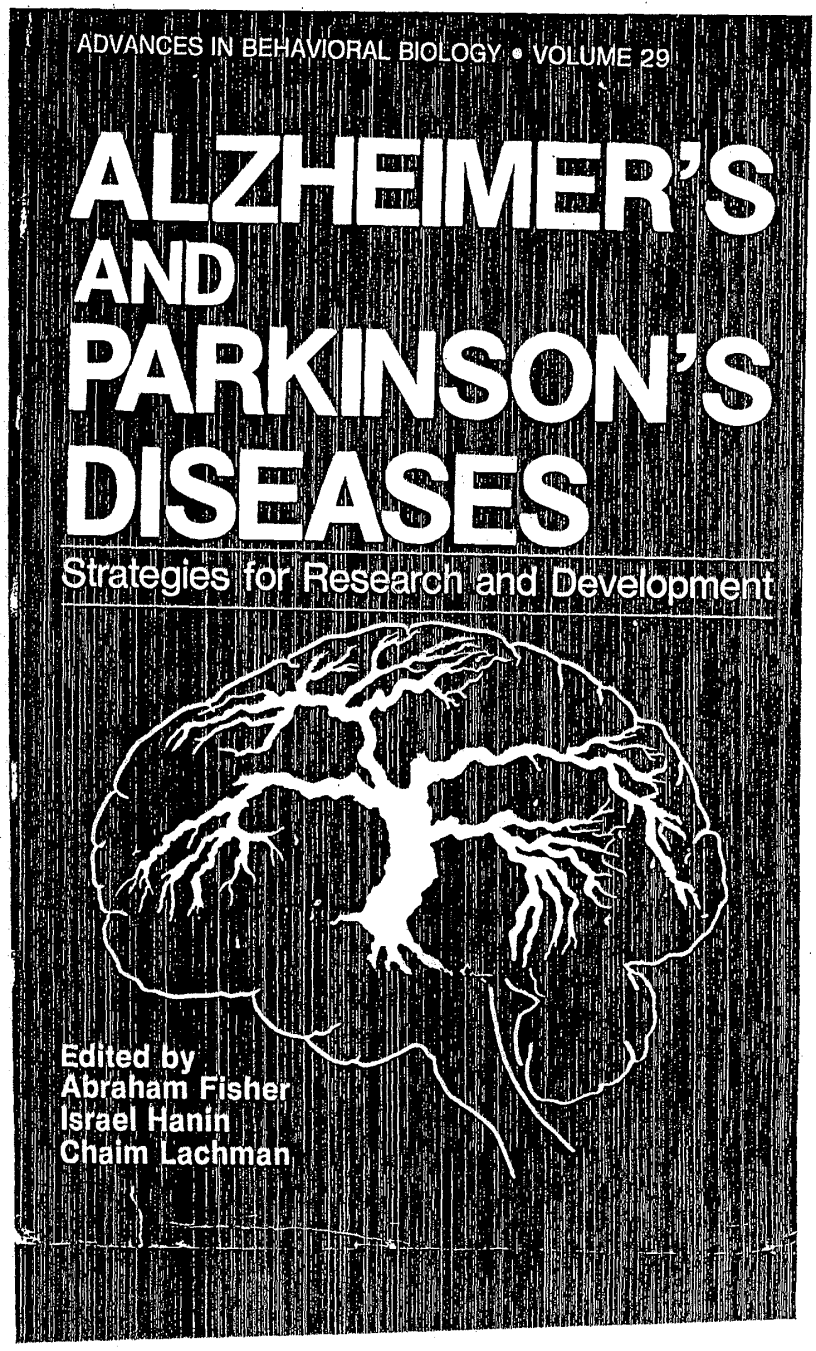
CERTIFICATE UNDER 37 CFR 1.8 (a)

I hereby certify that this amendment is being deposited with the United States Postal Service First class postage prepaid in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D. C. 20231, on December 9, 1988.

Richard T. Laughlin
Dated: 12/9/88



ALZHEIMER'S AND PARKINSON'S DISEASES

Strategies for Research and Development

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PHARMACOLOGICAL ACTIVITY OF NOVEL ANTICHOLINESTERASE AGENTS OF POTENTIAL USE IN THE TREATMENT OF ALZHEIMER'S DISEASE

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INTRODUCTION

In dementia of the Alzheimer type there is a selective loss in the cerebral cortex of choline acetyltransferase (CAT), the enzyme that synthesizes acetylcholine (ACh)[1,2]. The degree of dementia and memory impairment that occurs in this condition is well correlated with the decrement in cortical cholinergic transmission[3]. Moreover, scopolamine, a cholinergic antagonist, can cause memory impairment in normal individuals similar to that in aging[4]. These findings suggest that impaired cortical cholinergic transmission may be at least in part responsible for the symptomatology of Alzheimer disease. In support of this suggestion it was found that physostigmine, which prevents the destruction of ACh, can cause memory improvement in Alzheimer patients[5]. The extent of improvement of the symptomatology was closely related to the degree of inhibition of acetylcholinesterase (AChE) in the spinal fluid, and thus to the amount of physostigmine reaching the central nervous system[6].

As potential therapy for dementia, physostigmine has a number of disadvantages, the most serious of which is its low therapeutic ratio. In most studies in which any improvement in symptomatology was reported, the dose range in which this occurred was very narrow (1-2.5mg orally[6] or 0.25-0.5mg, i.v.[7]), with higher doses causing a decrement in performance or distressing side effects due to peripheral cholinergic overactivity. Another disadvantage is its low chemical stability[8] and short duration of action, which necessitate frequent dosing. Its oral bioavailability is also unpredictable, and it only appears to produce improvement in Alzheimer symptomatology by this route if it is given with lecithin[9].

The purpose of the present study was to synthesize anticholinesterase agents which readily reach the CNS after parenteral and oral administration; which have a higher therapeutic ratio than that of physostigmine, greater chemical stability, and a longer duration of action. These advantages should make them more suitable than physostigmine for the long term treatment of conditions associated with a deficit in cholinergic transmission in the central nervous system.

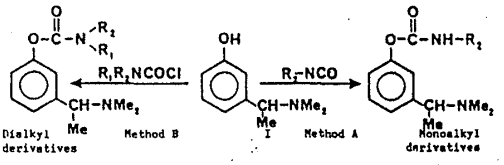
Apart from physostigmine, all of the carbamate anticholinesterases which are used medicinally, have a quaternary N-function and thus do not

penetrate the CNS to any significant extent[10]. Almost all the synthetic carbamates with a tertiary N were designed as insecticides, and have a monomethyl substituent on the N of the carbamate. They are thus relatively unstable at physiological pH and of short duration[10]. One such carbamate, miotine, has only been used clinically as a miotic[11]. The dimethyl analogue, has only been used as an insecticide[12]. The effect of other mono or dialkyl substitution on the N of the carbamate of this structure on AChE activity in vitro or in vivo does not appear to have been studied. Accordingly we prepared and tested a series of mono and dialkyl derivatives of miotine, the activities of some of which are described. (A patent has been applied for the novel structures). Particular emphasis is placed on their abilities to inhibit brain AChE and on their relative toxicities.

METHODS

Preparation of mono- and di-substituted phenyl carbamates

The N-monoalkyl and N,N-dialkyl substituted phenyl carbamates were synthesized from α-m-hydroxyphenylethyl-dimethylamine (I), which was itself prepared according to the procedure described by Stedman and Stedman[11] with minor modifications, as shown in the scheme below:



For the synthesis of the monoalkylphenyl carbamates, a 2-3 fold molar excess of the alkyl isocyanate was reacted with phenol I in dry benzene at room temperature overnight (see Scheme 1 method A). For the synthesis of the N,N-dialkyl-substituted phenyl carbamates, 1.5-2 fold molar excess of the corresponding carbamoyl chloride was allowed to react with phenol I in dry acetonitrile in the presence of a similar excess of sodium hydride (see Scheme 1 method B). The weak acidity of phenol I required the use of a strong base such as sodium hydride to produce the phenolate which acts as the nucleophile.

All carbamates were obtained as hydrochloride salts by saturating their etheral solutions with HCl(g). These salts were purified by recrystallization from ethanol-ether. Purity was assessed by t.l.c. on precoated silica gel plates, reversed-phase HPLC, elemental microchemical analysis and ^{1}H-n.m.r.

Measurement of antiAChE activity in vitro

Male mice (Sabra strain) weighing 30-40g were sacrificed by cervical dislocation and the whole brain minus cerebellum rapidly removed and weighed. The brains from 10 mice were homogenized in 1ml/100g wet weight phosphate buffer 0.1M pH 8.0, centrifuged at 12,000 rpm and the supernatant, discarded. The pellet was mixed with a similar volume as above of buffer 0.1M pH 8.0 containing 1% Triton using a Vortex Genie at maximum speed for 1 min. The mixture was centrifuged and the supernatant which contained most of the solubilized AChE was used for subsequent determinations of anticholinesterase activity.

The effect of at least three different concentrations of each inhibitor was measured on the rate of hydrolysis of 20 µl of 0.075M acetylthiocholine iodide by 25 µl of solubilized AChE. The enzyme was incubated with the inhibitor for periods ranging from 2-180 mins at 37°C before the addition of the substrate. The rate of hydrolysis was measured by the spectrophotometric method of Ellman et al.[13]. From these data the molar concentration of each agent that inhibited the activity of the enzyme by 50% (IC50) at the time of peak activity (30-120 min) was calculated.

Measurement of antiAChE activity in vivo

At least three doses of each drug were administered subcutaneously (s.c.) or orally to mice. Animals were sacrificed at different times ranging from 0.25 to 7 hours after drug administration. The presence or absence of side effects reminiscent of cholinergic hyperactivity (tremors, salivation, defecation, fasciculations, difficulty in breathing) were noted for each drug. The brain was rapidly removed at the given times stated above and the enzyme AChE extracted and solubilized as described in the previous section. The activity of the enzyme removed from drug treated mice was measured as described above and compared with that of mice given saline (control).

Assessment of acute toxicity

Male mice were given one of at least three different doses of each drug orally or s.c., a minimum of 10 mice being alotted to each dose. The number of animals that died in each group within 3 hours was determined, and from these data the LD50 (dose in µmoles/kg which was lethal to 50% of the mice) was computed.

Table 1. Relationship between chemical structure, relative hydrophobicity and molar refractivity of phenyl carbamates

Drug	R^1	R^2	Capacity factor (k')*	Mol. Refractivity
RA2 (miotine)	H	Me	0.5	5.65
RA6	H	Et	0.83	10.30
RA15	H	n-Pr	1.48	14.96
RA13	H	i-Pr	1.37	14.96
RA14	H	Allyl	1.33	14.49
RA12	H	c-Hexyl	6.17	26.69
RA10	Me	Me	1.33	11.30
RA7	Me	Et	2.33	15.95
RA8	Et	Et	4.33	20.60

* Capacity factor defined as ratio of difference between retention time of the compound and that of the unretained solute to that of the unretained solute on a reversed phase (C18) HPLC column (solvent; 70% of 0.1% aqueous TFA soln. + 30% methanol). This factor is a measure of the relative hydrophobicity of the compound.

This experiment was repeated in animals which had been pretreated 15 mins previously with either atropine methylnitrate (ATMN 5mg/kg) which block only peripheral muscarinic receptors[14] or atropine sulphate, (5mg/kg) which blocks both central and peripheral muscarinic receptors, and the anticholinesterase agents were injected s.c.

Measurement of antiAChE activity in different areas of rat brain

Male and female Sabra rats weighing 150-350g were injected s.c. with either saline, physostigmine 0.15mg/kg, RA6 1.0mg/kg. RA7 0.5mg/kg or RA15 0.5mg/kg (six animals were used for each treatment group). The cerebral cortex, hippocampus, corpus striatum and medulla oblongata were rapidly dissected on ice, weighed individually, homogenized in phosphate buffer and extracted and solubilized as described above for mouse brain. The activity of the enzyme from treated and control rats was also measured as described above.

The percent inhibition of AChE by each drug was computed for the different brain areas by comparison with the pooled mean of the control values (n=12) for each area.

Statistical analyses. Data from the experiment on the effects of drugs on AChE in different areas of rat brain were analysed by 2-way analysis of variance, followed by Neuman Keul's post hoc comparisons.

RESULTS

The relationship between the N alkyl substituents, relative hydrophobicity and molar refractivity is shown in Table 1. In general both the latter parameters increased as the size of the mono or disubstituted alkyl groups became larger.

Table 2. The effect of the novel compounds on AChE activity in mouse brain in vitro and in vivo

Drug	IC50 µM	Relative Potency to Physostigmine	ED50 µMoles/kg	Relative Potency to Physostigmine
Physostigmine	0.011	100	0.92	100
RA2	0.013	85	0.92	100
RA6	0.40	3	8.47	11
RA15	0.11	10	2.80	33
RA13	12.10	0.1	40.0	2
RA14	0.43	3	6.01	15
RA12	0.093	12	7.24	13
RA10	0.027	41	1.14	81
RA7	3.00	0.4	4.20	22
RA8	35.0	0.03	56.0	2

AntiAChE activity in mouse brain

The inhibitory activities of the novel carbamates and physostigmine on a solubilized preparation of AChE of mouse whole brain *in vitro* are summarized in Table 2. The monomethyl substituted derivative, RA2, (miotine), was found to be the most potent inhibitor of brain AChE, both *in vitro* and *in vivo*. It has a rapid onset of action which is of a relatively short duration (90-120 min *in vivo*) like that of physostigmine (Table 3). Increase in the size of the alkyl radical to ethyl (RA6), resulted in a large reduction (>30 fold) in *in vitro* activity, but only a 6-fold decrease, *in vivo*. Larger substituents, n-propyl, and c-hexyl proved to be more potent inhibitors than N-ethyl, or N-allyl, but less so, than N-methyl, while introduction of an i-propyl group resulted in a 1000-fold decrease in AChE activity. In general, all the novel monosubstituted carbamates were more active *in vivo* by factors of 2-20 times, than one would have expected from the activities on the isolated enzyme when compared to physostigmine or miotine. [Table 2].

Comparison of the data in Tables 1 and 2, reveals that there is no correlation between *in vitro* anticholinesterase activity (IC50) of the monosubstituted carbamates and any of the physical parameters examined, e.g. chain length in extended conformation, methyl (RA2), <ethyl (RA6), <n-propyl (RA15); molar refractivity, cf. c-hexyl (RA12), ethyl and i-propyl (RA13); hydrophobicity, of n-propyl and i-propyl.

The disubstituted carbamates were generally less active *in vitro* than the corresponding monosubstituted derivatives. Among the three analogues there appeared to be a negative correlation between inhibitory potency, and both hydrophobicity and molar refractivity volume.

Introduction of a second methyl group on the N of the carbamate caused only a small reduction in inhibitory activity. However, when one group was substituted by ethyl, (RA7) *in vitro* activity fell by 2 orders of magnitude. Surprisingly, this compound was considerably more potent than one would have expected from the *in vitro* data when it was injected into the whole animal Under these conditions its activity was only reduced to 1/3rd of that of the dimethyl derivative.

Table 3. Duration of action of carbamates on brain AChE in mice

Drug	Time of peak inhibition (min.)	% inhibition ± s.e. by ED50 at 3 hrs	ED50 oral / ED50 s.c.
Physostigmine	15	0	4.3
RA2	15	0	1.3
RA6	30-120	47±1	2.6
RA15	15-30	26±5	4.0
RA14	30	41±3	3.8
RA12	30-60	36±3	3.0
RA10	15	0	3.4
RA7	60-120	33±3	1.5
RA8	30-120	31±6	1.4

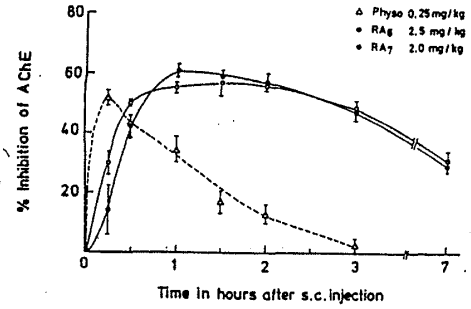


Fig. 1: Duration of inhibition of brain AChE after s.c. injection of physostigmine, RA6 and RA7 in mice

The diethyl substituted compound, RA8, proved to be a weak inhibitor, with an IC50 of only 35 µM. All the compounds having a substitutent larger than methyl, had a slower onset of action, both on the isolated solubilized enzyme and in the whole animal, and a longer duration of action in vivo, than methyl derivatives and physostigmine (Table 3). The latter drugs ceased to inhibit brain AChE 2-3 hours after injection, while all the novel compounds with alkyl substituents larger than methyl caused significant inhibition for 3-7 hours [Fig. 1].

The maximum inhibition of the brain AChE after oral administration of any dose of physostigmine, did not exceed 50%. This was achieved at about a 4 times larger dose than the ED50 after s.c. injection [Table 3]. Higher doses, caused marked respiratory distress, fasciculations and tremors. With the possible exception of RA10, a greater than 70% inhibition of brain AChE was obtained after oral administration of all the other compounds. The incidence of untoward symptoms due to cholinergic overactivity was also much lower with these compounds.

Acute toxicity

The acute toxicity of the anticholinesterase agents is shown in Table 4, when these were given alone or after pretreatment with ATMN or atropine. The therapeutic ratios, defined as the LD50/ED50, of all the compounds except RA2 were about 3 times greater than that of physostigmine, which was only 3.3. Blockade of peripheral muscarinic receptors by ATMN, caused a similar increase in LD50 (1.5-2.2 fold) in all the compounds. When muscarinic receptors in the CNS were also blocked by

atropine, the LD50 of physostigmine and the majority of the compounds rose by 2.2-3.5 fold. The disubstitued compounds, RA10 and RA7, however, showed a 6-11 fold increase in LD50.

AntiAChE activity in different areas of rat brain

The AChE activity of different areas of rat brain is shown in Table 5. While the cerebral cortex, hippocampus and medulla showed approximately similar amounts of enzyme activity, that in the striatum was about 10-fold higher.

Fig. 2 shows the effect of physostigmine and three novel carbamates on AChE activity in 4 areas of rat brain. The doses of the 4 drugs were chosen which gave the same degree of inhibition of AChE in the cerebral cortex. At these doses, RA6, RA7 and RA15 caused significantly less inhibition in the medulla ($P<0.05$) and RA7 caused a lower effect in the striatum, than in the cortex. RA6 and RA7 also produced significantly less inhibition in the medulla than did physostigmine. The effect of RA15 in the hippocampus was significantly greater than that of all the other drugs when given at a dose that inhibited the enzyme in the cortex to a similar extent.

DISCUSSION

In the present series of carbamate derivatives in vitro inhibition (IC50) of brain AChE varied 3000-fold from the most to least potent drug. In the mono-alkylated derivatives, no correlation was found between the IC50 values and hydrophobicity, molar refractivity, or length of the most extended conformation of the carbamate moiety. Thus, the largest substituent, c-hexyl, showed a much smaller decrease in inhibitory potency compared to miotine, than did the monoethyl derivative. On the other hand, introduction of an i-propyl resulted in a 1000-fold decrease in activity, while n-propyl, which has the same molar refractivity and hydrophobicity, was only 10 times less potent than miotine.

Table 4. Acute toxicity of carbamates in mice

Drug	LD50 µmoles/kg s.c.	Therapeutic ratio (LD50/ED50)	Degree of protection** afforded by pretreatment with	
			ATMN*	Atropine*
Physo.	3.0	3.3	1.8	3.0
RA2	4.50	4.9	1.8	2.4
RA6	95.7	11.3	1.5	2.7
RA15	30.5	10.9	1.5	3.0
RA14	64.8	10.8	1.8	2.2
RA12	41.5	9.8	1.2	3.5
RA10	12.4	10.9	1.6	5.8
RA7	46.0	11.0	2.2	10.9
RA8	>568	>10.0	-	

* Drug injected 15 min. after atropine methyl nitrate 5 mg/kg or atropine sulphate 5 mg/kg

** $\frac{\text{LD50 after ATMN or atropine pretreatment}}{\text{LD50 of drug alone}}$

Table 5. AChE activity in different areas of rat brain

Brain Area	μM of substrate hydrolysed per min per mg. tissue ± s.e.
Cerebral cortex (11)	2.73±0.09
Hippocampus (12)	3.43±0.09
Medulla (12)	5.55±0.27
Corpus striatum (11)	27.10±1.10

Furthermore, no clear correlation could be demonstrated between anti AChE activity of the carbamates on the isolated enzyme taken from mouse brain and that obtained ex vivo after injection of the drug into mice. All the novel carbamates were relatively much more active in vivo in relation to physostigmine or miotine, than in vitro. This discrepancy was especially evident in the disubstituted analogues, RA7 and RA8. These compounds were 50-60 times more effective in vivo than one would have predicted from the data on the isolated enzyme.

The relatively greater activity of the larger monoalkyl and dialkyl substituted drugs in the whole animal may be due to a greater chemical stability. It has previously been shown that monomethyl carbamates are much less stable that dimethyl derivatives at physiolgical pH[10]. The relatively long duration of enzyme inhibition (>7 hours) of all the larger alkyl derivatives in vivo, (compared with about 2 hours for physostigmine) suggests that they are chemically more stable at body pH and are more slowly metabolized.

Another reason for the greater in vivo activity of the RA compounds may be their higher lipid solubility, which should enable a greater proportion of the drug to reach the central nervous system. This property could also explain the more efficient absorption from the gastro-intestinal tract of several of these carbamates, particularly RA7 and RA8.

Comparison of the acute toxicity of the RA compounds with that of physostigmine in mice, showed the former to have considerably higher therapeutic ratios, 10-12, compared with 3.3 for physostigmine and 4.5 for miotine. Furthermore, signs of cholinergic overactivity, fasciculations, tremors, salivation and defecation were seen at the ED50 dose (which caused 50% inhibition of the whole brain enzyme) of physostigmine but not of the other carbamates. The greater therapeutic ratios of the RA compounds appears at first sight to be surprising since the mortality is a direct result of AChE inhibition, and is due to the presence of excess AChE in the medulla, which causes respiratory arrest[15]. This was demonstrated in the present study by pretreating the animals with atropine which prevents the centrally induced respiratory depression[14], and which raises the LD50 of all the monosubstituted carbamates by a factor of about 3. In the presence of such muscarinic blockade, death from overdose then results from respiratory muscle paralysis due to excess ACh at the neuromuscular junction. At this stage, no antidotes are effective and only artificial ventilation can prevent loss of life. The fact that the LD50 of RA7 can be increased 11-fold by muscarinic receptor blockade, demonstrates a relative lack of effect of this drug on somatic muscle. This is a distinct advantage in terms of its therapeutic potential.

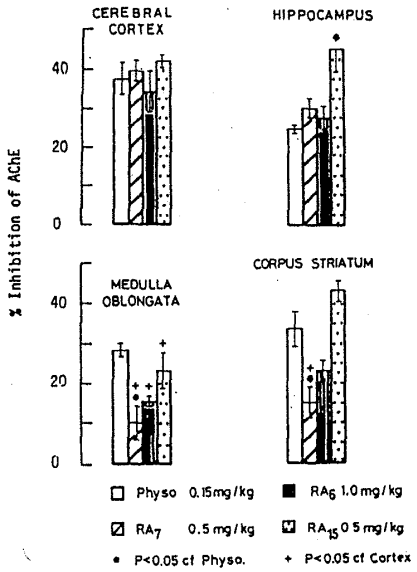


Fig. 2. Inhibition of AChE in different areas of rat brain by physostigmine and 3 novel carbamates
* Significantly different from physostigmine in same brain area P<0.05
+ Significantly different from value in cortex for same drug P<0.05

In order to explain the lower toxicity of the RA compounds an attempt was made to determine whether they have a selective effect in different brain areas. It was found that physostigmine inhibited AChE to the same extent in four areas in the rat brain in spite of the fact that these areas contain different amounts of enzyme. In contrast, RA6, RA7 and RA15 given in doses which blocked AChE in the cerebral cortex by 35-40%, caused significantly less inhibition in the medulla. The most striking difference was seen with RA7 which only reduced AChE in the medulla by 10%. Since the ED50 was determined in whole brain, of which the cerebral cortex contributes a major portion compared to the medulla, this differential effect of the drugs serves to explain their higher therapeutic ratio.

The selective effect may result from a difference in the distribution of the drugs to these brain areas. Alternatively, it may be due to the presence of AChE isoenzymes, which could have different affinities for the inhibitors. Such a differential sensitivity of multiple forms of AChE has been demonstrated for organophosphates[10]. It remains to be determined whether multiple forms of AChE are present in rat brain, and whether they are selectively inhibited by RA compounds.

The data from this study show that larger monoalkyl or dialkyl derivatives of miotine, possess several advantages over physostigmine for potential therapeutic application in conditions involving reduced cholinergic transmission in the cerebral cortex. If the therapeutic effect of these agents results from inhibition of AChE in this brain area, compounds RA6, RA15, RA14, RA12, RA10, RA7 and RA8 all have considerably higher therapeutic ratios than physostigmine and show fewer side effects at ED50 doses. This may be due to a selective inhibition in cortical areas sparing the medulla. RA7 and RA10 have an additional advantage in the fact that the lethal effects of drug overdose can be prevented by atropine. While the duration of significant enzyme inhibition after physostigmine is less than 2 hours, all the above drugs (except RA10) act for periods of 7 hours or more after a single injection. The longer duration is a distinct advantage in the treatment of chronic conditions such as Alzheimer's disease. Furthermore, RA6, RA7 and RA8 show a significantly more efficient oral absorption since their potencies when given by this route closely resemble those after parenteral administration.

Acknowledgement. This research was supported by a grant from the Israeli National Council for Research and Development No. 2248.

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549

185,451

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
07/185,451	04/25/88	ROSIN	M	469-102-1

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & FEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	**PAPER NUMBER**
126	6

DATE MAILED: 12/21/88

Below is a communication from the EXAMINER in charge of this application
COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☒ THE PERIOD FOR RESPONSE:

☐ is extended to run _____ from the date of the Final Rejection

☐ continues to run _____ from the date of the Final Rejection

☒ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date that the shortened statutory period for response expires as set forth above.

☐ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 12/12/88, has been considered with the following affect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:
   a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.
   b. ☐ They raise new issues that would require further consideration and/or search. (See Note).
   c. ☐ They raise the issue of new matter. (See Note).
   d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

   NOTE: _____

2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ Upon the filing of an appeal, the proposed amendment ☒ will be ☐ will not be, entered and the status of the claims in this application would be as follows:

   Allowed claims: 23+24
   Claims objected to: _____
   Claims rejected: 14-22+25

   However;
   a. ☐ The rejection of claims _____ on references is deemed to be overcome by applicant's response.
   b. ☐ The rejection of claims _____ on non-reference grounds only is deemed to be overcome by applicant's response.

4. ☒ The ~~affidavit~~ request for reconsideration has been considered but does not overcome the rejection, see attached sheet

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficient reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☐ Other

PTOL-303 (REV 3-86)

Applicants' evidence ("Advances in Behavioral Biology," Vol. 29, pages 539-549) has not been considered since a good and sufficient reason has not been presented why the evidence could not have been presented earlier. Moreover, it does not appear that such evidence would put the application in condition for allowance. Also, note MPEP 609.

The evidence presented in the specification has been considered but not found persuasive of patentability. Fisrt, while Aeschlimann (USP 1,905,990) refers to physostigmine, this compound is not representative of the compounds of the reference, e.g., compound of example 2. As such, it is considered that the compounds of Aeschlimann have not been compared. Second, it is not seen that miotine and the compound of Meltzer are structurally closer than the compounds of Aeschlimann. Third, the showing is not commensurate in scope with the claims. While compound RA6 is a homologue of miotine, the claims still read on other compounds that are just as structurally close or closer to the prior art compounds. For example, homologues and isomers of the Aeschlimann compounds wherein R1 and R2 are methyl, R3 is hydrogen and R4 and R5 are ethyl; the homologues of miotine wherein one or both of R4 and R5 is ethyl or R3 is methyl; and homologues and isomers of the Meltzer compound wherein R1, R2, R4 or R5 is ethyl or R3 is methyl. Fourth, the evidence of Talbe 3 would suggest that the Meltzer compound are comparable to the instantly claimed compounds. Fifth, the method claims read on the use of the prior art compounds represented by applicants to be inferior or

compounds structurally closer to the prior art compounds that have not been compared. Allegations as to duration stand unsubstantiated.

MShippen
703-557-3920

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

12.x 12/29/88, 3000 HG #7. 1/3/89 12 1/29/88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Rosin

Serial No.: 07/ 185,451 Group No.: Art Unit 126

Filed: 04/25/88 Examiner: Michael L. Shippen

For: Phenyl Carbamates

Commissioner of Patents and Trademarks

Washington, D. C. 20231

NOTICE OF APPEAL FROM THE PRIMARY EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant hereby appeals to the Board from the decision of the Primary Examiner dated October 11, 1988 finally rejecting claims 14 TO 22 & 25. Claims 23 & 24 were allowed.

The item(s) checked below are appropriate:

1.__ A petition and fee for extension of term for reply to the final rejection is attached.

2. X Appeal Fee
__X__ other than a small entity- fee $130.00
___ small entity- fee $ 65.00
____verified statement attached.
____verified statement filed on_____.

Fee $130.00

3. X Payment

__X__ Check attached for the sum of $ 130.00 .
____ Charge Account _____for any fee deficiency.

____Charge Account_____ the sum of $_____(and for any fee deficiency). A duplicate of this notice is attached.

Signature of Attorney

Reg No. 17,264

Tel. No. (201)539-0080

Richard T. Laughlin
129 Headquarters Plaza
Morristown, New Jersey 07960

070 01/13/89 185451 1 119 130.00 CK

CERTIFICATE OF MAILING (37 CFR.1.8a)

I hereby certify that this paper (along with along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: January 10, 1989

Richard T. Laughlin

Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960
(201) 539-0080

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
185,451	4/25/88	Rosin	

EXAMINER	
SHIPPEN	
ART UNIT	PAPER NUMBER
126	8

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) M. Shippen (3) _____

(2) Casper (4) _____

Date of interview 2/22/89

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: 18

Identification of prior art discussed: _____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: _____

① A comparison of claim 18 should be with closest prior art compounds; however, further evidence not already of record probably would not be considered in this case.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1—7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature

PTOL-413 (REV. 1-84)

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10X 1-31-89

RECEIVED 89 FEB 29 AM 9:29 GROUP 120

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: M. W. Rosin et al.

Serial No: 185,451 Art Unit: 126

Filing Date: April 25, 1988

Title: PHENYL CARBAMATES

Examiner: Michael L. Shippen

February 28, 1989 lau451al

AMENDMENT UNDER RULE 116

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR:

This is in response to the Office Action mailed on October 11, 1988 and setting a shortened statutory period for response of three months to expire on January 11, 1989. Applicants petition that, if required, the time for response be extended and the corresponding fee be charged. The Commissioner is hereby authorized to charge any additional fees which may be required to Acct. No. 11-0224. Applicants further respectfully request that this response be accepted as a bona fide effort to meet any potential response requirements outstanding and due in the above captioned matter.

OK To Enter 3/13/89

Please amend the application as follows:

1

IN THE CLAIMS:

Please cancel claims 14 to 17 without prejudice to their reintroduction at a later point in time.

18. (amended) [A compound of claim 14 which is] N-ethyl,N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate and [or] pharmacologically acceptable salts thereof.

Please cancel claims 19 to 22 and 25 without prejudice to their reintroduction at a later point in time.

REMARKS

Claims 14 to 25 were in the case. The present amendment cancels claims 14 to 17, 19 to 22 and 25 without prejudice to their reintroduction at a later point in time.

Applicants' attorney thanks the Examiner Michael L. Shippen for the interview kindly granted on February 22, 1989. The courtesies exchanged during the interview are very much appreciated. During the interview the claims and the references of record were considered. Applicants' attorney presented arguments distinguishing the composition disclosed in claim 18 of the instant application from the teachings of the references substantially as set forth below.

It appeared that the application provided some statements, which indicate that the N-ethyl,N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate of claim 18 is patentably distinguished over the art of record.

It was noted during the interview that the closest art of record appeared to be the compound code 1207 on page 177, first item of Table V of the journal article "Insecticidal Activity of Substituted Phenyl N-Methylcarbamates" by J. Meltzer and H. B. A. Welle in Ent. exp. & appl. 12 (1969), 169 -172. This compound is N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate. Whereas this reference compound has an active hydrogen atom left at the nitrogen of the carbamate group, the present invention has this hydrogen atom substituted by ethyl.

The reference "Inhibition of Activated Factors II, VII, IX, and X by Synthetic Organic Compounds Directed against the Active-Site Seryl Residue" by J. A. v. d. Woerd-de Lange et al. in Haemostasis 10, 315 -347 (1981) also lists as compound #37 on page 332 the same compound as the above reference, that is N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

It is submitted that the compound of claim 18 is clearly distinguished from the compound recited in these two

references by the ethyl group substituting a hydrogen atom at the carbamate nitrogen atom.

This compound of the references is further recited in the specification of the applicants and listed in the tables of the present application as a reference compound labelled Miotine or Miotine HCl for the hydrochloric acid salt (compare Page 19, Table 1, second item; Page 20, Table 2, second item; and Page 21, Table 3, second item).

The same Tables contain the compound of claim 18 designated as RA_7 HCl (Table 1) or RA_7 (Tables 2 and 3). As stated on Page 17, lines 15 to 25, the acetylcholinesterase inhibition was determined after subcutaneous administration. While the potency of the reference compound miotine is 5 percent on a relative scale of 100 percent referring to the activity of physostigmine, the invention compound of claim 18 was found to have a potency of 41 or more than eight times as large.

Table 3 compares the acute toxicity of carbamates in mice. This toxicity was determined as set forth in the specification on page 17, line 26 to page 18, line 14. The first data column of Table 3 shows that the lethal dose of the reference compound Miotine was 4.5, whereas the invention compound of claim 18 exhibited a lethal dose of

46, which is more than ten times as large. Thus the side effects of the invention compound of claim 18 appear to be much less than those of the reference compound Miotine. Furthermore, data column 2 of Table 3 indicates that the degree of protection afforded by pretreatment with atropine has only a value of 2.4 for the reference compound Miotine, whereas the invention compound of claim 18 has a value of 10.4, which is more than four times as large. Data column 3 of Table 3 calculates the therapeutic ratio and finds that the reference compound Miotine has a therapeutic ratio of 4.9, whereas the invention compound of claim 18 has a therapeutic ratio of of 12.4, which is more than 2.5 times as high.

The applicants' specification provides on pages 22 to 26 a comparison of compounds according to the invention to reference compounds. The last two paragraphs on page 23 show a general superiority of certain compounds including the compound of claim 18 over the reference compound Miotine. Page 24, lines 4 and 5 demonstrates that the invention compound of claim 18 shows a longer time effectivenessas compared to other compounds considered including the reference compound Miotine. This leads to particular advantages in the treatment of certain diseases

as stated on page 24, lines 8 to 14. Furthermore, page 24, lines 15 to 23 discloses that there is a more effective absorption of the invention compound of claim 18 as compared to the reference compound Miotine.

In view of the clear structural difference of the invention compound of claim 18 as compared with what appears to be the closest reference Miotine, as well as in view of the advantageous pharmacological properties of the invention compound of claim 18 over the reference Miotine, it is respectfully submitted that the compound of claim 18 N-ethyl,N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate is clearly patentable over Miotine and the art of record.

The present amendment is intended to present a claim which is deemed to be in better form for appeal.

It is submitted that a large part of the present submission is inherently contained in the application. Therefore, it was not appropriate to present this focused consideration prior to the cancellation of claims 14 to 17, 19 to 22 and 25.

The present amendments were not presented earlier since they are in response to the specific combination of references performed in the Final Rejection. The way of combination of references in the Final rejection is

unexpected and new to the Applicants.

The present amendment is deemed to remove and/or simplify issues which would otherwise require consideration in an appeal. The present amendment is further submitted to adopt suggestions gathered from the Office Actions.

The present amendment is believed not to present any new issues since the claim is substantially based on a previously presented claim and since the application provides clear support for particular advantages associated with the invention compound of claim 18.

The present amendment is cancelling the finally rejected claims 14 to 17, 19 to 22 and 25 in order to place the application in better condition for appeal.

It is submitted that the amendment is a bona fide attempt to advance the prosecution by amendments to the claims seeking to overcome rejections based on the applied prior art.

It is submitted that the present amendment complies with observations made in the Final Rejection.

Reconsideration of all outstanding rejections is respectfully requested.

Thus, the Applicants believe that this case is now in condition for allowance and a Notice of Allowance is

solicited. If the Office in any respect finds that this amendment is not complete to place the application in condition for allowance, then an interview with Applicants' attorney is respectfully requested. It is submitted that such an interview would be particularly appropriate because applicants have narrowed the issues substantially during prosecution of this application.

Entry of the present amendment is respectfully requested. The claim as presently submitted is deemed to be in form for allowance and an early notice of allowance is earnestly solicited.

Respectfully submitted,

M. W. Rosin et al.

By: _____

Horst M. Kasper, their attorney
13 Forest Drive, Warren, N.J. 07060
(201)757-2839; Reg.No. 28559
Docket No.: lau451

*%FAMEND(lau451(February 28, 1989(rep

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

185,451

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
07/185,451	04/25/88	ROSIN	M	469-102-1

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	10

DATE MAILED: 03/14/89

NOTICE OF ALLOWABILITY

PART I.

1. ☒ This communication is responsive to 2/28/89.
2. ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. ☒ The allowed claims are 18, 23+24.
4. ☐ The drawings filed on _____ are acceptable.
5. ☒ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has [_] been received. ☒ not been received. [_] been filed in parent application Serial No. _____, filed on _____
6. ☐ Note the attached Examiner's Amendment.
7. ☐ Note the attached Examiner Interview Summary Record, PTOL-413.
8. ☐ Note the attached Examiner's Statement of Reasons for Allowance.
9. ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
10. ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. ☐ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. ☐ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:

- _ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- _ Reasons for Allowance
- _ Notice of References Cited, PTO-892
- _ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

Michael L. Shippen
MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

OL-85 (REV. 4-86)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

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Washington, D.C. 20231

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

All communications regarding this application should give the serial number, date of filing, name of applicant, and batch number.

Please direct all communications to the Attention of "OFFICE OF PUBLICATIONS" unless advised to the contrary.

The application identified below has been examined and found allowable for issuance of Letters Patent. PROSECUTION ON THE MERITS IS CLOSED.

	SC/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT		DATE MAILED
	07/185,451	04/25/88	003	SHIPPEN, M	126	03/14/89

First Named Applicant: ROSIN, MARTA W.

TITLE OF INVENTION: PHENYL CARBAMATES

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
	469-102-1	560-115.000	R05	UTILITY	NO	$560.00	06/14/89

The amount of the issue fee is specified in 37 C.F.R. 1.18. If the applicant qualified for and has filed a verified statement of small entity status in accordance with 37 C.F.R. 1.27, the issue fee is one-half the amount for non-small entities. The issue fee due printed above reflects applicant's status as of the time of mailing this notice. A verified statement of small entity status may be filed prior to or with payment of the issue fee. However, in accordance with 37 C.F.R. 1.28, failure to establish status as a small entity prior to or with payment of the issue fee precludes payment of the issue fee in the amount so established for small entities and precludes a refund of any portion thereof paid prior to establishing status as a small entity.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE as indicated above. The application shall otherwise be regarded as ABANDONED. The issue fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office. Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of the notice of allowance, the issue fee is charged to the deposit account at the time of mailing of this notice in accordance with 37 C.F.R. 1.311. If the issue fee has been so charged, it is indicated above.

In order to minimize delays in the issuance of a patent based on this application, **this Notice may have been mailed prior to completion of final processing.** The nature and/or extent of the remaining revision or processing requirements may cause slight delays of the patent. In addition, **if prosecution is to be reopened, this Notice of Allowance will be vacated and the appropriate Office action will follow in due course.** If the issue fee has already been paid and prosecution is reopened, the applicant may request a refund or request that the fee be credited to a deposit account. However, applicant may request that the previously submitted issue fee be applied. If abandoned, applicant may request refund or credit to a deposit account.

In the case of each patent issuing without an assignment, the complete post office address of the inventor(s) will be printed in the patent heading and in the Official Gazette. If the inventor's address is now different from the address which appears in the application, please fill in the information in the spaces provided on PTOL-85b enclosed. If there are address changes for more than two inventors, enter the additional addresses on the reverse side of the PTOL-85b.

The appropriate spaces in the ASSIGNMENT DATA section of PTOL-85b must be completed in all cases. If it is desired to have the patent issue to an assignee, an assignment must have been previously submitted to the Patent and Trademark Office or must be submitted not later than the date of payment of the issue fee as required by 37 C.F.R. 1.334. Where there is an assignment, the assignee's name and address must be provided on the PTOL-85b to ensure its inclusion in the printed patent.

Advance orders for 10 or more printed copies of the prospective patent can be made by completing the information in Section 4 of PTOL-85b and submitting payment therewith. If use of a deposit account is being authorized for payment, PTOL-85c should also be forwarded. The order must be for at least 10 copies and must accompany the issue fee. The copies ordered will be sent only to the address specified in section 1 or 1A of PTOL-85b.

☑ Note attached communication from the Examiner.

☐ This notice is issued in view of applicant's communication filed _____

IMPORTANT REMINDER

Patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. See 37 CFR 1.20 (e)—(j).

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Duration
pkb
5/22/89

April 21, 1989

COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, DC 20231

Re: U.S. PATENT APPLICATION
SERIAL NO. 185,451
Our file No. 469-101

Dear Sirs:

The enclosed articles have come to our attention in connection with the above identified application.

We believe the claims are allowed over these references since the articles only relate to the compounds we had indicated previously on Compound I.

Respectfully submitted,

Richard T. Laughlin

Richard T. Laughlin
182

Enclosures

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DEAR CLIENT:

THIS IS THE BEST COPY AVAILABLE, OF THE ATTACHED PAGE(S), DUE TO THE CONDITION OF THE SOURCE DOCUMENT.

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SINCERELY,

Mike Dunlap

FILE HISTORY SPECIALIST

PATENT IMAGING CORP.

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CXLIII. STUDIES ON THE RELATIONSHIP BETWEEN CHEMICAL CONSTITUTION AND PHYSIOLOGICAL ACTION.

IV. THE INHIBITORY ACTION OF CERTAIN SYNTHETIC URETHANES ON THE ACTIVITY OF ESTERASES.

By EDGAR STEDMAN and ELLEN STEDMAN.

From the Department of Medical Chemistry, University of Edinburgh.

(*Received June 26th, 1932.*)

PART III [Stedman and Stedman, 1931] of this series of communications was concerned with an examination of the action towards liver esterase of the group of urethanes which had previously been shown to behave pharmacologically as "parasympathetic stimulants," and it was demonstrated that, while such urethanes possessed the power of inhibiting the action of this enzyme to a very high degree, other urethanes and bases did not produce a similar effect. It was therefore concluded that a relationship between constitution and inhibitory action towards liver esterase existed analogous to that between constitution and physiological action in the same group. This result afforded exceedingly strong, if not conclusive, evidence in favour of Loewi and Navratil's [1926] views regarding the mechanism of the action of physostigmine on the heart, and further appeared to indicate the nature of the enzyme which, according to Engelhart and Loewi [1930] and to Matthes [1930], brings about the destruction of acetylcholine and of the vagus substance. It is clear from Loewi's work, however, that the liver is not normally directly responsible for the destruction of the vagus substance, which, in his original experiments, was shown to be caused by aqueous extracts of the frog's heart. It therefore appeared to be desirable to examine the inhibitory action of the above group of urethanes towards serum esterase, particularly as Engelhart and Loewi, Matthes, and Plattner have shown that both whole blood and serum are capable of destroying acetylcholine. At the same time the actions of the urethanes on two other enzymes, namely pancreatic lipase and phosphatase, the normal substrates of which are esters, have been examined in order to determine the extent to which the inhibitory action of the urethanes is specific.

PANCREATIC LIPASE.

Enzyme preparation. The pancreatic lipase was prepared from pig's pancreas according to Willstätter and Waldschmidt-Leitz's method [1923]. The minced glands were desiccated by successive treatment with acetone and ether and the fibrous material was separated from the dry preparation by pounding it in a mortar and then shaking it through a fine sieve. The fine powder so obtained was extracted for 4 hours at 30° with 87 % glycerol (32 cc. for 2 g. of powder), centrifuged, and the extract stored in this form. Immediately before use, 15 cc. of this extract were diluted to 60 cc. with water, and again centrifuged to remove a fine precipitate which separated.

Hydrolysis of olive oil. The influence of miotine hydrochloride on the hydrolysis of olive oil by pancreatic lipase was first examined. Into each of two small stoppered bottles 2·5 g. of olive oil were weighed and 2 cc. of ammonia-ammonium chloride buffer (p_H 8·9) were then added. The contents of one bottle were now treated with 11 cc. of the above enzyme extract and those of the other with 11 cc. of the same extract containing 5 mg. of miotine hydrochloride which had been dissolved in it one hour previously. Each bottle was shaken for 3 minutes to emulsify the oil and then placed in a thermostat at 30° for one hour. The contents of each bottle were now washed with 100 cc. of rectified spirit into a flask, 20 cc. of ether added to dissolve the oil and the solutions titrated with 0·727*N* alcoholic potassium hydroxide, using thymolphthalein as indicator. In each case 6·8 cc. of the alkali were used. A control, using 11 cc. of water in place of the enzyme solution, required 2·4 cc. of the alkali. It is clear that miotine exerted no inhibitory action.

Hydrolysis of tributyrin. In order to follow the hydrolysis of tributyrin, the stalagmometric method described in Part III was employed. The above diluted extract (1 cc.) of pancreatic lipase was mixed with 2 cc. of phosphate buffer (p_H 7·9) and diluted to 10 cc. with water. One cc. of this preparation was treated with 12 mg. of miotine hydrochloride dissolved in 1 cc. of water and allowed to stand for one hour, when 1 cc. of the mixture was used in a hydrolysis experiment. For the control, 1 cc. of water was employed in place of the solution of miotine hydrochloride. The following figures represent the diminution in the drop number in successive periods of 20 minutes: control, 15, 23; in the presence of miotine, 14, 23. Miotine is thus without inhibitory action on the hydrolysis of tributyrin by pancreatic lipase.

Hydrolysis of methyl butyrate. The method employed was at first identical with that used in Part III with liver esterase, except that the reaction mixture contained a high percentage of glycerol which activates as well as stabilises the lipase. Into a 100 cc. graduated flask were introduced 50 cc. of a 50 % solution of glycerol and 20 cc. of buffer (1 part 2·5*N* NH_3:2 parts 2·5*N* NH_4Cl; p_H 8·9) and the mixture was then warmed to 30° in a thermostat. One cc. of methyl butyrate was dissolved as completely as possible in this by shaking, when 20 cc. of the diluted extract of pancreatic lipase were added and the volume was

made up to 100 cc. with water. The flask was again placed in the thermostat, 20 cc. of the mixture being immediately withdrawn, run into a mixture of 25 cc. of 0·2 *N* hydrochloric acid with 20 cc. of water and titrated with 0·2 *N* sodium hydroxide, using bromocresol purple as indicator. Similar volumes of the mixture were withdrawn at intervals of 20 minutes and titrated in the same way. In the experiment designed to test the inhibitory action of miotine, 12 mg. of the hydrochloride were dissolved in 25 cc. of the diluted extract of the enzyme and allowed to stand for one hour; 20 cc. of this solution were then used in a hydrolysis experiment. The following results were obtained, the figures representing the number of cc. of 0·2 *N* alkali required to titrate the acid liberated in 20 cc. of the reaction mixture in 20, 40 and 60 minutes respectively: control, 1·75, 2·7, 3·4; in the presence of miotine, 1·15, 1·85, 2·35. A small inhibitory effect is apparent, although it is much smaller in magnitude than with liver esterase.

Willstätter and Memmen [1924] have shown that calcium oleate exerts a marked activating action on the hydrolysis of methyl butyrate by pancreatic lipase. Another experiment was therefore carried out in the presence of this activator. The procedure was identical with that outlined above except that 2 cc. each of 2 % sodium oleate and calcium chloride solutions were added to the reaction mixture after the addition of the enzyme but before making up to volume. In view of the activation caused by this addition, only 5 cc. of the diluted glycerol extract of lipase were used. Nevertheless the same amount of miotine was employed. The following figures are typical of the results obtained: control, 2·25, 4·1, 5·05; in the presence of miotine, 1·7, 3·15, 4·25. Owing to the fact that the hydrolysis of methyl butyrate by pancreatic lipase does not take a linear course, possibly because of the changes which occur in the p_H of the solution, it is not possible to calculate in a simple manner the percentage inhibition produced by the miotine. Nevertheless it is clear from the figures that the inhibition caused by the miotine in this experiment is of the same order of magnitude as that produced by the same quantity in the absence of calcium oleate, although only one fifth of the amount of enzyme was required in the latter experiment.

An experiment similar to that last described was also carried out with the hydrochloride of the methylurethane of *m*-dimethylaminophenol, this particular urethane being chosen because it had proved to be the most active of the urethanes examined in inhibiting the hydrolysis of methyl butyrate by liver esterase. About 10 mg. of the urethane were employed, the remaining details being identical with those described above. The results are shown by the following figures: control, 2·05, 3·9, 4·75; in the presence of the urethane, 1·45, 2·9, 3·85. A small inhibitory effect is again apparent.

In view of the possibility that the glycerol, necessarily present in the above solutions, might diminish the inhibitory action of the urethanes, the influence of glycerol on the inhibitory action of miotine towards liver esterase was examined. An acidified and dialysed extract of liver powder, similar to those

described in Part III, was employed, the hydrolysis of methyl butyrate being followed under the conditions described in that paper with the modification that 50 cc. of water were replaced by 50 cc. of 50 % glycerol. As inhibitor, 0·01 mg. of miotine hydrochloride was employed. The following titration figures were obtained: control (without glycerol), 1·85, 3·8, 5·5; control (with glycerol), 1·85, 3·65, 5·45; in the presence of miotine (without glycerol), 0·55, 1·5, 2·2; in the presence of miotine and glycerol, 0·55, 1·4, 2·0. It is evident that the glycerol was without influence either on the activity of the esterase or on the inhibitory activity of miotine. The experiment further serves to illustrate the much greater sensitivity of liver esterase to miotine. With only a thousandth part of the amount used with pancreatic lipase a greater inhibition was produced.

Kidney phosphatase.

The preparation of kidney phosphatase employed was obtained by Erdtman's method [1927]. 600 g. of pig's kidneys were minced, suspended in 500 cc. of water to which much toluene had been added, and incubated for 2 days at 37°. The mixture was then filtered through a fine metal strainer, and the turbid filtrate treated with 1½ litres of rectified spirit. The precipitate so produced was filtered, stirred with 500 cc. of alcohol and again filtered, this process being repeated twice. It was then similarly treated with 500 cc. of ether, dried in the air and ground in a mortar. Extracts of the phosphatase were prepared by shaking 4 g. of this dry powder with 80 cc. of $N/40$ ammonia for 1½ hours and removing the solid material in the centrifuge.

The substrate employed was sodium glycerophosphate. To a mixture of 10 cc. of 5 % sodium glycerophosphate, 10 cc. of buffer (ammonia-ammonium chloride, p_H 8·9) and 60 cc. of water, previously warmed to 30° in a thermostat, were added 10 cc. of the above solution of phosphatase. The total volume was then made up to 100 cc. with water and the solution replaced in the thermostat. 20 cc. of the mixture were immediately withdrawn and run into 10 cc. of 10 % trichloroacetic acid. The free phosphate was then estimated in 25 cc. of the filtrate by precipitating it as phosphomolybdic acid, dissolving the latter after filtration in 10 cc. of 0·21N NaOH and titrating the excess alkali with 0·0995N nitric acid. Similar withdrawals and estimations were made at fixed intervals. A typical experiment will suffice to illustrate that miotine is without action on kidney phosphatase. 12 mg. of miotine hydrochloride were dissolved in 12 cc. of the enzyme solution and, after an interval of one hour, the phosphatase activity of 10 cc. of the solution was determined, a control being carried out simultaneously. The following figures represent the cc. of nitric acid required to neutralise the excess alkali in estimations on samples withdrawn immediately and after 80 minutes respectively: control, 18·2, 8·9; with miotine, 18·6, 8·9.

Serum esterase.

In order to examine the inhibitory action of the various urethanes towards serum esterase, the serum of the guinea-pig was chosen, since the blood of this

animal is known to contain a relatively high amount of the enzyme. 2–3 cc. of the serum, the exact volume depending upon the esterase activity of the sample employed, were treated with 2 cc. of phosphate buffer of p_H 7·9 and the mixture was diluted to 10 cc. with water. One cc. of this solution was mixed with 1 cc. of a solution of the urethane under examination and allowed to stand for about an hour, when the esterase activity of 1 cc. of the mixture was determined by the stalagmometric method described in Part III. In the control experiment, the solution of the urethane was replaced by an equal

Table I. *Inhibition of serum esterase by various urethanes.*

Substrate: tributyrin. T = 20°. p_H = 7·9.

Inhibitor*	Final conc. of inhibitor ($M \times 10^{-5}$)	Decrease in drop number in 20 mins.	Decrease in drop number in 40 mins.	Percentage inhibition
Phenyl series:				
Control	—	11	21	—
m-HCl	4	5	11	48
p-HCl	4	8	16	24
o-HCl	4	8	15	29
Control	—	13	25	—
m-MeI	400	5	9	64
p-MeI	400	1	4	84
o-MeI	400	1	3	88
Mixline series:				
Control	—	11	23	—
m-HCl	400	1	2	91
,,	40	5	10	57
p-HCl	400	2	4	83
,,	40	4	10	57
o-HCl	400	1	3	87
,,	40	9	19	17
Control	—	14	27	—
m-MeI	4000	2	3	89
,,	400	11	22	19
p-MeI	4000	1	3	89
,,	400	13	23	15
o-MeI	4000	1	2	93
,,	400	9	17	37
Benzyl series:				
Control	—	12	23	—
m-HCl	40	2	5	78
,,	4	8	16	30
p-HCl	40	3	7	70
,,	4	9	18	22
o-HCl	40	6	12	48
Control	—	14	26	—
m-MeI	400	8	17	35
p-MeI	400	6	14	46
o-MeI	400	7	13	50

* *Phenyl series*: methylurethanes of the isomeric dimethylaminophenols.
Mixline series: methylurethanes of the isomeric α-hydroxyphenylethyldimethylamines.
Benzyl series: methylurethanes of the isomeric hydroxybenzyldimethylamines.

volume of water. The results obtained are given in Table I. The urethanes employed were identical with those used in Part III and are indicated by the same abbreviated names.

Liver esterase.

In view of the fact that serum from the guinea-pig was used in the above experiments it was thought that it would be of interest to examine the inhibitory actions of the various urethanes on the liver esterase from the same species. A preparation of this esterase was therefore made from a number of guinea-pig's livers using the same procedure as was employed in Part III in connection with liver esterase from the pig. The activity of the preparation towards methyl butyrate, however, proved to be much smaller than that from the pig and it was not, therefore, possible to follow the hydrolysis of simple esters by the technique employed in Part III. Despite its smaller activity towards methyl butyrate, this preparation nevertheless proved to be at least as active towards tributyrin as was the esterase from pig's liver. The experiments recorded in Table II were therefore carried out, using this substance as

Table II. *Inhibition of guinea-pig's liver esterase by various urethanes.*

Substrate: tributyrin. T = 20°. p_H = 8·0.

Inhibitor	Final conc. of inhibitor ($M \times 10^{-4}$)	Decrease in drop number in 20 mins.	Decrease in drop number in 40 mins.	Percentage inhibition
Phenyl series:				
Control	—	14	26	—
m-HCl	40	0	0	100
p-HCl	40	9	18	31
o-HCl	40	7	16	38
Control	—	14	27	—
m-MeI	400	0	13	52
p-MeI	400	3	8	70
o-MeI	400	7	16	41
Miotine series:				
Control	—	11	22	—
m-HCl	40	4	8	64
p-HCl	40	6	14	36
o-HCl	40	4	8	64
Control	—	13	24·5	—
m-MeI	400	5	10·5	57
p-MeI	400	5	11	55
o-MeI	400	5	10·5	57
Benzyl series:				
Control	—	13	24	—
m-HCl	40	3	8	67
p-HCl	40	7	16	33
o-HCl	40	4	9	63
Control	—	14	27	—
m-MeI	400	4	9	67
p-MeI	400	5	12	56
o-MeI	400	6	13	52

substrate. The preparations of the esterase employed were obtained by extracting the desiccated liver powder with dilute ammonia, acidifying this extract with acid and, after removing the precipitate thus produced, dialysing for about 3 days in collodion membranes. In the various experiments recorded,

1·5–3 cc. of such an extract were mixed with 2 cc. of phosphate buffer of p_H 8·0 and the mixture was diluted to 10 cc. with water. One cc. of this solution was mixed with 1 cc. of water or of a solution of the urethane under examination, allowed to stand for about an hour, and 1 cc. of the mixture used in a hydrolysis experiment. The results obtained with the various urethanes are recorded in Table II.

Summary and discussion.

In so far as they show that the hydrolysis of tributyrin by the esterase from the liver of the guinea-pig is inhibited by small concentrations of the urethanes which have been shown to behave as parasympathetic stimulants, the above experiments constitute an extension to the liver esterase from a second species of the results obtained with the pig and reported in Part III of this series. While we have not carried out any extensive experiments with liver esterases from other species, we have nevertheless submitted those from a few to a preliminary examination and have found in each case that their activity is inhibited by miotine when present in concentrations of the same order of magnitude as employed in the above experiments. It would therefore appear legitimate to conclude that the urethanes of the type under consideration inhibit the activity of liver esterases in general and irrespective of the species from which they are derived.

Our experiments further demonstrate that the inhibitory action of the urethanes is not restricted to the liver enzyme but extends to the esterase which is present in the blood-serum of the guinea-pig and of certain other species. In view of the fact that it has been shown by the authors mentioned in the introduction that both whole blood and serum are capable of destroying small amounts of acetylcholine, a process which is inhibited by physostigmine, this result was, perhaps, to be expected. Nevertheless, it must be emphasised that the experiments here recorded have been carried out using tributyrin as substrate. While it would seem probable that the same enzyme is concerned in both processes, this cannot at present be regarded as definitely established. There exists, indeed, some evidence to the contrary. Thus, Takahashi [1930], has recently compared the lipolytic activities of the sera from a number of species, using tributyrin as substrate, and has found that they fall in the following order: rabbit > cat > horse > man > dog > cattle. This order is in general agreement with the results of earlier workers and also with some unpublished experiments which we have carried out. On the other hand, the order in which the defibrinated blood from a number of species destroys acetylcholine is, according to Galehr and Plattner [1927], man > pig ≧ cattle > dog > horse > rabbit > cat. The latter series is practically the reverse of that which holds for the hydrolysis of tributyrin and hence suggests either that different enzymes are concerned in the two processes or that some other factor, hitherto unrecognised, is involved. It is unlikely that a solution of this aspect of the problem will be possible until the blood-enzymes have been purified and

concentrated. Unfortunately such purification is attended with considerable difficulty on account of the quantity and ready solubility of the serum proteins. We are nevertheless at present engaged in an attempt to effect this purification.

Although the possibility thus exists that the hydrolysis of tributyrin and the destruction of acetylcholine are brought about by different serum enzymes, our results appear quite definitely to indicate that the enzymes, if specifically different, are of the same general nature. We have now established that the activities of both liver and serum esterases are inhibited by small concentrations of urethanes of the miotine type. Nevertheless, the above results show that the activity of pancreatic lipase, an enzyme which, although it resembles liver esterase in hydrolysing simple esters, differs from the latter enzyme in attacking fats relatively more rapidly, is not inhibited by miotine when its substrate is either a fat, in the form of olive oil, or tributyrin. When, however, its substrate is methyl butyrate, a simple ester, its action is inhibited by miotine, although a concentration of the drug which is high compared with that required with serum or liver esterase is necessary. Similarly, the hydrolysis of glycerophosphoric acid by kidney phosphatase, a process involving an ester of a different type from that attacked by liver esterase, is unaffected by miotine. It thus appears that the inhibitory action of the urethanes with which we are concerned is directed mainly towards true esterases, and it is therefore probable that the destruction of acetylcholine is brought about by an enzyme of the same nature.

Regarding the nature of the inhibitory action of the drugs in question, there appears little to be added to our comments in Part III. It was pointed out in that communication that the urethanes are esters and that combination between them and the enzyme probably involves the same mechanism as with the normal substrate, in which case the resulting inhibition would be due to the inability of the enzyme to hydrolyse the urethane. Some chemical evidence which supports this view may be mentioned. In Part I [1926] of this series it was mentioned that when aqueous solutions of certain of the urethanes are boiled, decomposition occurs with the production of methyl *iso*cyanate. This decomposition was erroneously referred to as hydrolysis, but it is clear, as pointed out by Aeschlimann and Reinert [1931] in a paper concerned with the pharmacology of urethanes of the same type, that no hydrolytic process is involved. This can be seen from the following equation:

$$N(CH_3)_2.CH(CH_3).C_6H_4.O.CO.NHCH_3 = N(CH_3)_2.CH(CH_3).C_6H_4.OH + CH_3CNO.$$

If, then, despite their structures as esters, these urethanes are not normally hydrolysed by ordinary chemical reagents but undergo a decomposition of a different nature, it is not difficult to understand the inability of esterases to bring about their destruction. The inhibitory action would be a direct consequence of this, provided the urethanes were endowed with high affinities for the enzyme.

The general relations between the nature of the urethane and its inhibitory activity towards liver esterase observed in Part III and discussed in that paper have again been encountered in the experiments described above and require no further comment. The order of activity of isomeric urethanes appears, however, to differ according to the source of the enzyme. While it would be in accordance with other observations that the enzymes from different species should exhibit differences in this respect, it is more difficult to understand why the esterases from the liver and serum of the same species should show similar differences. The obvious explanation, that the two latter enzymes are different substances, may be correct. We feel, however, that it would be wiser at the present time not to lay too much stress upon these small differences, which might conceivably be caused by impurities which are present in the enzyme solutions.

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CXXIX. STUDIES ON THE RELATIONSHIP BETWEEN CHEMICAL CONSTITUTION AND PHYSIOLOGICAL ACTION.

III. THE INHIBITORY ACTION OF CERTAIN SYNTHETIC URETHANES ON THE ACTIVITY OF LIVER ESTERASE.

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Parts I and II [Stedman, 1926; 1929, 1] of this series of communications have dealt with a new relationship between chemical constitution and physiological action. It has been shown that certain synthetic urethanes possess in common with the alkaloid physostigmine, which is also a urethane, the power of producing a constriction of the pupil when instilled into the eye. As a result of this work, as well as of extensions thereof published elsewhere [Stedman and Stedman, 1929; 1931], it has been concluded that substances which can be classed as phenyl esters of carbamic acids and which possess a basic group will, in general, exhibit similar miotic properties. Constitutional factors which repress, or tend to repress, such properties have already been discussed. Until recently the pharmacological examination of the synthetic urethanes had not extended beyond their action on the eye and it was not therefore certain that they would resemble physostigmine in other physiological properties. One of the urethanes, namely, the methylurethane of *α-m*-hydroxyphenylethyldimethylamine, which had already been shown to possess marked miotic properties and had been named miotine [Stedman, 1929, 2] on that account, has, however, now been submitted to a complete, and three others to an extensive although less complete, pharmacological examination [White and Stedman, 1931]. Miotine has thus been shown to possess physiological properties which are qualitatively identical with those of physostigmine, and similar results have been obtained with the other urethanes as far as they have been examined. The above relationship between chemical constitution and physiological action is therefore not limited to miotic activity but embraces all the known physiological actions of physostigmine. Now, Loewi and Navratil [1926] have attributed the action of physostigmine on the heart to its power of inhibiting the destruction of acetylcholine by an agent which they have shown to be present in the heart and in aqueous extracts of this organ. A similar agent is present in the blood of certain species [Galehr and

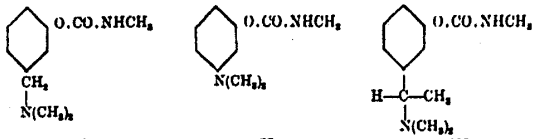
Plattner, 1927]. Loewi and Navratil consider this agent to be an esterase, a view which has been disputed by Plattner and Galehr [1928]. The arguments of Plattner have, however, been disposed of in a recent paper by Engelhart and Loewi [1930], who have brought forward convincing evidence of the enzymic nature of the substance causing the destruction of acetylcholine. Evidence leading to the same conclusion has also been published by Matthes [1930]. In view of the similarity between the actions of miotine and physostigmine on the heart, and in particular of the fact that miotine, like physostigmine, sensitises the vagus and potentiates the action of acetylcholine, it might be expected that miotine would also inhibit the destruction of acetylcholine by the agent in question. That this is actually the case has been shown by Dr Matthes, who kindly compared the action of miotine with that of physostigmine during his investigation of the inhibition by the latter of the destruction of acetylcholine by serum. The problem thus arises as to whether urethanes of the type discussed above, which have been shown to possess similar physiological properties, also share the property of inhibiting the action of the serum-enzyme. Another problem is, however, involved. In the investigations both of Loewi and his co-workers and of Matthes, the destruction of acetylcholine by the solutions under examination has been measured by biological methods, and no experiments appear to be recorded which indicate whether the enzyme which is responsible for this destruction will hydrolyse simple esters, or whether its action is specific towards acetylcholine. If the enzyme is a true esterase it should be capable of hydrolysing simple esters, in which case its activity, as well as the inhibition thereof by drugs, could be followed by titration methods. It appeared to us that these problems could be most conveniently, although indirectly, solved by examining the influence of the urethanes on the activity of the enzyme present in the liver and which is known to hydrolyse simple esters more readily than glycerides. The results of such an investigation are recorded in the present communication.

Materials.

Esterase preparations. All the esterase solutions employed in these experiments have been prepared from pigs' livers by the method of Willstätter and Memmen [1924, 2]. The liver, obtained direct from the slaughter house, was finely minced, desiccated by successive treatment with acetone and ether, and the dry preparation powdered and sieved. Solutions of the enzyme were prepared, as required, by extracting 2 g. of this fine powder with 100 cc. of 0·025 *N* ammonia for 1 hour and removing the insoluble material in the centrifuge. In the earlier experiments no further purification of the esterase was attempted. In the later work, however, it was found to be advantageous carefully to acidify the ammoniacal extract with acetic acid, and, after removing the precipitate so produced, to dialyse the clear solution in collodion membranes for 3–4 days, a procedure similar to that first described by

Willstätter, Bamann and Waldschmidt-Graser [1928] and subsequently employed by other workers.

Substances examined for inhibitory action. The urethanes with a pharmacological action similar to that of physostigmine which have been examined for inhibitory action comprise three series of isomeric compounds, namely, the methylurethanes of *o*-, *m*- and *p*-hydroxybenzyldimethylamine, the *o*-, *m*- and *p*-dimethylaminophenyl esters of methylcarbamic acid, and the methylurethanes of the isomeric *α*-hydroxyphenylethyldimethylamines. The structures of the *m*-isomerides in these three series are represented in formulae I, II and III respectively. These compounds have been examined both in the



I II III

form of their hydrochlorides and methiodides, the preparation of which has been effected by the methods of Stedman [1926; 1929, 1] and Stedman and Stedman [1929]. In the case of the *o*-isomerides, the hydrochlorides of which are hygroscopic, the base was dissolved in the calculated quantity of hydrochloric acid immediately before use. It should be noted that the urethane represented by formula III is the substance which has been named miotine. As will be evident from the formula, miotine and its position isomerides contain an asymmetric carbon atom. Unfortunately attempts to resolve these compounds have not so far met with success; the examination of their inhibitory actions towards liver esterase has therefore necessarily been carried out with the racemic substances. It will be convenient in the following pages to refer to the isomeric compounds corresponding with formula III as *o*-, *m*- and *p*-miotine, and to those corresponding with formulae I and II as the methylurethanes of the benzyl and phenyl series respectively.

In addition to urethanes with a physostigmine-like action, the methylurethane of choline iodide, $CH_3NH.CO.OC_2H_4N(CH_3)_3I$ [Stedman, 1929, 1], has been examined as an example of an aliphatic urethane. Some tests have also been made with the following alkaloids: pilocarpine, arecoline, ricinine. The two former were employed as nitrate and hydrobromide respectively, these salts being obtained from commercial sources. The ricinine was prepared from castor-oil seeds; since this alkaloid is soluble in water it was employed in the form of the free base.

Pilocarpine and arecoline were examined because the pharmacological actions of these alkaloids are similar to that of physostigmine. Ricinine does not exhibit such similarities. It occurs, however, in the castor-oil seed associated with a lipase, and since physostigmine also occurs in a seed, the Calabar

bean, it was thought that if the two alkaloids were found to resemble one another with respect to their inhibition of the activities of enzymes, this might form the basis of an explanation of the functions of alkaloids in general. As will be shown below, ricinine does not inhibit the activity of liver esterase; its possible influence on the activity of ricinus lipase, the enzyme with which it is associated naturally, has not yet been investigated.

Experiments with methyl butyrate as substrate.

Technique. The activity of the esterase preparations has been determined by a method essentially similar to that developed by Willstätter and Memmen [1924, 1] for pancreatic lipase and subsequently adapted by the same authors [1924, 2] for use with liver esterase. For convenience of measurement, 1 cc. of substrate (methyl butyrate) was used for each 100 cc. of reaction mixture, as recommended by Bamann [1929]. The general procedure was as follows: 20 cc. of ammonia-ammonium chloride buffer (1 part 2·5 *N* NH_3 : 2 parts 2·5 *N* NH_4Cl; p_H 8·9) contained in a 100 cc. measuring flask were diluted with 65–70 cc. of water, the exact volume depending on the volume of enzyme solution subsequently added, and the mixture brought to 30° in a thermostat. 1 cc. of methyl butyrate was then added and the flask shaken vigorously until the ester had dissolved completely. To the clear solution a measured volume (5 or 10 cc.) of the enzyme preparation was added, the total volume of the mixture brought to 100 cc. by the addition of water, and the whole thoroughly mixed. 20 cc. of the mixture were immediately withdrawn, run into a mixture of 25 cc. of 0·2 *N* hydrochloric acid with 20 cc. of water, and titrated with approximately 0·2 *N* sodium hydroxide, using bromocresol purple as indicator. Similar titrations were made at intervals of 20 minutes. With this procedure and using a suitable quantity of enzyme the amount of hydrolysis was approximately proportional to the time.

The enzyme solution consisted, in the earlier experiments, when ammoniacal extracts of liver powder were employed, of 5 cc. of a mixture of 5 cc. of the extract with 1 cc. of water, the latter being replaced by 1 cc. of a solution of the substance under examination in the experiments designed to test the inhibitory action of this substance. In the later experiments, in which the acidified and dialysed esterase preparation was employed, 5 cc. of enzyme solution were mixed with 5 cc. of ammonia-ammonium chloride buffer (of above composition but diluted 1 in 10), 2 cc. of water or solution added, and 10 cc. of this mixture used for the hydrolysis.

Activity of esterase preparations. The activity of the esterase preparations made from different livers was fairly constant. The following figures, which represent the number of cc. of 0·194 *N* alkali required to titrate the acid liberated in 20 cc. of the reaction mixture in 20, 40 and 60 minutes respectively, using 4·17 cc. of an ammoniacal extract of liver powder in 100 cc. of reaction mixture, may be taken as typical: 2·3, 4·75, 7·25. This result was

obtained on May 19th, 1930, with a fresh extract of a liver powder (L.P. 2) prepared on April 23rd, 1930, and almost identical values were obtained on June 10th, 1930, with a similar extract from the same powder. These facts are recorded in detail because of the following remarkable phenomenon which was observed with an extract of this powder but which has not so far been encountered with other preparations. On June 23rd, 1930, a fresh ammoniacal extract of L.P. 2 was rendered just acid to litmus by the addition of 0·5 *N* acetic acid. The precipitate, which formed slowly, was centrifuged off and the clear solution tested for activity under precisely the same conditions as those employed for the above ammoniacal extract, when the following figures were obtained: 4·45, 8·25, 8·95. Owing to the unexpectedly high activity of this extract the hydrolysis of the substrate was virtually complete in the experimental period of 1 hour, with the result that the reaction did not take the usual linear course. It is therefore not possible accurately to compare the activities of the different extracts of L.P. 2, but it is clear from the figures quoted that the activity of the acidified extract was at least twice as great as that of the ammoniacal extracts previously prepared from the same weight of the same powder. Dialysis of the acidified extract for 4 days was accompanied by an apparent slight diminution in activity, as is shown by the following titration figures: 3·4, 6·7, 8·35; this, however, was mainly due to the dilution which occurred during dialysis. The increase in activity observed on acidification can doubtless be explained by the removal of an inhibitory substance; from the difficulty in reproducing this experiment it appears that a very critical adjustment of conditions, probably of p_H, is necessary for the precipitation of this inhibitor.

Influence of time of contact between inhibitor and enzyme on inhibitory action. Rona and Bach [1920], in their studies on the inhibitory action of atoxyl on various lipases, have shown that the maximum inhibitory effect of this substance is not developed unless it is left for a certain period of time in contact with the enzyme before the addition of the substrate. A number of preliminary experiments having demonstrated that urethanes of the miotine type exert a pronounced inhibitory action on the hydrolysis of methyl butyrate by liver esterase, the influence of this time of contact of the inhibitor with the enzyme was investigated. For this purpose, 1 cc. of a solution of miotine hydrochloride, containing 1·2 mg./cc., was added to each of 7 test-tubes containing 5 cc. of an ammoniacal extract of liver powder. After varying intervals of time 5 cc. of the mixture were employed for a hydrolysis experiment. The results are given in Table I. The figures indicate that, under the conditions employed, the maximum inhibitory action of miotine hydrochloride is not exerted unless it has been in contact with the esterase for 2 hours prior to the addition of the substrate. The difference between the maximum inhibitory action and that produced after contact for 1 hour is, however, very small and almost falls within the limits of experimental error. It has therefore been considered sufficient, when comparing the inhibitory activities of the various

Table I. *Influence on inhibitory action of time of contact of inhibitor with enzyme.*

Substrate: methyl butyrate. Inhibitor: miotine hydrochloride. T = 30°.

Time of contact (mins.)	Titration figures (cc. of 0·104 *N* NaOH required to titrate acid liberated in 20, 40 and 60 mins.)			Percentage inhibition
Control (no inhibitor)	2·5	5·15	7·75	—
0·1	1·8	3·7	5·6	28
15	0·9	1·95	3·0	61
30	0·75	1·5	2·25	71
45	0·6	1·25	1·9	75
60	0·5	1·1	1·7	78
120	0·4	0·9	1·5	81
330	0·4	0·9	—	81

urethanes, to allow the inhibitor to stand in contact with the enzyme for 1 hour before use.

The figures in Table I give some further important information. Miotine is an ester of methylcarbamic acid and might therefore be expected to suffer, like other esters, a more or less rapid hydrolysis when in contact with liver esterase. In the above experiments, however, it is clear that no appreciable hydrolysis has occurred in a period of 5½ hours. In this connection it should be mentioned that, although the hydrolysis experiments were carried out at 30°, the mixtures of miotine and esterase were allowed to stand at room temperature before addition to the reaction flask.

Some experiments on the influence of the time of contact of enzyme and inhibitor on the inhibitory action of the latter were also made under somewhat different conditions. Although the procedure employed in these experiments was not adopted in much of the subsequent work, the results are recorded here since they are not entirely without interest. In the preceding experiments the enzyme and inhibitor were in contact under conditions of concentration widely different from those obtaining in the final reaction mixture. In order to render these conditions more nearly comparable the following modified series of experiments was carried out. A solution of 1·2 mg. of miotine hydrochloride in 1 cc. of water was added to each of a series of flasks containing the diluted buffer mixture and enzyme (ammoniacal extract) previously warmed to 30°. After varying intervals of time, 1 cc. of methyl butyrate was added and rapidly dissolved by shaking, the volume adjusted to 100 cc., and the titrations were performed as before. The results are expressed in Table II. Unfortunately a quantitative comparison cannot be made between the results of Tables I and II since not only were different enzyme preparations employed but the amounts of inhibitor used were slightly different. Nevertheless it is evident that the inhibitory action of miotine hydrochloride is of the same order of magnitude in the two sets of experiments. The most striking point of difference is the definite indication of a destruction of the miotine in the second set after 4 hours' contact with the enzyme. We are inclined, however, to attribute this destruction to the alkalinity of the

Table II. *Influence on inhibitory action of time of contact of inhibitor with enzyme.*

Substrate: methyl butyrate. Inhibitor: miotine hydrochloride. T = 30°.

Time of contact (mins.)	Titration figures (cc. of 0·194 *N* NaOH required to titrate acid liberated in 20, 40 and 60 mins.)			Percentage inhibition
Control (no inhibitor)	1·95	4·00	6·1	—
15	0·7	1·5	2·35	61
30	0·6	1·25	1·7	72
45	0·4	1·0	1·4	77
60	0·4	0·85	1·3	79
120	0·45	1·0	1·6	74
240	0·8	1·55	2·45	60

solution, combined with the fact that the enzyme and inhibitor were maintained at 30° during the whole period of contact, rather than to the action of the esterase.

Inhibitory actions of various urethanes. A number of the urethanes exhibiting miotic activity were examined for inhibitory action under fairly wide ranges of concentration of inhibitor. The results are collected in Table III.

Table III. *Inhibitory action of various urethanes with miotic activity.*

Substrate: methyl butyrate. T = 30°. Initial p_H = 8·9.

In the three instances marked (a), 10 cc. of the extract were employed owing to the diminution in the activity of the liver powder.

The solution used in the experiment marked (b) was prepared by extracting only 1·5 g. of liver powder with 100 cc. of *N*/40 ammonia.

Inhibitor	Liver preparation	Final conc. of inhibitor (mg. per 100 cc.)	Titration figures			Percentage inhibition
m-Miotine HCl	L.P. 2	Control	2·55	5·15	7·8	—
		10	0·2	0·5	0·9	89
		1	0·45	1·0	1·45	81
		0·1	1·25	2·6	4·05	48
		0·01	2·1	4·3	6·55	16
		0·001	2·4	4·8	7·3	6
o-Miotine HCl	L.P. 5 (a)	Control	2·15	4·35	6·5	—
		10	0·55	1·0	1·45	78
		1	1·6	3·3	4·9	25
		0·1	1·8	3·75	5·9	11
p-Miotine HCl	L.P. 2	Control	2·35	4·75	7·1	—
		10	0·0	0·3	0·45	94
		1	0·25	0·55	0·85	88
		0·1	0·9	1·5	2·55	64
		0·01	1·65	3·3	5·0	30
		0·001	2·35	4·5	6·85	4
m-Miotine MeI	L.P. 2	Control	2·45	5·0	7·5	—
		10	0·25	0·5	0·85	90
		1	1·25	2·4	3·75	51
		0·1	2·15	4·4	6·75	11
		0·01	2·15	4·8	7·2	4
		0·001	2·3	4·8	7·3	—
o-Miotine MeI	L.P. 5 (a)	Control	2·0	4·2	6·4	—
		10	0·25	0·7	1·1	83
		1	1·3	2·5	3·6	44
		0·1	2·05	4·1	6·2	3

Table III (*contd.*).

Inhibitor	Liver preparation	Final conc. of inhibitor (mg. per 100 cc.)	Titration figures			Percentage inhibition
p-Miotine MeI	L.P. 5 (b)	Control	1·75	3·4	5·2	—
		10	0·15	0·35	0·65	86
		1	0·85	1·55	2·35	55
		0·1	1·4	3·0	4·5	14
		0·01	1·6	3·25	5·0	—
		0·001	1·45	3·3	5·0	—
HCl of *o*-urethane (phenyl series)	L.P. 6	Control	2·5	5·1	7·7	—
		10	0·2	0·6	1·0	87
		1	0·6	1·2	1·85	77
		0·1	1·5	3·05	4·75	38
		0·01	2·05	4·15	6·4	17
		0·001	2·2	4·55	7·05	8
HCl of *m*-urethane (phenyl series)	L.P. 2	Control	2·45	4·85	7·5	—
		10	0·0	0·1	0·3	96
		1	0·35	0·5	0·75	90
		0·1	1·15	2·1	3·25	57
		0·01	1·8	3·55	5·45	27
		0·001	2·1	4·6	6·8	9
HCl of *p*-urethane (phenyl series)	L.P. 2	Control	2·3	4·75	7·4	—
		10	0·25	0·5	0·85	89
		1	0·7	1·35	2·0	73
		0·1	1·7	2·25	3·95	33
		0·01	2·2	4·4	6·65	11
		0·001	2·2	4·55	7·0	6
MeI of *m*-urethane (phenyl series)	L.P. 2	Control	2·45	5·1	7·6	—
		10	0·75	1·5	2·4	69
		1	1·7	2·35	3·95	37
		0·1	2·15	4·55	6·9	11
		0·01	2·25	4·75	7·2	5
		0 001	2·35	4·8	7·3	—
MeI of *p*-urethane (phenyl series)	L.P. 5 (a)	Control	2·1	4·4	6·55	—
		10	0·6	1·15	1·7	74
		1	1·35	2·65	4·0	39
		0·1	2·2	4·3	6·45	—
MeI of *o*-urethane (benzyl series)	L.P. 2	Control	2·35	4·75	7·3	—
		10	0·2	0·45	0·75	90
		1	1·15	2·3	3·4	53
		0·1	2·3	4·6	7·0	4
		0·01	2·4	4·75	7·25	—

In all these experiments the general technique described earlier was utilised, ammoniacal extracts of desiccated liver powder being employed as esterase solution in each case. Since this work was carried out over an extended period of time, different liver preparations, as indicated in Table III, were necessarily employed owing to the moderately rapid deterioration which such preparations undergo. The results demonstrate clearly the marked inhibitory action which urethanes of the type examined exert upon the activity of liver esterase. A detailed comparison of the figures shows further that, in general, the inhibitory activity of the hydrochlorides of the tertiary bases is greater than that of the corresponding quaternary iodides. While this is not universally the case, particularly in the higher concentrations, the inhibitory activities of the methiodides consistently decrease, with increasing dilution of the inhibitor, at a much greater rate than do those of the hydrochlorides of the tertiary

bases, with the result that at low concentrations the latter are uniformly more active. This difference between the two types of salts is emphasised because it is probably related to the mechanism of the inhibitory action.

Examination of other types of compounds for inhibitory activity. The following compounds were tested for inhibitory activity under conditions similar to those used in the preceding experiments: methylurethane of choline iodide, hydrochloride of α-*m*-hydroxyphenylethyldimethylamine, pilocarpine nitrate, arecoline hydrobromide, atropine hydrochloride and ricinine. The titration figures need not be recorded in detail since in no case was any inhibitory action observed in the highest concentrations examined, namely, 10 mg. per 100 cc. of reaction mixture.

Influence of p_H *on inhibitory action.* The technique adopted for the experiments in which methyl butyrate was employed as substrate is not very suitable for the investigation of p_H effects. Even when relatively large amounts of buffer are employed, the acid liberated during the hydrolysis is sufficient to cause some change in p_H; if the solution is thereby brought to the acid side of neutrality, some loss in the activity of the esterase may occur with the result that the hydrolysis of the substrate will no longer proceed at a constant rate. This effect was actually observed in a number of experiments. In the experiment quoted in Table IV, which was carried out at p_H 6·8, proportionality between the amount of hydrolysis and the time was maintained;

Table IV. *Influence of* p_H *on the inhibitory action of miotine hydrochloride.*

Substrate: methyl butyrate. T = 30°.

p_H	Esterase preparation	Conc. of inhibitor (mg. per 100 cc.)	Titration figures			Percentage inhibition
6·8	Acidified ammoniacal	Control	1·65	3·4	5·0	—
(phosphate buffer)	extract of L.P. 5	10	0·5	1·25	2·0	60
		1	1·45	2·0	4·35	13
		0·1	1·0	3·2	4·85	3
		0·01	1·75	3·4	4·85	—
		0·001	1·7	3·5	5·15	—
8·9	Ammoniacal extract	Control	1·85	3·8	5·75	—
(ammonia buffer)	of L.P. 5	12	0·05	0·25	0·45	92
		1·2	0·35	0·75	1·15	80
		0·12	1·0	2·25	3·5	39
		0·012	1·65	3·3	4·9	15
		0·0012	1·85	3·6	5·45	5

this experiment therefore serves to illustrate the general influence of p_H on the inhibitory action of miotine hydrochloride. For comparison, the results of an experiment at p_H 8·9 carried out under similar conditions are included in the same table. The technique described in connection with the experiments quoted in Table II was employed for both experiments. The phosphate buffer used consisted of a mixture of equal parts of *M*/3 Na_2HPO_4 and *M*/3 NaH_2PO_4, 70 cc. being employed in each 100 cc. of reaction mixture. When this buffer was employed, phenolphthalein was used as indicator for the titrations.

Unfortunately the enzyme solutions employed in these experiments, although prepared from the same liver powder, were of different degrees of

purity; the results are not therefore strictly comparable. Nevertheless, two important conclusions can be drawn from these experiments: firstly, the inhibitory action of miotine hydrochloride is smaller on the acid than on the alkaline side of neutrality; secondly, the inhibitory action falls off with decreasing concentration of inhibitor much more rapidly in acid than in alkaline solution.

Comparison of the inhibitory activities of isomeric urethanes. The urethanes which have been shown above to inhibit the hydrolysis of methyl butyrate by liver esterase are members of three series of isomeric compounds, with their corresponding methiodides. The results do not, however, necessarily indicate the relative inhibitory activities of the position isomerides within a given series or even of members of different series, for, as previously mentioned, the inhibitory activities of the various compounds were examined on different esterase preparations, and since the latter were made from different liver powders at varying times after their preparation they possessed different activities and probably contained varying amounts of natural inhibitors. On the assumption that any natural inhibitors which may be present in the enzyme preparation enter into competition with the urethanes, it is clear that in experiments designed to compare the activities of different compounds it is not sufficient to employ the same esterase activity; the comparison can only be regarded as valid in those cases where the same esterase extract has been employed. Some experiments, recorded in Table V, have consequently been carried out in which a comparison has been made of the inhibitory activities of the members of each series of urethanes towards the same enzyme preparation. Finally, the hydrochlorides of the *m*-isomerides of the three series have been compared under the same conditions as a measure of the relative activities of the three series of compounds. In all cases the ammoniacal extracts of the liver powders were purified by acidification with acetic acid followed by dialysis.

From the magnitude of the titration figures it is evident that the percentage inhibition shown in the last column of Table V must be subject to an error of a few per cent. Bearing this in mind, the more significant results revealed by a study of this Table may be summarised as follows.

Miotine series. The inhibitory activities of the hydrochlorides of the *m*- and *p*-isomerides are identical, that of the *o*-isomeride being considerably smaller; the inhibition produced by the latter is, in fact, slightly smaller than that caused by the two former when present in one-tenth of its concentration. No very great difference can be observed between the activities of the methiodides, the *m*- and *p*-isomerides showing about equal activity and the *o*-compound exhibiting a slightly greater effect. It is noteworthy and exceptional that the inhibitory activity of the hydrochloride of the *o*-isomeride is somewhat smaller than that of its methiodide in the same molar concentration.

Phenyl series. The activities of the hydrochlorides are definitely in the order $m > p > o$. The methiodides follow the same order; the *m*- and *p*-compounds show, however, practically identical activities.

Table V. *Comparison of inhibitory activities of various urethanes.*

Substrate: methyl butyrate. T = 30°. Initial p_H = 8·0.

Inhibitor (methylurethane)	Esterase preparation	Final conc. of inhibitor (molar × 10^{-6})	Titration figures			Percentage inhibition
Miotine series:	L.P. 6	Control	1·75	3·5	5·3	—
m-HCl		1/30	0·25	0·65	1·75	80
"		1/300	1·15	2·4	3·7	30
p-HCl		1/30	0·4	0·8	1·2	77
"		1/300	1·25	2·45	3·85	27
o-HCl		1/3	0·75	1·55	2·3	57
"		1/30	1·5	2·95	4·5	15
m-MeI		1/3	0·7	1·35	1·9	63
"		1/30	1·65	3·2	4·9	8
p-MeI		1/3	0·75	1·15	1·85	65
"		1/30	1·4	2·9	4·4	17
o-MeI		1/3	0·4	0·8	1·25	77
"		1/30	1·45	2·9	4·3	19
Phenyl series:	L.P. 6	Control	1·6	3·35	5·1	—
m-HCl		1/300	0·15	0·45	0·7	86
p-HCl		"	0·8	1·65	2·6	49
o-HCl		"	1·0	2·05	3·2	37
m-MeI		1/30	0·9	1·75	2·55	52
p-MeI		"	0·75	1·6	2·55	50
o-MeI		"	1·3	2·6	4·0	22
Benzyl series:	L.P. 7	Control	2·4	4·75	6·85	—
m-HCl		1/300	0·8	1·75	2·55	63
p-HCl		"	1·35	2·75	3·95	42
o-HCl		"	1·85	3·6	5·7	17
m-MeI		1/30	1·45	2·8	4·3	37
p-MeI		"	0·75	1·5	2·25	67
o-MeI		"	1·25	2·55	3·85	44
All series:	L.P. 7	Control	2·15	4·55	6·75	—
m-Benzyl-HCl		1/3000	1·95	3·8	5·85	13
m-Phenyl-HCl		"	1·15	2·45	3·6	47
m-Miotine-HCl		"	1·5	3·1	4·7	30
	L.P. 7	Control	2·4	4·75	6·85	—
m-Benzyl-HCl		1/300	0·8	1·75	2·55	63
m-Phenyl-HCl		"	0·1	0·35	0·45	93
m-Miotine-HCl		"	0·35	0·6	0·8	88

Benzyl series. In this series the order for the hydrochlorides is $m > p > o$, and for the methiodides $p > o > m$.

Comparison of the three series. The inhibitory activities of the hydrochlorides of the *m*-isomerides in equimolar concentrations are definitely different, the order being phenyl > miotine > benzyl.

The results in Table V may be further utilised in order to direct attention to the varying activities of the urethanes with different esterase preparations. Thus, to take the most marked case, the concentrations of miotine hydrochloride required to inhibit the acidified and dialysed extracts of two esterase preparations (L.P. 6 and L.P. 7) to exactly the same extent (30 %) are in the ratio 1:10, the extract from L.P. 6 requiring a final concentration of 1/300,000 *M* and that from L.P. 7 one of 1/3,000,000 *M*. The different responses of these enzyme preparations are doubtless related to their histories, which are as follows: the liver powder L.P. 6 was prepared on January 9th,

1931. An ammoniacal extract of this was made on January 14th and its activity tested by the standard procedure used in this investigation, when the following titration figures were obtained: 2·3, 4·6, 7·0. Practically identical values were obtained on the same day following the acetic acid treatment. The acid solution was then dialysed for 5 days, the dialysed solution giving, on January 19th, titration figures of 2·05, 4·05, 6·05. The diminution in activity shown by these figures is only apparent and can be accounted for by the slight dilution which the extract had undergone during dialysis. On the following day, however, the titration figures for the dialysed extract had fallen to 1·75, 3·5, 5·3. Thus, in 24 hours the activity had diminished about 12 %, and it was this extract with lowered activity which was employed for the comparison of the inhibitory activities of the urethanes of the miotine series. We are of opinion that this loss in activity can be attributed to the acidity caused by the relatively long period of dialysis, and we have found that, with the highly permeable collodion bags which we employ, such losses consistently occur when the process of dialysis is extended beyond 3 or, at most, 4 days.

The preparation L.P. 7 was made on February 18th, 1931. An ammoniacal extract was prepared on February 20th, and this was acidified and dialysed until February 23rd. In view of the fact that this extract, which was more sensitive to miotine hydrochloride than the one referred to above, proved to be stable, it appears probable that the presence of inactivated esterase in the enzyme solutions diminishes the inhibitory activity of urethanes of the type under discussion.

Experiments with tributyrin as substrate.

Technique. In order to follow the hydrolysis of tributyrin by liver esterase the stalagmometric method devised by Rona and Michaelis [1911] and subsequently employed by Willstätter and Memmen [1923] in connection with pancreatic lipase has been utilised. A straight stalagmometer with a water value of 81 at 20° was employed throughout the experiments.

In using this method it is frequently considered sufficient to work at room temperature without any special temperature control. At the time our experiments were carried out, however, the temperature of our laboratory fluctuated considerably and tended to be somewhat low, falling at times to below 10°. At such temperatures the activity of the enzyme is naturally considerably diminished, and it was therefore considered advisable to use a thermostat. The reaction mixture, contained in a small beaker, was placed in a bath at 20° and the stalagmometer supported directly over the beaker by means of a suitable stand. By raising the beaker and applying gentle suction at the top of the stalagmometer the mixture could be drawn into the latter. The beaker was then again lowered and the drops from the stalagmometer allowed to fall into it. By this arrangement fluctuations in the temperature of the liquid actually in the stalagmometer could not be prevented, but the

bulk of the reaction mixture was maintained constantly at 20°, thereby permitting a uniform action of the esterase.

The remaining procedure was as follows: 50 cc. of a saturated solution of tributyrin were mixed with 5 cc. of $M/3$ phosphate buffer (p_H 7·9 or 6·8) and the mixture brought to 20°. 1 cc. of a mixture of 1 cc. of esterase solution with 1 cc. of water in the control experiment, or of 1 cc. of esterase solution with 1 cc. of a solution of the urethane in the experiments designed to measure the inhibitory action of the latter, was then added and the drop number immediately measured. Two further counts were made at intervals of 20 minutes. As in the experiments with methyl butyrate, the enzyme was left in contact with the urethane for 1 hour before mixture with the substrate.

All the esterase solutions employed were prepared from ammoniacal extracts of liver powders acidified with acetic acid; in some cases the extracts were dialysed. In each case, 1 to 5 cc. of the enzyme preparation, the actual volume depending on its activity, were mixed with 2 cc. of $M/3$ phosphate buffer of the same p_H as that employed in the hydrolysis experiments, and diluted to 10 cc. with water.

Willstätter and Memmen [1923] have pointed out that commercial preparations of tributyrin frequently contain an impurity, with a saponification number corresponding with that of dibutyrin, which renders them unsuitable for use, without extensive purification, in the stalagmometric estimation of lipolytic activity. As a criterion of purity they recommend the preparation of two saturated aqueous solutions of the sample, one by shaking 5 to 10 g. of the tributyrin with 200 cc. of water, and the other by shaking 3 drops with a similar volume of solvent. If the tributyrin is pure, the two solutions should give the same drop number. The experiments recorded in this communication have been made with one delivery of B.D.H. tributyrin. When this was submitted to the test described above, the two saturated aqueous solutions gave identical drop numbers. According to the above criterion the sample was therefore quite pure and was employed in our experiments without further treatment.

Influence of p_H *on inhibitory action.* The study of the inhibition of the hydrolysis of tributyrin by liver esterase by urethanes of the type considered in this series of communications has been carried out partly with the object of ascertaining if the inhibitory action of such urethanes is independent of the type of substrate employed and partly because, owing to the sensitivity of the stalagmometric method, only relatively small amounts of tributyrin need be employed as substrate. This renders the method eminently suitable for a study of the influence of p_H on the inhibitory process, since no difficulty is experienced in buffering the small amounts of acid which are liberated by the action of the enzyme. The inhibitory activities of the hydrochlorides and methiodides of each member of the three series of urethanes mentioned above have therefore been examined, using a range of concentrations of inhibitor, at two different acidities, namely, p_H 7·9 and 6·8. Table VI gives some typical

Table VI. *Influence of* p_H *on the inhibitory action of various urethanes.*

Substrate: tributyrin. T = 20°.

Inhibitor	Final conc. of inhibitor (mg. per 56 cc.)	p_H 7·9 Decrease in drop number in 20 mins.	p_H 7·9 Decrease in drop number in 40 mins.	p_H 6·8 Decrease in drop number in 20 mins.	p_H 6·8 Decrease in drop number in 40 mins.
m-Miotine HCl	0	14	25	12	20
	10	0	1	1	1
	1	1	1	5	8
	0·1	0	1·5	9	16
	0·01	8	17	10	18
	0·001	13	24	—	—
m-Miotine MeI	0	0·5	10	9	18
	10	0	1	5·5	11
	1	2	4	8	16
	0·1	7·5	15·5	—	—
HCl of *p*-urethane (benzyl series)	0	10	20	11	20
	10	0	0	0	0
	1	0	0·5	0·5	1
	0·1	1·5	3	9	15
	0·01	8	15	9	17
MeI of *p*-urethane (benzyl series)	0	9·5	18·5	8·5	15·5
	10	0	0	0·5	1·5
	1	1	1·5	2	3·5
	0·1	5	10	8	16
HCl of *m*-urethane (phenyl series)	0	0·5	17·5	7	14·5
	1	1	1	1	1·5
	0·1	1	1·5	0	1
	0·01	0	1	1	2
	0·001	1	3	2	4
	0·0001	4	9	7	14
MeI of *m*-urethane (phenyl series)	0	9	19	8·5	15·5
	10	0	0	2	2
	1	0	0	5	9
	0·1	2	4	—	—
	0·01	7	14	—	—

results. Similar results were obtained with the isomerides of the urethanes mentioned in Table VI; since, however, the same enzyme preparation was not employed with all the compounds, these results do not necessarily indicate correctly the relative inhibitory activities of the various compounds and are not therefore quoted. It should be mentioned in connection with these results that the same enzyme preparation was always employed in the experiments with a given urethane, although, owing to the smaller activity of the esterase at p_H 6·8 a greater volume of the enzyme preparation was used in the more acid solution. Thus in the experiments with the hydrochloride of the methylurethane of *p*-hydroxybenzyldimethylamine in which the enzyme showed the same activity, corresponding with a decrease of 20 in the drop number in 40 minutes, at the two acidities, 33 % more enzyme was employed at p_H 6·8 than at 7·9. In every instance quoted in Table VI the inhibitory activity of a given urethane is smaller at p_H 6·8, *i.e.* on the acid side of neutrality, than at p_H 7·9, and the same effect was consistently observed with the remaining urethanes. As an example, the results with the hydrochloride of the methylurethane of *p*-hydroxybenzyldimethylamine, mentioned above, may be taken,

since identical esterase activities, as shown by the control experiments, were employed at the two acidities. In this case, the amounts of inhibitor required to diminish the activity of the enzyme by 25 %, *i.e.* to diminish the decrease in drop number from 20 to 15, were 0·01 and 0·1 mg. at p_H 7·9 and 6·8 respectively. The inhibitory activity of the urethane at p_H 6·8 was thus only one-tenth of that which it exhibited at p_H 7·9. Ratios of the same order of magnitude were obtained with the other urethanes. To what extent the partial inactivation of the enzyme which takes place in acid solution, and which necessitated the employment of larger volumes of the preparation at p_H 6·8, contributes to this result cannot at present be determined.

Attention should further be directed to the results obtained with the hydrochloride of the methylurethane of *m*-dimethylaminophenol. Reference to Table VI will show that 0·0001 mg. of this substance produced, at p_H 7·9, an inhibition of the activity of the liver esterase of approximately 50 %. Since, in the final reaction mixture, the inhibitor was contained in a volume of about 50 cc., the final concentration of this substance required to diminish the activity of the esterase by this amount was about 1:500,000,000. This is the greatest activity which has been observed with any of the urethanes so far examined. It agrees with other results obtained with the same urethane, which has, in fact, proved to be the most active inhibitor amongst the three series of compounds investigated.

Comparison of the inhibitory activities of isomeric urethanes. In order to obtain a direct comparison of the activities of the members of a given series of urethanes, it was necessary, for reasons stated in connection with the similar experiments carried out with methyl butyrate as substrate, to measure the inhibitory activities on the same enzyme preparation. The results are recorded in Table VII. Different esterase solutions were employed for the three series, so that the figures recorded do not necessarily give a comparison of the activities of members of different series. In the benzyl and phenyl series, an interval of some days elapsed between the experiments with the hydrochlorides and methiodides. The enzyme had meanwhile undergone slight deterioration, as shown by the figures for the second control.

The order of inhibitory activity of the various isomerides on liver esterase, using tributyrin as substrate, is, according to the results of Table VII as follows.

Miotine series. The hydrochlorides do not show large differences in activity. Nevertheless they appear to be definitely in the order $m > o > p$. With the methiodides, which are considerably less active, the *o*-compound exhibits the greatest activity; the *m*- and *p*-isomerides are, within the limits of experimental error, equally active.

Benzyl series. The hydrochlorides of this series show greater differences in activity, the order being quite definitely $o > m > p$. The methiodides again show little difference in activity; the order $p > o > m$ is indicated by the results.

Table VII. *Comparison of the inhibitory activities of isomeric urethanes.*

Substrate: tributyrin. T = 20°. p_H = 7·0.

Inhibitor	Final conc. of inhibitor (molar × 10^{-7})	Decrease in drop number in 20 mins.	Decrease in drop number in 40 mins.	Percentage inhibition
Miotine series:	Control	11	22	—
m-HCl	40	0	2	91
"	4	5	10	55
p-HCl	40	3·5	7·5	68
"	4	6	12	47
o-HCl	40	1	3·5	80
"	4	5	10·5	52
m-MeI	400	2	3	87
p-MeI	400	2	4	83
o-MeI	400	0	1·5	91
Benzyl series:	Control	12	23	—
m-HCl	400	1	2·5	87
"	40	8	16	30
p-HCl	400	4	8·5	61
"	40	10	20	13
o-HCl	400	0	1	96
"	40	3	6	84
	Control	11·5	21	—
m-MeI	400	7	15	29
p-MeI	400	6	12	43
o-MeI	400	6·5	13·5	38
Phenyl series:	Control	13	27	—
m-HCl	4	1	2	90
p-HCl	4	5	10	63
o-HCl	4	5	10·5	59
	Control	11·5	21	—
m-MeI	40	8	16	24
p-MeI	40	8	16	24
o-MeI	40	3	7	67

Phenyl series. In this series the *m*-hydrochloride is considerably more active than the *o*- and *p*-compounds, which can scarcely be distinguished from one another in activity. Of the methiodides, the *o*-compound is the most active, the *m*- and *p*-isomerides possessing the same activity. It should be noted that the order of inhibitory activity of both the hydrochlorides and methiodides of this series is identical with that of the miotine series.

Discussion.

The foregoing results have established definitely that urethanes of the type which have been shown in earlier papers to possess very specific physiological properties also possess the common property of inhibiting in low concentration the activity of liver esterase. Of the nine urethanes examined, all exhibit this property to a marked degree, and not only do they do so in the form of their hydrochlorides but their methiodides are similarly, although usually somewhat less, active. It appears legitimate to conclude that a relationship exists between chemical constitution and inhibitory activity towards liver esterase analogous to that between constitution and physiological action in the same group. In conformity with this conclusion, the methylurethane of choline

iodide, a substance containing many structural features in common with the active urethanes but lacking the essential phenyl group, is inactive. Similarly, the hydrochloride of *α*-*m*-hydroxyphenylethyldimethylamine, the phenol from which miotine is prepared and which contains the phenyl and basic but not the urethane group, produces no inhibition in relatively high concentration.

Whether these results hold for the enzyme, present in blood-sera from certain species and in tissue extracts, which is normally responsible for the destruction of acetylcholine, has not yet been determined. Nevertheless, the fact that urethanes of the type which possess physiological properties similar to those of physostigmine also inhibit, in minute concentration, the hydrolysis by liver esterase of a simple ester such as methyl butyrate, affords substantial support to the view that the above-mentioned serum-enzyme, the activity of which towards acetylcholine has been shown by Loewi and his co-workers and by Matthes to be inhibited by physostigmine, is a true esterase such as is present in the liver and is not one which acts specifically towards acetylcholine.

It is not proposed to discuss in detail the question, which now arises, as to whether the physiological activity of urethanes of the miotine type is an indirect result of the inhibitory action which they exert upon esterases, since this problem has already been considered from many aspects by White and Stedman [1931], who have pointed out that while much of the activity of miotine, in particular its toxicity, may probably be attributed to its inhibition of the destruction of acetylcholine, other mechanisms are possibly also involved. One fact, which supports the suggestion, originally made by Loewi and Navratil [1926], that the action of physostigmine on the heart is due to its inhibitory action on the acetylcholine-destroying enzyme, may, however, be mentioned. It has been shown above that arecoline, in relatively high concentration, does not inhibit the activity of liver esterase. Now this alkaloid belongs, pharmacologically, to the physostigmine group of drugs and might therefore be expected to resemble physostigmine in its behaviour towards esteruses. That it does not do so is in complete accord with some unpublished pharmacological experiments by Dr A. C. White, to whom we are indebted for information regarding his results, according to which arecoline, unlike physostigmine, does not potentiate the action of acetylcholine on the vagus.

It is not to be expected, even on the assumption that the whole of the physiological activity of urethanes of the miotine type is attributable to the resulting accumulation of acetylcholine in the organism, that an exact parallelism will exist between the magnitude of their physiological activities and that of their inhibitory activities towards esterases, for the drugs will be subject to many influences in the organism which are eliminated in *in vitro* experiments with enzymes. Thus, a property of a drug, such as adsorbability, which may render it particularly efficient in inhibiting the activity of an esterase, may actually prevent it from reaching the enzyme in the organism. Similarly, stability will be of greater influence in *in vivo* than in *in vitro* experiments, since in the former case the drug is subjected to the action of

more destructive agents. That no such parallelism does, in fact, exist follows from our experiments; the order of the inhibitory activity of various urethanes towards liver esterase in no way corresponds with the previously published provisional order for their miotic activities.

Perhaps the most interesting questions which call for consideration in connection with our experiments are those concerning the nature of the inhibitory action and its bearing on the mechanism of the normal action of esterases. Of a group of compounds possessing a common structural feature, every member which has been examined has been found to inhibit, in minute concentration, the activity of liver esterase. These inhibitory substances, like the normal substrate of the enzyme, are esters. Nevertheless they are either not attacked, or are only hydrolysed extremely slowly, by the esterase. The suggestion thus arises that the urethanes combine with the esterase in the same way and by means of the same mechanism as do simple esters, but that the affinity between urethane and enzyme is enormously greater than that between simple ester and enzyme. Hence the esterase-urethane compound is formed preferentially and, owing to the inability of the enzyme to decompose the urethane, the hydrolysis of the normal substrate is prevented. The latent period which occurs in the hydrolysis of ethyl mandelate by liver esterase when esters of certain keto-acids are present has been similarly explained by Willstätter, Kuhn, Lind and Memmen [1927], and further experiments in support of this explanation have been published by Bamann and Schmeller [1930]. On the above basis the relative inhibitory activities of the various urethanes will be a measure of their affinities for the esterase, and any regularity which is observed between inhibitory and other properties should give a clue as to the nature of the affinity between the urethanes and esterase, and hence of the nature of the forces normally responsible for the formation of enzyme-substrate compound. Now, the following regularities have, in fact, been noted in our experiments.

(1) In a given series of urethanes the isomerides show inhibitory activities which, although usually different, are of the same order of magnitude.

(2) Of the three series examined, the hydrochlorides of the phenyl series are outstanding with respect to their high inhibitory activities.

(3) The methiodides are, in general, considerably less active than the hydrochlorides of the corresponding tertiary bases.

(4) The inhibitory activity of a given urethane is considerably smaller at p_H 6·8 than in slightly alkaline medium.

We believe that these results can be best interpreted by assuming that the affinities in question are, in the first instance, of the nature of adsorptive forces. If we postulate (*a*) that the urethane group confers high adsorbabilities on the inhibitory substances, (*b*) that the adsorbability and therefore inhibitory activity is increased by any factor which tends to lower the solubility, (*c*) that the free bases and not the salts are the active inhibitors, our reasons for this belief will be at once clear. In accordance with postulates (*b*) and (*c*),

any factor which tends to convert the readily soluble salts into the sparingly soluble bases, thus diminishing the solubility and increasing the concentration of the active constituent, would be expected to assist adsorption and hence increase inhibitory activity. Increase in alkalinity is such a factor, and as shown above this increases the inhibitory activity. Similarly diminishing the basic strength of the compounds would constitute another such factor; corresponding with this the compounds which are the weakest bases, namely, the methylurethanes of the phenyl series, show the greatest inhibitory activity. Further, solubility is increased by converting the tertiary bases into their quaternary ammonium salts, the free bases of which are readily soluble in water. This should diminish adsorbability and hence inhibitory activity, as is actually the case.

Nevertheless, adsorption is clearly not the only process involved. The possession of surface activity of a high order will not necessarily confer upon a compound the marked inhibitory activity shown by the above urethanes. Moreover, it has been shown by Willstätter, Kuhn and Bamann [1928] that esters of *d*- and *l*-mandelic acids exhibit different affinities towards esterases, while Murray and King [1930] have demonstrated that certain enantiomorphic alcohols inhibit the activity of liver esterase to different degrees. Optical isomerides would be expected to possess identical adsorbabilities; it is therefore almost certain that some factor other than adsorption is also involved. We are of opinion that, following adsorption, the inhibitor enters into especial relation with the enzyme, the exact nature of which is not at present clear, and it is probably this second stage of the combination process which is the cause of the different inhibitory activities of isomeric urethanes. One fact which has been elicited in our experiments tends to support this view. In examining the inhibitory activities of the various urethanes towards liver esterase, it has been found that the general effect of those factors which we have interpreted above as exerting their influence by producing changes in adsorbability is the same whether the substrate is methyl butyrate or tributyrin. The relative activities of isomeric urethanes vary, however, with the nature of the substrate, a result which cannot be explained on the basis of adsorption.

Summary.

1. Urethanes of the type which have previously been shown to possess physiological properties of the same kind as physostigmine have been found to possess the common property of inhibiting the activity of liver esterase. A relationship thus exists between chemical constitution and inhibitory action towards liver esterase analogous to that between constitution and physiological activity in the same group.

2. This inhibitory action is exerted in high dilution. Thus, the hydrochloride of the *m*-dimethylaminophenyl ester of methylcarbamic acid, which

is the most active urethane examined, produced an inhibition of 50 % in the hydrolysis of tributyrin by liver esterase when in a final concentration of about 1:500,000,000. The same urethane inhibited the hydrolysis of methyl butyrate to about the same extent when in a final concentration of $\frac{1}{2} \times 10^{-6} M$, which corresponds with one of about 1:13,000,000.

3. Three series of isomeric urethanes, which have been termed the methylurethanes of the phenyl, benzyl and miotine series respectively, have been examined for inhibitory activity in the forms both of their hydrochlorides and methiodides. The relative activities of the isomerides, when methyl butyrate is employed as substrate, are as follows.

Hydrochlorides. Phenyl series: $m > p > o$; benzyl series: $m > p > o$; miotine series: $m = p > o$.

Methiodides. Phenyl series: $m \geqq p > o$; benzyl series: $p > o > m$; miotine series: $o > p \geqq m$.

Using tributyrin as substrate the following orders, which differ from those above, are obtained:

Hydrochlorides. Phenyl series: $m > p \geqq o$; benzyl series: $o > m > p$; miotine series: $m > o > p$.

Methiodides. Phenyl series: $o > p = m$; benzyl series: $p > o > m$; miotine series: $o > p = m$.

4. The inhibitory activity is, in every case, greater in slightly alkaline medium than at p_H 6·8.

5. In general, the methiodides exert a considerably smaller inhibitory effect than do the hydrochlorides.

6. The relative inhibitory activities of the various urethanes do not correspond with their relative miotic activities. It is pointed out that such correspondence is not to be expected even on the assumption that the whole of the physiological activity of the urethanes is due to their inhibitory action on esterases.

7. The mechanism of the inhibitory action is discussed.

Part of the expense of this investigation has been met by grants from the Earl of Moray Research Fund of this University.

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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

185,451

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/185,451	04/25/88	ROSIN M	[illegible]

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	12

DATE MAILED: 05/24/89

The amendment filed 4/25/89 under Rule 312 has been considered, and has been:

1. ☐ entered.
2. ☐ entered as directed to matters of form not affecting the scope of the invention (0.3311).
3. ☒ disapproved. A report appears below.
4. ☐ entered in part. A report appears below.

By Direction of the Commissioner

Report: The information disclosure statement filed April 25, 1989 fails to comply with the provisions of MPEP 609 because it is not accompanied by:

(a) A proposed amendment canceling or further restricting at least one independent claim and narrowing the scope of protection sought;

(b) A timely affidavit under 37 CFR 1.131 with respect to the material cited; or

(c) A statement by the applicant or his attorney or agent that, in the judgment of the person making the statement, the information cited (1) raises a serious question as to the patentability of the claimed subject matter, or (2) is closer than that of record, or (3) is material to the examination of the application as defined in 37 CFR 1.56(a) and is filed with an explanation of why the information disclosure statement was not earlier presented e.g., information recently cited in a corresponding foreign patent application. It has been placed in the application file, but the information referred to therein has not been considered as to the merits and will not be cited on the patent as a result of this information disclosure statement or prior art citation. Applicant is advised that the filing of the above-noted paper may not satisfy the duty of disclosure requirement of 37 CFR 1.56 insofar as any material information is referenced in the above-noted paper.

Shippen:st PLEASE FURNISH YOUR ZIP CODE IN ALL CORRESPONDENCE
A/C 703-557-3920
5/23/89

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

FORM PTOL-271 (REV. 4/81)

UNITED STATES DEPARTMENT OF COMMERCE
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SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT		ATTORNEY DOCKET NO.
07/185,451	04/25/88	ROSIN	M	469-102-1

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MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	13

DATE MAILED: 07/27/89

NOTICE OF ABANDONMENT

This application is abandoned in view of:

1. ☐ Applicant's failure to respond to the Office letter, mailed _____ .
2. ☐ Applicant's letter of express abandonment which is in compliance with 37 C.F.R. 1.138.
3. ☐ Applicant's failure to timely file the response received _____ within the period set in the Office letter.
4. ☒ Applicant's failure to pay the required issue fee within the statutory period of 3 months from the mailing date of 5-12-89 of the Notice of Allowance.
   - ☐ The issue fee was received on _____ .
   - ☐ The issue fee has not been received in Allowed Files Branch as of _____ .

   In accordance with 35 U.S.C. 151, and under the provisions of 37 C.F.R. 1.316(b), applicant(s) may petition the Commissioner to accept the delayed payment of the issue fee if the delay in payment was unavoidable. The petition must be accompanied by the issue fee, unless it has been previously submitted, in the amount specified by 37 C.F.R. 1.17 (l), and a verified showing as to the causes of the delay.

   If applicant(s) never received the Notice of Allowance, a petition for a new Notice of Allowance and withdrawal of the holding of abandonment may be appropriate in view of Delgar Inc. v. Schuyler, 172 U.S.P.Q. 513.
5. ☐ Applicant's failure to timely correct the drawings and/or submit new or substitute formal drawings by _____ as required in the last Office action.
   - ☐ The corrected and/or substitute drawings were received on _____ .
6. ☐ The reason(s) below.

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PTO-1432 (REV. 5-83)

FORM PTO-875 (REV 1-86)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

PATENT APPLICATION FEE DETERMINATION RECORD

SERIAL NO.	FILING DATE
185451	4-25-88

APPLICANT (FIRST NAMED): Weinstock Moshe Noam ET AL

CLAIMS AS FILED - PART I

FOR:	NO. FILED		NO. EXTRA		SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
BASIC FEE						$170	OR		$340
TOTAL CLAIMS	12	-20=	*		X6=	$	OR	X12=	$
INDEP. CLAIMS	4	-3=	*	1	X17=	$	OR	X34=	$ 34
MULTIPLE DEPENDENT CLAIM PRESENT					X55=	$	OR	X110=	$
					TOTAL	$	OR	TOTAL	$ 374

* If the difference in col. 1 is less than zero, enter "0" in col. 2

CLAIMS AS AMENDED - PART II

	(1)		(2)	(3)	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	x5=	$	OR	x10=	$
INDEP.	*	MINUS	***	=	x15=	$	OR	x30=	$
D FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$	OR	+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	x5=	$	OR	x10=	$
INDEP.	*	MINUS	***	=	x15=	$	OR	x30=	$
D FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$	OR	+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA					
TOTAL	*	MINUS	**	=	x5=	$	OR	x10=	$
INDEP.	*	MINUS	***	=	x15=	$	OR	x30=	$
D FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+50=	$	OR	+100=	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest No. Previously Paid For" (Total or Indep.) is the highest number found in the appropriate box in Col. 1.

PTO 1130 (REV 8 87) 185451

PALM III APPLICATION FILE DATA CODING SHEET

U.S. DEPARTMENT OF COMMERCE—PATENT & TM OFFICE

PREPARED BY: B Gross

DATE: 5-26-88

FORMAT NO. 2 Serial No.	TYPE APPL	FILING DATE Month	Day	Year	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWINGS	ASGT?	TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE RECEIVED	SECURITY CASE?	FOREIGN LICENSE?
07	1	04	25	88	2	126	560	0	N	12	04	0	0374	N	Y

FORMAT NO. 3 ATTORNEY DOCKET NUMBER (12 spaces)	Atty.'s Reg. Nos.	FORMAT NO. 4 Applicant's Name & Address	FORMAT NO. 5 Title of Invention	FORMAT NO. 6&7 Correspondence Address
469-102-1	☑ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET	☑ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 8

		CONTINUITY CODE	PARENT APPLICATION SERIAL NUMBER	PARENT FILING DATE Month Day Year	STATUS CODE	PARENT PATENT NUMBER
	RECORD 801	03 2	06835466	03 03 86	2	
	RECORD 802		0			
	RECORD 803		0			
	RECORD 804		0			
	RECORD 805		0			
	RECORD 806		0			
	RECORD 807		0			
	RECORD 808		0			
☐ MORE ON SUPPLEMENTAL CODING SHEET	RECORD 809		0			
	RECORD 810		0			

FORMAT NO. 9

		COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FILING DATE Month Day Year
	RECORD 901	IL X	74497	03 05 85
FOREIGN PRIORITY CLAIMED ☑ YES ☐ NO	RECORD 902			
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	RECORD 904			
	RECORD 905			
	RECORD 906			
☐ APPLICATION PAPERS	RECORD 907			
	RECORD 908			
	RECORD 909			
☐ MORE ON SUPPLEMENTAL CODING SHEET	RECORD 910			

PATENT NUMBER

APPLICATION SERIAL NUMBER
185,451

APPLICANT'S NAME (PLEASE PRINT)
Rosin, et al

IF REISSUE, ORIGINAL PATENT NUMBER

INTERNATIONAL CLASSIFICATION (INT. CL. 4)

C	07	C		125/067

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
560	115

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
560	32	136		
544	162			
546	226			
514	234	330	484	487
514	490			

GROUP ART UNIT
126

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)
Michael L. Shippen

PTO 270 (10-84)

ISSUE CLASSIFICATION SLIP

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[redacted]

SEARCHED

Class	Sub.	Date	Exmr.
560	32 115 163	9/88	[illegible]
544	162		
546	226		
514	234 330 487 484 490		
	update	3/89	[illegible]

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
560	115 136	3/89	[illegible]

SEARCH NOTES

	Date	Exmr.
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INDEX OF CLAIMS

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SYMBOLS

✓	Rejected
=	Allowed
- (Through numeral)	Cancelled
+	Restricted
N	Non-elected
I	Interference
A	Appeal
O	Objected

[redacted]

APPROVED FOR LICENSE ☐

INITIALS TL

185451

Entered or Counted

ABANDONED

CONTENTS

Received or Mailed

1. Application papers.
2. Amdt A Apr. 25 1988
3. Amdt B Apr. 25 1988
4. Final Ej 3 Months 10-11-88 9-29
5. Amdt C (~~rec~~) Dec. 12 1988 RECEIVED
6. Advisory Action 12-21-88 AUG 01 1988 12-20
7. Appeal Jan. 13 1989 GROUP 120
8. Interview Summary 2/22/89
9. Amdt D (~~rec~~) Feb. 28 1989
10. Ng allow March 14, 1989 3-14
11. Ltr w/Ref 4/25/89
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JUMBO

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4948807

SERIAL NUMBER (Series of 1987)	PATENT DATE	PATENT NUMBER
07 320700	AUG 14 1990	4948807

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP UNIT	EXAMINER
07/320,700	03/08/89	~~560~~ 514	H5 11	126	Shippen

APPLICANTS

MARTA W. ROSIN, JERUSALEM, ISRAEL; MICHAEL CHOREV, JERUSALEM, ISRAEL; ZEEV TASHMA, JERUSALEM, ISRAEL.

CONTINUING DATA********

VERIFIED

THIS APPLN IS A CON OF 07/185,451 04/25/88 ABN

WHICH IS A CON OF 06/835,466 03/03/86 ABN

FOREIGN/PCT APPLICATIONS********

VERIFIED

ISRAEL 74497 03/05/85

ILX

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged (Examiner's initials)	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☒ yes ☐ no / ☒ yes ☐ no		ILX	0	1	1	$ 340.00	4691022

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TITLE

PHENYL CARBAMATES

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78

LR

PARTS OF APPLICATION FILED SEPARATELY Petition Granted 7/19/89

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE	CLAIMS ALLOWED	
3-7-90	Assistant Examiner / Docket Clerk	Total Claims 4	Print Claim 1

ISSUE FEE		DRAWING		
Amount Due	Date Paid	Sheets Drwg.	Figs. Drwg.	Print Fig.
620.00	6/4/90	0	0	—

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126
Primary Examiner

ISSUE CLASSIFICATION		ISSUE BATCH NUMBER
Class 514	Subclass 484	D51

Label Area

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PTO-436A

07 320700

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
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FEE RECORD SHEET

140 03/14/89 07/320700 1 101 340.00 CK

PTO-1556
(5/87)

07 320700

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Case 118-6848

PHENYL CARBAMATES

The present invention relates to novel phenyl carbamates which are useful as pharmaceutical compositions. The invention further relates to pharmaceutical compositions having anticholinesterase activity.

Acetylcholine is a major neurotransmitter which is found in all parts of the body. Any reduction in its activity, either as a result of neuronal damage, degeneration etc. or as induced by drugs or toxins, causes marked changes in the function of the organism. Acetylcholine itself has an extremely short half life, since it is rapidly hydrolysed at its site of action and in plasma by specific cholinesterase enzymes. Drugs that inhibit acetylcholinesterase, markedly increase and prolong the action of acetylcholine, thereby enhancing cholinergic transmission. Three such agents are used clinically, i.e., physostigmine, a naturally occurring alkaloid, and two synthetic analogues, neostigmine and pyridostigmine. The latter two agents are strongly ionised at physiological pH and therefore are only poorly absorbed from the gastro-intestinal tract, and do not penetrate the central nervous system to any significant extent. Physostigmine is absorbed after

oral administration and readily enters the brain. As a therapeutic agent it has several disadvantages. It is chemically unstable and must be prepared in solution with an antioxidant, and protected from light. It has a relatively short half-life (20-40 mins) thereby necessitating frequent administration. The latter is of particular importance when the drug is to be administered chronically. It has a low therapeutic ratio, a value of 3-5 being reported in the majority of studies in laboratory animals, and a small therapeutic window, i.e. small range of dose in which it can be given without the accompaniment of side effects. Although physostigmine is absorbed from the gastro-intestinal tract, this is reported to be irregular and unpredictable, and therefore it is usually preferred to administer the drug parenterally. This is a serious drawback if it is to be used chronically on an outpatient basis.

There are a number of clinical and pathological conditions which are associated with cholinergic under-activity which can be improved by the administration of an anticholinesterase agent. These include reduction in cholinergic transmission induced by a variety of exogenous substances acting in the peripheral, or central nervous system. Peripherally acting agents are gallamine, d-tubocurarine and pancuronium, which are used as muscle relaxants. Their action can readily be overcome by an anticholinesterase drug. Drugs which interfere with central cholinergic transmission are numerous, anticholinergic, atropine-like drugs including antiparkinson drugs, tricyclic antidepressants, neuroleptics, opiate analgesics, benzodiazepines and some types of general anaesthetics. So far the only agent that has proved to be of any value in reversing the effects of the latter group of drugs is physostigmine. In all reported cases of drug overdose or lack of recovery when the agent was used peri-operatively, physo-

stigmine is usually administered parenterally, and administration is repeated every 20-30 minutes as required.

Chronic treatment with neuroleptics often results in tardive dyskinesias. The widespread use of agents having anticholinesterase activity for the treatment of schizophrenia makes this side effect an ever increasing possibility. Physostigmine injected intravenously produces a significant but short lived improvement in a proportion of patients.

A number of pathological and degenerative diseases has also been shown to be associated with a reduction or loss of cholinergic transmission. This includes myasthenia gravis and Eaton Lambert syndrome in which there is an interference with neuromuscular transmission.

A selective loss of choline acetyltransferase (the enzyme that synthesises acetylcholine) has been found in specific brain regions of patients with pre-senile dementia of the Alzheimer type. These include the frontal and temporal cortex, hippocampus, amygdala, caudate nucleus, substantia innominata. Degeneration of cholinergic neurons in some of these areas appears to be associated with the aphasia, apraxia, agnosia and loss of short term memory that occurs in Alzheimer's disease. A similar type of dementia is also found in patients with Down's syndrome that survive to the age of 40 years and show similar cholinergic deficits. There is also a loss of cholinergic transmission in the caudate nucleus and putamen of patients with Huntingdon's chorea. Physostigmine injections have also been of some benefit in this condition. Treatment with a centrally acting anticholinesterase should also prove to be beneficial in Friedrich's ataxia.

There are two major classes of potent inhibitors of the enzyme cholinesterase. The first group was modelled primarily on the natural alkaloids physostigmine (a carbamate) and an inhibitor of cholinesterase, and d-tubocurarine, an antagonist of acetylcholine. The second group consists of various organophosphorus compounds, such as diisopropylfluorophosphonate, paraxon etc. The vast majority of the compounds of both these series were designed primarily as insecticides. In the first group of carbamate derivatives, almost all of the potent insecticides are monomethyl carbamates lacking a charged nitrogen function. This enables the molecule to penetrate rapidly the insect cuticle and fatty nerve sheath. The dimethyl derivatives are slightly less potent but are particularly toxic to houseflies and aphids. The monomethyl derivatives tend to be unstable in solution and hydrolyse readily at physiological pH. This greatly limits their biological action in mammals and makes them less suitable as pharmaceutical or therapeutic agents.

The organo-phosphorus group of compounds causes irreversible inhibition of cholinesterase and other serine containing enzymes, which, together with their high relative toxicity, virtually precludes their use in pharmaceutical preparations. The only exception is echothiopate, a quaternary ammonium organophosphorus compound, employed in eye drops for the treatment of glaucoma.

The synthetic anticholinesterase agents currently employed as pharmaceuticals all contain a charged nitrogen function and can be broadly classified into 3 groups.

1) Reversible inhibitors which contain a charged nitrogen function attached to an aromatic ring, e.g. edrophonium.

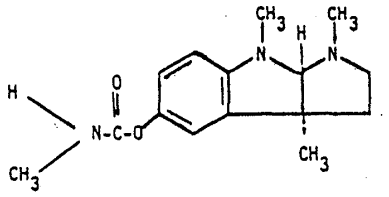
2) Dimethyl carbamates with an aromatic or heterocyclic ring containing a charged nitrogen, neostigmine, pyridostigmine.

3) Bisquaternary structures, e.g. Demacarium, Ambenonium. These agents tend to be more selective inhibitors of acetylcholinesterase than butyrylcholinesterase, compared with the monoquaternary molecules.

The pharmaceutical application of the quaternary anticholinesterase agents is limited because of their poor penetration through cell membranes. They are therefore used for actions outside the central nervous system, and are usually given parenterally, since they are not reliably absorbed from the gastrointestinal tract. Edrophonium, neostigmine and pyridostigmine and the bisquaternary analogues are used in anaesthetic practice for the reversal of the action of muscle relaxants. They are also used for the treatment of myasthenia gravis, and paralytic ileus.

Physostigmine is the only potent anti-cholinesterase agent which has been used clinically to treat conditions in which an elevation of brain acetylcholine activity is desired. These include, Alzheimer's disease, tardive dyskinesia, Down's syndrome and Huntingdon's chorea. Physostigmine is also used to reverse the effects of overdose of anticholinergic agents, anti-Parkinson drugs, benzodiazepines and opiate analgesics.

Physostigmine is a natural alkaloid extracted from calabar beans and the seeds of the vine Physostigma venenosum and has the formula



There is a need to provide new carbamate derivatives which show greater chemical stability than physostigmine.

Furthermore there is a need to provide new compounds which inhibit acetylcholinesterase in the brain for periods exceeding 3 nours but not more than 12 hours after a single administration.

There is also a need to provide new compounds which will be completely and reliably absorbed after oral administration.

There is also a need to provide new compounds which will be relatively less toxic than physostigmine. This means that the therapeutic ratio, defined as

$$\frac{\text{dose to produce therapeutic effect}}{\text{dose to produce mortality in 50 \% of animals}}$$

should be significantly higher than those of physostigmine and that the incidence and severity of side effects should be less than those of physostigmine at therapeutic doses.

There is also a need to provide new compounds which can be given orally or parenterally to treat chronic conditions in which it is

desired to raise cholinergic activity in the central nervous system. These include, Alzheimer's disease, Down's syndrome, Huntingdon's chorea, Friedrich's ataxia.

There is also a need to provide compounds that can be given parenterally at the end of operations, and anaesthetic procedures, to restore wakefulness, respiration and cardiovascular parameters to normal, after the use of anticholinergic, opiates, benzodiazepines, neuroleptics and general anaesthetics, thereby shortening the stay of patients in the recovery room.

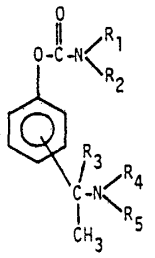
There is also a need to provide compounds that can be given together with narcotic analgesics to patients suffering from severe pain, e.g. traumatic, post-operative, or due to carcinomatosis etc. in order to reduce the side effects (respiratory depression, somnolence, constipation and urinary retention) commonly encountered with narcotics, without impairing their analgesic potency.

There is also a need to provide compounds that can be given to patients receiving antipsychotic drugs, which have developed tardive dyskinesias, in order to diminish or abolish the latter syndrome, without exascerbating the psychosis.

According to the present invention it has now been surprisingly found that certain novel and known phenyl carbamates also inhibit acetylcholinesterase in the mammalian brain after administration to provide systemic activity, e.g. oral or parenteral administration.

Thus according to the present invention there is now provided a pharmaceutical composition adapted to produce anticholinesterase

activity in the central nervous system of mammals comprising a compound of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

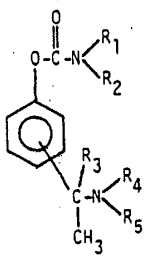
R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor. Hereinafter these compounds are called compounds of the invention.

Especially preferred are pharmaceutical compositions having anticholinesterase activity in the central nervous system of mammals, wherein the dialkylaminoalkyl group is in the meta position, and R_4 and R_5 are both methyl.

Certain compounds falling within the above formula have previously been described i.e. the m disubstituted compound in which R_1 and R_3 = H and R_2, R_4 and R_5 = methyl which is known as Miotine(R) was claimed to be an insecticide and a myopic agent for use in eye drops. The m disubstituted compound in which R_1 and R_2 are methyl, R_3 is H and R_4 and R_5 are methyl has been described as an insecticide. The p and o disubstituted derivatives in which R_1 and R_3 = H and R_2, R_4 and R_5 = CH_3 have been shown to inhibit a preparation of liver cholinesterase. The m disubstituted derivative in which R_1 = H and R_2, R_3, R_4 and R_5 = CH_3 has also been shown to inhibit liver cholinesterase.

The remaining compounds are believed to be novel and thus the present invention also provides novel phenyl carbamate derivatives of the general formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

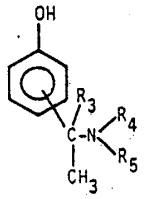
R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

Preferred compounds of the above formula are N-ethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate, N-allyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N-butyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-methyl, N-propyl-3[1-(dimethylamino)ethyl]phenyl carbamate and N-ethyl, N-methyl-3[1-(dimethylamino)isopropyl]phenyl carbamate.

As indicated, the invention also includes the pharmacologically acceptable salts of these compounds such as the acetate, salicylate, fumarate, phosphate, sulphate, maleate, succinate, citrate, tartrate, propionate and butyrate salts thereof.

The compounds of formula I can be prepared by amidating a compound of formula II

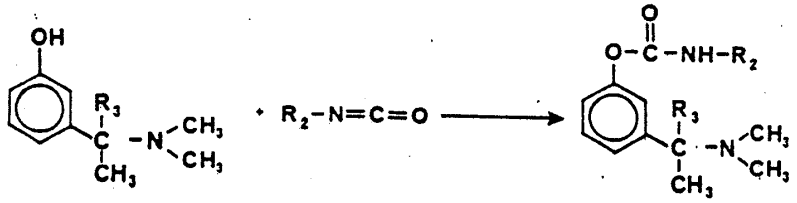


II

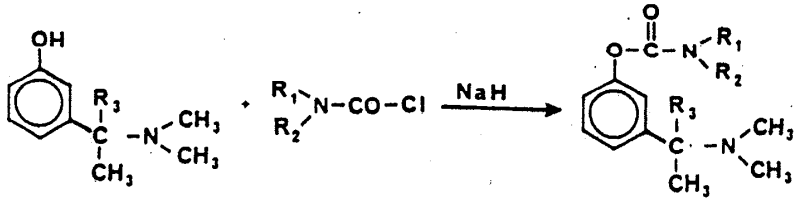
wherein R_3, R_4 and R_5 are as defined above.

The process can be effected in conventional manner, e.g. by reacting the compound of formula II with an appropriate isocyanate if a compound wherein R_1 is hydrogen is desired, or with an appropriate carbamoyl halogenide, e.g. as described below in processes A and B.

PROCESS A:



PROCESS B:



PROCESS A:

A stirred suspension of α-m-Hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in benzene (0.2 - 0.3 g/ml) is treated with 2.5 - 3 fold molar excess of the isocyanate. After stirring for 15 - 24 hours at ambient temperature the reaction mixture is connected to a rotovaporator (20 mm Hg). The residue obtained is dissolved in dry ether (25 ml) and the solution, which is ice cooled, is saturated with dry HCl (g). The formed precipitate (the anticipated carbamate) is filtered off, washed with dry ether (25 ml) and dried to constant weight in a dessicator over KOH pellets under high vacuum (0.1 mm Hg).

PROCESS B:

A solution of α-m-hydroxyphenylethyldimethylamine or α-m-hydroxyphenylisopropyldimethylamine in dry acetonitrile (0.1 - 0.5 M) is reacted with 50 - 70 % molar excess of the corresponding carbamoyl chloride in the presence of 200 % molar excess of NaH dispersion (50 - 80 % in mineral oil). The reaction mixture is left to stir at ambient temperature for 15 - 24 hours. Removal of the acetonitrile under reduced pressure (20 mm Hg) is followed by the addition of water (10 - 25 ml). The pH of the aqueous solution is adjusted to pH = 11 by the addition of the appropriate amount of NaOH 0.1 N followed by extraction with ether (3 x 25 ml). The combined organic phases are washed with brine (25 ml) dried over $MgSO_4$ anhydride which is then filtered off. The ice cooled etheral filtrate is saturated with a stream of HCl (g) resulting in the formation of a heavy precipitate (the anticipated carbamate) which is collected by filtration, washed with dry ether (20 ml) and dried to constant weight in a desiccator under high vacuum (0.1 mm Hg) over KOH pellets.

The compounds of the invention e.g. in free form or salt form can be utilized by formulating one or more of them in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. A compound or mixture of compounds of formula (I) or physiologically acceptable salt(s) thereof is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as mangnesium stearate; a sweetening agent such as sucrose, lactose or saccarin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other mterials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavour.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice by dissolving or suspending the active substance in a vehicle such as water for injection.

Buffers, preservatives, antioxidants and the like can be incorporated as required.

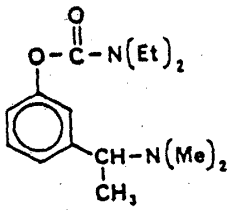
Preferred antioxidants for use with the compounds of the present invention include sodium metabisulphite and ascorbic acid.

While the invention will now be described in connection with certain preferred embodiments in the following examples, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars described are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the invention.

EXAMPLE 1

0.5 g (3.03 mmole) of α-m-hydroxyphenylethyldimethylamine are dissolved in 15 ml of dry acetonitrile and 0.70 g (5.2 mmole) of diethylcarbamylchloride are added to the mixture with stirring. This is followed by NaH 150 mg (50 %) of dispersion. The reaction mixture is stirred overnight at 25 - 30 ° C. Removal of acetonitrile under reduced pressure is followed by addition of water (10 ml) and adjustment of the pH to 11. The product is extracted in ether, which is washed by brine, dried over $MgSO_4$ and filtered. Upon addition of HCl (g) precipitation occurs immediately, the product is filtered off, washed by dry ether and dried in a desiccator under high vacuum over KOH pellets.

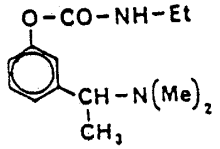
The carbamate is obtained as a white powder 640 mg (80 %) mp. 137 - 138 ° and identified as N,N-diethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, having the formula



EXAMPLE 2

0.75 g (4.55 mmol) of α-m-hydroxyphenylethyldimethylamine are suspended in benzene (3 ml) and 0.898 g of ethylisocyanate are added to the mixture with stirring. After stirring 12 hours at room temperature the solvent is removed under reduced pressure.

The residue obtained was dissolved in dry ether. Introduction of dry HCl gas into the reaction mixture causes a heavy precipitation. The product is filtered off, washed with ether and dried in a desiccator over KOH pellets. The carbamate is obtained as a white powder 800 mg (75 %) mp. 177 - 179 ° C and identified as N-ethyl-3[1-(dimethylamino)ethyl]phenyl carbamate having the formula



The compounds of the present invention are useful as pharmaceuticals. In particular they show the following activities in vitro and in vivo in the tests specified below.

The values are correct when taken in comparison with the standard drug physostigmine.

IN VITRO EXPERIMENTS:

Tests for anticholinesterase activity

A solubilized preparation of acetylcholinesterase was prepared from mouse whole brain (minus cerebellum). The brain was homogenized with (100 mg/ml) phosphate buffer; pH 8.0, centrifuged, the supernatant discarded, and the pellet mixed with a similar volume as above of buffer pH 8.0 plus 1 % Triton; mixed, centrifuged and the supernatant which contained most of the solubilized enzyme, was used for the subsequent determinations of anticholinesterase activity.

The activity of the enzyme (rate of hydrolysis of substrate, acetylthiocholine) was measured using at least 4 different concentrations of substrate, and at least 3 different concentrations of each inhibitor. The enzyme was incubated with inhibitor for periods ranging for 2 - 180 mins. at 37 ° C, substrate was then added, and its rate of hydrolysis measured by the spectrophotometric method of Ellman et al. (1961).

The molar concentration of each agent that inhibited the activity of the enzyme by 50 % (IC_{50}) at the peak time of activity (15 - 60 min) was calculated from this data and recorded in Table 1 hereinafter. The compounds in general produce a significant inhibition from about 10^{-5} to about 10^{-8} molar.

IN VIVO EXPERIMENTS:

a) Assessment of acetylcholinesterase inhibition

The effect of each compound on brain acetylcholinesterase in vivo was measured, after subcutaneous or oral administration to mice. Animals were sacrificed, at different times ranging from 0.25 - 8 hours after drug administration. The brain was rapidly removed, and the enzyme acetylcholinesterase extracted and solubilized with 0.1 % Triton, and its ability to hydrolyse acetylthiocholine assessed as described above (in vitro experiments), in comparison with the enzyme removed from mice injected with normal saline. The compounds have in general a potency of from about 2% to about 90% that of physostigmine.

b) Assessment of acute toxicity

Mice were given one of at least three different doses of each compound, orally or subcutaneously, a minimum of 10 mice allotted to each dose. The number of animals which died at

each dose within 3 hours was determined. From these data, the LD_{50} (dose in mg/kg which was lethal to 50 % of the mice) was computed.

This experiment was repeated after the animals had been pretreated with atropine sulphate, which blocks both peripheral and central muscarinic receptors. The data from these experiments enabled the assessment of the relative degrees of toxicity of the carbamates which result from excessive activation of muscarinic receptors, and from respiratory muscle paralysis, which is insensitive to this blocking agent.

The incidence and degree of side effects was noted for each dose of drug, starting with the lowest that caused any significant (> 20 %) inhibition of whole brain acetylcholinesterase.

c) Antagonism of the somnolent and respiratory depressant effects of opiates

Different doses of the carbamate compounds were injected intravenously with morphine in rabbits. Respiration rate, arterial blood gas tensions and pH were monitored continuously before and after drug administration for 4 - 5 hours. In another series of experiments the effect of the anticholinesterase drugs was assessed on the analgesic effect of opiates in rabbits after application of a nociceptive stimulus, i.e. electrical stimulation of the sciatic nerve.

All specific examples of formula I' mentioned hereinbefore, e.g. on specification page 10, and after especially Tables 1 to 3, are prepared in analagous manner to Example 1 when R_1 and R_2 are each other than hydrogen and Example 2 when one of R_1 and R_2 are hydrogen. They are thus obtained as hydrochloride salts (except where otherwise specified). The specific compounds have metal substitutions.

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Table 2

Anticholinesterase activity of compounds in mouse brain compared to that of physostigmine

Compound	Relative potency to physostigmine after subcut. (s.c.) administration	Relative potency to physostigmine after oral administration	% cholinesterase inhibition 3 hours after s.c. administration
Physo-stigmine	100	100	0
Miotine	100	300	5
RA_6	11	19	35
RA_{15}	33	32	37
RA_{14}	15	22	35
RA_{13}	2	5	-
RA_5	36	29	30
RA_{12}	13	17	37
RA_{10}	81	92	7
RA_7	25	57	41
RA_8	2	5	32
RA_4	13	29	25

Table 3

Acute toxicity of carbamates in mice

Compound	LD_{50} µmoles/kg s.c.	Degree of* protection afforded by pretreatment with atropine	Therapeutic ratio LD_{50}/ED_{50} s.c.	LD_{50} oral / LD_{50} s.c.
Physostigmine	3.0	3.0	3.3	4.1
Miotine	4.5	2.4	4.9	1.2
RA_6	96	2.6	11.9	2.1
RA_{15}	31	4.1	11.1	4.5
RA_{14}	69	8.0	11.5	4.4
RA_{13}	65	4.5	1.6	1.1
RA_5	19	5.8	7.6	5.0
RA_{12}	42	3.8	5.8	3.6
RA_{10}	14	5.0	12.7	9.7
RA_7	46	10.4	12.4	1.2
RA_8	> 568	-	> 10.0	-
RA_4	72	4.9	10.0	1.7

*Ratio of LD_{50} after pretreatment with atropine sulphate 5 mg/kg to LD_{50} of drug alone.

The data in Tables 1 and 2 demonstrate that somewhat larger quantities are required of all the drugs of the RA series than of physostigmine to inhibit the enzyme acetylcholinesterase. However, a comparison of the data in Table 1 with that in Table 2, shows that compounds RA_5, RA_6, RA_{15}, RA_{14}, RA_{10}, RA_7 and RA_8 are all relatively more active in vivo compared to physostigmine than one would expect from the in vitro data. This greater in vivo potency is particularly marked when the drugs are administered orally. This relatively greater in vivo activity may be due to:

a) greater chemical stability

b) a slower metabolic degradation or/and excretion

c) a higher lipid solubility, enabling a greater proportion of the drug to gain access to the enzyme in the central nervous system

d) more efficient absorption from gastro-intestinal tract.

For the purposes of their therapeutic application it is of little importance if one needs to give the drug (to human subjects) at a dose of 1 - 2 mg (physostigmine) or 2 - 50 mg that may be required of the compounds of the RA series. What is important is the safety of the drugs and the presence and severity of side effects that may occur at therapeutic doses. A commonly-used measure of drug safety is the therapeutic index - or LD_{50}/ED_{50}

$$\frac{\text{Dose to kill 50 \% of animals}}{\text{Dose to cause the desired therapeutic effect}}$$

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The duration of significant brain enzyme inhibition (> 30 %) induced by physostigmine (ED_{50} dose) is less than 2 hours. Compounds RA_4, 5, 6, 7, 8, 12, 14, 15 all act for more than 3 hours at their respective ED_{50} doses and RA_6 and RA_7 still causes significant inhibition (36 %) after 7 hours. Since none of these drugs caused noticeable side effects at the ED_{50} doses, an even longer duration of action may be achieved by giving between 50 and 100 % larger doses. The longer duration of action is a distinct advantage, particularly if the drugs are to be administered chronically to subjects suffering from neurological and behavioural conditions associated with a deficit in cholinergic transmission in the central nervous system, e.g. Alzheimer's disease, tardive dyskinesias, Huntingdon's chorea, Down's syndrome and Friedrich's ataxia.

The better the absorption of the drug after oral administration the more closely the LD_{50} given by this route resembles that after subcutaneous injection. Table 3 shows that RA_6, 13, 7 and 4 are more efficiently absorbed from the gastro-intestinal tract than is physostigmine. The ED_{50} of RA_8 after oral administration is the same as that after S.C. injection, indicating a much better oral bioavailability than that of physostigmine. The higher oral bioavailability of these compounds may be a considerable advantage for their clinical use.

RA_{10}, RA_6, RA_{14} and RA_{15} produce significant antagonism of the respiratory depressant effects of morphine in rabbits for periods lasting between 3 - 5 hours depending on the drug and the dose administered. The analgesic activity of morphine is not reduced by the RA compounds. Muscle fasciculations are not evident at the doses of drugs administered. Physostigmine (0.1 - 0.2 mg/kg) antagonizes the respiratory depressant effect of morphine for

30 - 60 mins only and fasciculations are marked at the higher dose.

These findings show that the RA compounds may be given together with morphine to obtain adequate analgesia without significant degrees of respiratory depression.

The most preferred compounds of the RA series are RA_4, RA_5, RA_6, RA_{15}, RA_{14}, RA_7 and RA_8, all of which produce inhibition of brain acetylcholinesterase after parenteral administration of significantly longer duration than that induced by physostigmine or miotine. These compounds also have a greater safety margin (therapeutic ratio) than physostigmine. RA_4, 6, 7 and 8 also show better bioavailability after oral administration than physostigmine. In addition, the acute toxicity (lethality) induced by RA_7 can be decreased more than 10-fold and that of RA_{14} more than 8-fold by the antidote atropine, compared to only a 3-fold decrease for physostigmine and miotine.

The compounds of the invention are therefore useful for the treatment of senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Down's syndrome and Friedrich's ataxia.

For these indications, the exact dosage will of course vary depending upon the compound employed, mode of administration and treatment desired. The compounds may be administered by any conventional route, non-oral or preferably orally.

In general, satisfactory results are obtained when administered at a daily dosage of from about 0.05 to 10 mg/kg animal body weight. For the larger mammals, an indicated total daily dosage

is in the range from about 0.5 to about 25 mg of the compound, conveniently administered in divided doses 2 to 4 times a day in unit dosage form containing for example from about 0.1 to about 12 mg of the compound or in sustained release form.

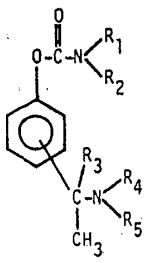
The compounds may be administered in similar manner to known standards for use in these utilities. The suitable daily dosage for a particular compound will depend on a number of factors such as its relative potency of activity.

The compounds according to the invention may be administered in free base form or as a pharmaceutically acceptable acid addition salt. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free forms.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative embodiments and examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is, therefore, desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come with the meaning and range of equivalency of the claims are, therefore, intended to be embraced therein.

WHAT IS CLAIMED IS:-

1. A pharmaceutical composition adapted to produce anticholinesterase activity in the central nervous system comprising a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

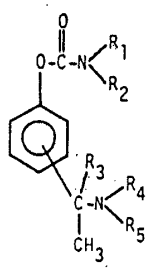
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor.

2. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering a therapeutically effective amount of a compound of formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

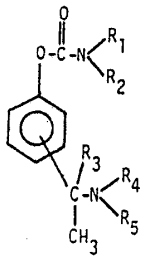
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

or a pharmacologically acceptable salt thereof.

3. A phenylcarbamate of formula I'



I'

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoalkyl group is in the meta, ortho or para position,

and pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R_2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the ortho or para position when R_1 and R_3 are both hydrogen R_2 is not methyl.

4. A compound of claim 3 wherein the dialkylaminoalkyl group is in meta position and R_4 and R_5 are both methyl.

5. A compound of claim 3 which is N-ethyl-3-[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

6. A compound of claim 3 which is N-propyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

7. A compound of claim 3 which is N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

8. A compound of claim 3 which is N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

9. A compound of claim 3 which is N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

10. A compound of claim 3 which is N-allyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

11. A compound of claim 3 which is N-butyl-3[1-(dimethylamino)-ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

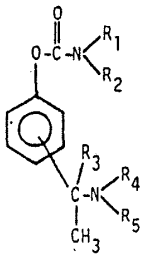
12. A compound of claim 3 which is N-methyl, N-propyl-3[1-dimethylamino)ethyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

13. A compound of claim 3 which is N-methyl, N-ethyl-3[1-dimethylamino)isopropyl]phenyl carbamate or a pharmacologically acceptable salt thereof.

PHENYL CARBAMATES

Abstract of the disclosure

Phenyl carbamates of the general formula



I

wherein R_1 to R_5 are as defined in the claims, are useful as pharmaceuticals.

3700/WY/ER

07 320700

Case 469-104

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Names Applicant: Rosin	
U. S. Serial No.:	Art Unit 126
Filed: 03/08/89	Michael L. Shippen
Title: Phenyl Carbamates	Examiner

AMENDMENT

Hon. Commissioner of Patents and Trademarks
Washington, D. C. 20231

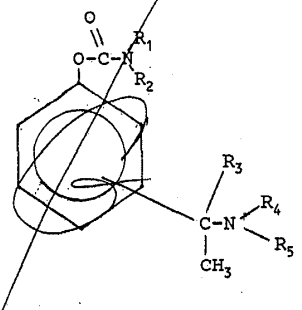
Dear Sir:

Please amend the above identified application as

IN THE CLAIMS

Cancel 1 to 13 and substitute the following:

14. A phenylcarbamate of formula



wherein

R_1 is hydrogen, lower alkyl, cycolohexyl, allyl or benzyl,

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with nitrogen to which they are attached form a morpholino or piperidino radical,

R_3 is hydrogen or lower alkyl,

R_4 and R_5 are the same or different and each is a lower alkyl, and the dialkylaminoakyl group is in the meta, ortho or para position,

pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3 is hydrogen, R_1 is neither hydrogen nor methyl, and when R2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R5 are both methyl and having the dialkyl amino group in the ortho or para position when R_2 and R_3 are both hydrogen and R_2 is not methyl.

27
~~15~~. The compound of claim 14 wherein the dialkylaminoalkyl group is in meta position and R_4 and R_5 are both methyl.

28
~~16~~. The compound of claim 14 which is N-ethyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

29
~~17~~. The compound of claim 14 which is N-propyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

30
~~18~~. The compound of claim 14 which is N-ethyl, N-methyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

31
~~19~~. The compound of claim 14 which is N,N-diethyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

32
~~20~~. The compound of claim 14 which is N-butyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

E' cont'd

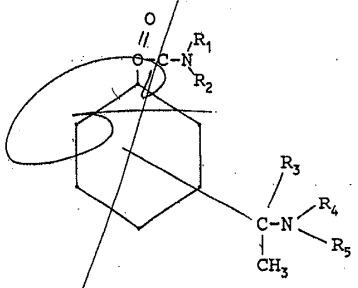
33
~~21~~. The compound of claim 14 which is N-methyl, N-propyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

34
~~22~~. The compound of claim 14 which is N-methyl, N-ethyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

35
~~23~~. The compound of claim 14 which is N-cyclohexyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

24. The compound of claim 14 which is N-allyl-3-[1-(dimethylamino)-ethyl] phenyl carbamate or a pharmacologically acceptable salt thereof.

25. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering a therapeutically effective amount of a compound of the formula



wherein R_1 is hydrogen, lower alkyl, cycolohexyl, allyl or benzyl, R_2 is hydrogen, methyl, ethyl or propyl, or R_1 and R_2 together with nitrogen to which they are attached form a morpholino or piperidino radical, R_3 is hydrogen or lower alkyl, R_4 and R_5 are the dialkylaminoakyl group is in the meta, ortho or para position, pharmacologically acceptable salts thereof, provided that for compounds wherein R_4 and R_5 are both methyl and having the dialkylamino group in the meta position, when R_2 is methyl and R_3

is hydrogen, R_1 is neither hydrogen nor methyl, and when R2 and R_3 are methyl, R_1 is not hydrogen, and for compounds wherein R_4 and R_5 are both methyl and having the dialkyl amino group in the ortho or para position when ~~R1 and~~ R_3 are both hydrogen and R_2 is not methyl.

REMARKS

The claims in the application are claims 14 to 25. Claims 23 and 24 were indicated as allowable in the parent application.

Volume 29 of Advances in Behavioural Biology brought to the attention of the Examiner was published approximately the middle of 1986. The Weinstock et al. article, p. 539 to 549, was reported at the 30th OHOLO Biol. Conference in Eilat, Israel on March 24 to 27, 1985. This conference had previously been mis-identified as the 3rd. and the date of November instead of March.

The article in "Advances in Behavioral Biology" is not prior art. The evidence of this article has not been presented earlier because it is basicly the same data as is included in the patent application specification. The article is cited now in order to show that the data was presented in a recognized scientific publication.

The compounds of Aeschlimann have not been compared because it is believed that they are not as close to miotine and to the Meltzer compound than the RA compounds of Table 1 which all have the dialkylaminoalkyl group in the meta position, whereas the only compound specifically mentioned in Aeschlimann with a dialkyl group

has the ortho configeration. The therapeutic ratio of RA10 is comparable to the claimed compounds as can be seen by reference to Table 3 but RA10 has a short duration of action compared to all the other RAs tested as can be seen by reference to Table 2.

For the reasons given hereinabove it is believed that all of the present claims are allowable.

Respectfully submitted,

Richard T. Laughlin
Attorney for Applicant

CERTIFICATE UNDER 37 CFR 1.8 (a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D. C. 20231, on March 8, 1989.

Richard T. Laughlin
Dated: March 8, 1989

CERTIFICATION

This is to certify that the attached copy is a true copy of United States patent application Ser. No. 185,451 filed April 25, 1988, entitled PHENYL CARBAMATES as filed in the United States Patent and Trademark Office on that day.

Loretta L. Dascoll
Loretta L. Dascoll
Notary Public

Dated: March 8, 1989

LORETTA L. DASCOLL
A Notary Public of New Jersey
My Commission Expires June 3, 1991

4/5

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/320,700	03/08/89	ROSIN M	4691022

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

All fees must be paid pursuant to the new fee schedule published at 54 F.R. 6893 effective April 17, 1989.

000

DATE MAILED: 05/09/89

NOTICE OF IMPROPER FWC FILING UNDER 37 CFR 1.62
NO FILING DATE GRANTED

The above identified application was deposited under 37 CFR 1.62 as a file wrapper continuing application but is improper and has not been granted a filing date for the reasons shown below:

_____ 1. The application does not include the correct serial number including filing date or series code of the prior application.

_____ 2. The application, which is not a continuation-in-part, was not filed by the same or less than all the inventors named in the prior application and no petition for correction of inventorship was filed.

_____ 3. The application, which is a continuation-in-part, does not identify the names of all the inventors (37 CFR 1.41 (a)). The application uses "et al" but only one inventor was named in the prior application.

__✓__ 4. The filing included a new specification or copies of a specification from the prior application. Under 37 CFR 1.62, all changes are required to be made in the form of an amendment to the prior application as it exists at the time of filing the application under 37 CFR 1.62. Therefore, it is unclear whether filing under 37 CFR 1.60 or 1.62 was intended.

_____ 5. The request does not include an orginal signature of the inventor(s), assignee of the entire interest, or registered attorney or agent.

_____ 6. The application was not filed before the payment of the issue fee, abandonment of, or termination of proceeding on the prior application:

_____ a) The issue fee was paid on the prior application on _____.

_____ b) The prior application was abandoned, or proceedings terminated on _____.

_____ c) The prior application was abandoned by the filing of application serial number _____ on _____, under 37 CFR 1.62.

_____ 7. The prior application was not complete as set forth in CFR 1.51(a).

Any request for review of this matter should be made by way of petition directed to the attention of the Office of the Assistant Commissioner for Patents. Such petition must be accompanied by the appropriate fee (37 CFR 1.17 (h)). If the petition alleges that no defect exists, a request for refund of the petition fee may be included. Applicant has TWO MONTHS from the date of this notice to submit a petition to avoid further processing as an improper application with no filing date granted.

Special Handling Branch
ONIAR, Application Processing Division
(703) 557-3831

PTOL-457 (REV. 6/88) PART 1--OFFICE COPY

120 124 Asst #2 Com for Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Rosin

Serial No. 320,700

Filed: 03/08/89

For: Phenyl Carbamates

Office of Assistant
Commissioner For Patents

Commissioner of Patents and Trademarks

Washington, D. C. 20231

PETITION FOR ASSIGNING A FILING DATE

By a letter dated May 9, 1989 Applicants were informed that a filing date of the subject patent application would not be granted because:

> The filing included a new specification or copies of a specification from the prior application. Under 37 CFR 1.62, all changes are required to be made in the form of an amendment to the prior application as it exists at the time of filing the application under 37 CFR 1.62. Therefore, it is unclear whether filing under 37 CFR 1.60 or 1.62 was intended.

This Petition request that the filing date be granted.

As applicants understand the basis for refusal it is grounded on the belief that applicants did not indicate whether it was the intention of applicants to abandoned the parent application and therefore the new application be an application under 37 CFR 1.62 or to keep the parent application pending under 37 CFR 1.60. It is applicants position that the application meet each of the requirements of 37 CFR 1.62 and that it was clearly indicated in

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the papers filed that this was the intention of applicants. It is not seen how the application could be refused the filing date.

At the time of filing, applicants, through their attorney, filed a certified copy of the parent application (See the attached Exhibit A) along with an amendment amending the claims of the parent application. Further the document filed at that time entitled "REQUEST FORM FOR FILE WRAPPER CONTINUING APPLICATION UNDER 37 CFR 1.62" would seem to clearly indicate that it was the intention to file under 37 CFR 1.62 and not 1.60. Further in the paragraph which appears in the middle of page 2 of that document applicants request the abandonment of the parent application. In view of these filings the application had to be under 37 CFR 1.62 and not under 37 CFR 1.60. Still further in the amendment accompanied the application it was also clear that the provisions of 37 CFR 1.62 were being followed. Additionally in paragraph numbered 6 in the conveying form it was requested to amend the specification to state that the application is a "continuation" of the parent application. In view of these filings it is not seen what else applicants could have done to meet the requirements of 37 CFR 1.62 and it is believed that they did comply with all of the requirements.

It is respectively requested that in view of the aforesaid comments that this petition be granted and that the application be given the filing date of March 8, 1989. In view of the fact that all of the requirements were meet, it is requested that the Petition fee be refunded.

Respectively submitted,

Richard T. Laughlin
Attorney for Applicants
Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960
201-539-0080

CERTIFICATE OF MAILING (37 CFR.1.8a)

I hereby certify that this paper (along with along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: May 19, 1988

Richard T. Laughlin

Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960
(201) 539-0080

CERTIFICATION

This is to certify that the attached copy is a true copy of United States patent application Ser. No. 185,451 filed April 25, 1988, entitled PHENYL CARBAMATES as filed in the United States Patent and Trademark Office on that day.

Loretta L. Dascoll
Loretta L. Dascoll
Notary Public

Dated: March 8, 1989

LORETTA L. DASCOLL
A Notary Public of New Jersey
My Commission Expires June 3, 1991

"EXHIBIT A"

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

#47

JUL 19 1989

COPY MAILED

JUL 19 1989

ASSISTANT
COMMISSIONER'S OFFICE

Richard T. Laughlin
Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, NJ 07960

In re Application of :
Marta W. Rosin et al :
Serial No. 07/320,700 : DECISION ON PETITION
Filed March 8, 1989 :
For: PHENYL CARBAMATES :

This is a decision on the petition filed May 24, 1989 requesting that the above-identified application be treated as a proper continuation application under 37 CFR 1.62.

When the application was filed, it included a form requesting a continuation under 37 CFR 1.62 but the proper procedures were not followed for filing a FWC application.

Accordingly, in response to a Notice of Improper FWC Filing Under 37 CFR 1.62 mailed May 9, 1989, the present petition was filed requesting the PTO to accept the application as one filed under 37 CFR 1.62.

When petitioners originally filed a request for a continuation application under 37 CFR 1.62, they submitted a certified copy of the prior application along with the request. Such a copy is usually filed with a request for an application under 37 CFR 1.60 (see 37 CFR 1.60(b)). 37 CFR 1.62(a) states that

> "An application filed under this section will utilize the file wrapper and contents of the prior application to constitute the new continuation..."

Therefore, petitioners' intentions were unclear and resulted in the need for special handling of the application. Thus, the present petition and petition fee were necessary to correct applicants' filing error.

Since the petition clearly states that it was applicants' intention to file the application under 37 CFR 1.62, the copy of the prior application filed on March 8, 1989 is considered withdrawn and will not be entered or used in the prosecution of the present case.

The petition is granted.

The application is being forwarded to Application Branch for further processing as a continuation application under 37 CFR 1.62 of application Serial No. 185,451, with a filing date of March 8, 1989.

R. Franklin Burnett
Special Assistant to the
Assistant Commissioner for Patents

3:10-ae-101-A

07 320700
PTO-3

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this transmittal form and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this March 8, 1989 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number B14219802 addressed to the "Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: March 8, 1989

Richard T. Laughlin

REQUEST FORM FOR FILE WRAPPER CONTINUING APPLICATION UNDER 37 CFR 1.62

469-102-2

Prior Application: Ser. No. 185,451
Filed 04/25/88
Entitled: Phenyl Carbamates 501

Group Unit 126
Examiner: Michael L. Shippen

Commissioner of Patents and Trademarks
Box F W C
Washington, D. C. 20231

This a Request for filing a [] continuation-in-part [X] continuation [] divisional application under 37 CFR 1.62 of prior application Serial No. 185,451, filed on 04/25/88, entitled Phenyl Carbamates which in tern was a continuation of application Ser. No. 835,466 filed March 3, 1986 with the same title, by the following named inventor(s):

Full Name of Inventor	Family Name	First Given Name	Second Given Name
	Rosin 4000	Marta	W.
Residence/Post Ofice	City	State/Foreign Country of	
9 Herzog Str.,	Jerusalem, ILX	Israel	
Citizenship.	Isreal		

1

Full Name of Inventor	Family Name	First Given Name	Second Given Name
	Chorev 40300	Michael	

Residence/Post Ofice	City	State/Foreign Country of
135/4 Feinstein,	Jerusalem, ILY	Israel

Citizenship	Isreal

Full Name of Inventor	Family Name	First Given Name	Second Given Name
	Tashma 40300	Zeev	

Residence/Post Ofice	City	State/Foreign Country of
2 Shahal	Jerusalem ILY	Isreal

Citizenship	Isreal

The above identified prior application in which no payment of the issue fee, abandonment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application. (note: 37 CFR 1.60 may be used for applications where the prior application is not to be abandoned.)

1. [] Enter the amendment previously filed on _____ under 37 CFR 1.116 but unentered, in the prior application.

2. [X] A preliminary amendment is enclosed.

The filing fee is calculated on the basis of the claims existing in the prior application as amended at 1 and 2 above.

CLAIMS CALCULATIONS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5)
	TOTAL CLAIMS	-20=		X $12.00=	$
	INDEPEN-DENT CLAIMS	-3=		X $34.00=	

	MULTIPLE DEPENDENT CLAIM(S)(IF APPLICABLE)		+$110.00=	
			BASIC FEE	$340.00

Total of above $340.00

Reductions by 1/2 for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28). If applicable, verified statement must be attached.	-

TOTAL = $340.00

3.[] The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required, or credit any overpayment to Deposit Account No. _____.

4. [X] A check in the amount of $ 340.00 is enclosed.

5.[] A new oath or declaration is included since this application is a continuation-in-part which discloses and claims additional matter.

6.[X] Amend the specification by inserting before the first line the sentence:

This application is a continuation of application Serial No. 185,451, filed on 04/25/88, entitled Phenyl Carbamates which in turn was a continuation of application Ser. No. 835,466 filed March 3, 1986, both now abandoned

7.[] A verified statement claiming small entity status is enclosed. (necessary even if a statement was filed in the prior application).

8. [X] Priority of application Serial No. 74497 filed on 5/5/85 in Israel is claimed under 35 U.S.C. 119.

9. [X] The prior application is assigned of record to Proterra AG and the assignment is recorded in the U. S. Patent and Trademark Office at reel 4545, Frame 863.

10.[] The power of attorney in the prior application is to: Richard T. Laughlin.

11.[] Also enclosed

Address all future communications to: (May only be completed by applicant, or attorney or agent of record)

Richard T. Laughlin, Esq. 601
Laughlin, Markensohn, Lagani & Pegg 602

3

2

129 Headquarters Plaza 701 702
Morristown, New Jersey 07960
Tel. No. (201) 539-0080

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application be it either this application or a prior application in the same file wrapper, the Patent and Trademark Office may provide similar information or access to all the other applications in the same file wrapper.

DATE March 8, 1989

Richard T. Laughlin
Attorney of Record

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Rosin

Serial No. 320,700 — Group Art Unit: 126

Filed: 03/08/89 — M. Shippen, Examiner

For: Phenyl Carbamates

AMENDMENT

Commissioner of Patents and Trademarks
Washington, D. C. 20231

Dear Sir:

Please amend the above identified application as follows:

IN THE CLAIMS

Cancel all of the claims and substitute the following claims:

~~14~~. N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

~~15~~. N-allyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

~~16~~. N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate and pharmacologically acceptable salts thereof.

~~17~~. A method of treating a subject suffering from senile dementia, Alzheimer's disease, Huntingdon's chorea, tardive dyskinesias, hyperkinesia, mania, acute confusion disorders, Friedrich's ataxia and Down's syndrome, which comprises administering to such a subject a therapeutically effective amount of a compound selected from the group consisting of N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N-allyl-3[1-

(dimethylamino)ethyl]phenyl carbamate, N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate, and pharmacologically acceptable salts thereof.

REMARKS

Claims 14, 15 and 16 were allowed in the parent application. Claim 17 is a method claim directed to the use of the compounds of the other claims and should be allowable for the same reasons that the product claims were allowed.

It is respectfully requested that the claims remaining in the application be allowed.

Respectfully submitted,

Richard T. Laughlin
Attorney for Applicants
129 Headquarters Plaza
Morristown, New Jersey 07960
201-539-0080

CERTIFICATE OF MAILING (37 CFR.1.8a)

I hereby certify that this paper (along with along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: July 26, 1989

Helen S. Lowenstein

Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960
(201) 539-0080

320,700

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/320,700	03/08/89	ROSIN M	1691022

RICHARD T. LAUGHLIN
LAUGHLIN, MARKENSOHN, LAGANI & PEGG
129 HEADQUARTERS PLAZA
MORRISTOWN, NJ 07960

EXAMINER	
SHIPPEN, M	

ART UNIT	PAPER NUMBER
126	20

DATE MAILED: 09/26/89

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 3/8/89 7/31/89 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire THREE month(s), ____ days from the date of this letter. Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 35-41 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 35-41 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☒ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☒ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

The claims presented in the papers filed March 8, 1989 and July 31, 1989 have been renumbered in accordance with 37 CFR 1.126.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 38-41 are rejected under 35 U.S.C. § 102(a or b) as being anticipated by Weinstock, or the 30th OHOLO Biol Conference wherein the Weinstock paper was presented, or any published abstract of the paper presented at that conference. Applicants state in the paper filed March 8, 1989 that the Weinstock article was published "approximately in the middle of 1986." The instant application is entitled to the benefit of a parent application filed on March 3, 1986. From applicants' statement the actual publication date of the Weinstock reference is unclear. It may in fact be earlier than the effective filing date in the United States making it prior art under 35 USC 102 (a). The conference was clearly held before the effective filing date of the instant application make it prior art under 35 USC 102 (a), note Massachusetts Institute of Technology v. AB Fortia, 227 USPQ 428.

Any abstract of the article presented at the meeting would also constitute prior art under 35 USC 102 (a) and if published more than one year before the United States effective filing date would it would be prior art under 35 USC 102 (b). In their response applicants should indicate the earliest actual publication date of the Weinstock article known to them. They should also identify any such abstracts cited above and the actual publication dates thereof (a copy should also be supplied).

Applicants' priority date is noted; however, the right of priority has not been perfected under 35 USC 119 and as such the claims are not entitled to the priority date, also note MPEP 201.15.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 38-41 are rejected under 35 U.S.C. § 103 as being unpatentable over Weinstock, or the 30th OHOLO Biol Conference wherein the Weinstock paper was presented, or any published abstract of the paper presented at the conference. The references are applied as above. If they do not anticipate the claims they at least render the claims obvious.

The remaining references are cited as of interest. It is noted that the rejections of the claims over such prior art was overcome in the parent application USSN 185,451.

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

MShippen
September 25, 1989

TO SEPARATE, H[illegible] TOP AND BOTTOM EDGES, SNAP–APART AND P[illegible] ARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER	
NOTICE OF REFERENCES CITED	320,700	126		20
	APPLICANT(S) Rosin, etal			

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
X	A	1905990	4-1933	Aeschlimann	560	32	
X	B	2208485	7-1940	Aeschlimann	560	136	
X	C	2493710	1-1950	Aeschlimann	560	136	
X	D	2362508	11-1944	Stevens	560	136	
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
X	L	1037753	7-1956	Germany	Fitch	560	136		
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

X	R	Stedman, Biochemical Journal, 20 p719-34 (1926)
X		Wasserman, Proc. Natl. Acad. Sci., USA, 79 p4810-4 (1982)
X	S	Worden, J. Agr. Food Chem., 13 p200-4 (1965)
X		Berry, Biochem. Pharmacol., 20 p3236-8 (1971)
X	T	Weinstock, Advances in Behavioral Biology, 29 p539-49 (1986)
X	U	Lange, Haemostasis, 10 p315-47 (1981)
X		Meltzer, Entomol. Exp. Appl., 12 p169-82 (1969)

EXAMINER	DATE
M. Shippen	9/22/89

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

12C 9-26-89

041 Micheal Shippen 126

#21 12-1-89

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Rosin	:	
Serial No. 320,700	:	Group Art Unit: 126
Filed: 03/08/89	:	M. Shippen (Micheal)
For: Phenyl Carbamates	:	Examiner

AMENDMENT

Commissioner of Patents and Trademarks
Washington, D. C. 20231

Dear Sir:

This is in response to the Official Action of September 26, 1989.

The article appearing in Advances in Behavioral Biology, 29 p 539-49 (1986) was a report of an oral lecture delivered by Professor Weinstock-Rosin at a Congress which took place in Eilat during the period of March 24th through March 27, 1985 which was three weeks after the convention date. There is no publication date of the article and the only information is the copyright date of 1986 appearing in the volume. Professor Weinstock-Rosin has examined Volume 29 and can give no better date. No written material or abstracts were distributed at or before the conference and the first written material was the aforementioned article.

In view of this information it is submitted that the references relied on are subsequent to the convention date of the Israel patent application filed on March 5, 1985, and therefore can

not anticipate the invention as defined in the claims. For these reasons reconsideration of the rejection is respectively requested.

Respectfully submitted,

Richard T. Laughlin
Attorney for Applicants
129 Headquarters Plaza
Morristown, New Jersey 07960
201-539-0080

CERTIFICATE OF MAILING (37 CFR.1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: November 15, 1989

Richard T. Laughlin
Laughlin, Markensohn, Lagani & Pegg
129 Headquarters Plaza
Morristown, New Jersey 07960
(201) 539-0080

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Rosin :
:
Serial No.: 320,700 : Group No.: Art Unit 125
:
Filed: 03/08/89 : Examiner: Michael L. Shippen
:
For: Phenyl Carbamates :

Commissioner of Patents and Trademarks

Washington, D. C. 20231

AMENDMENT

This is in further response to the Official Action of September 26, 1989.

Attached hereto are the priority documents which is a certified copy of the priority application which was filed in Israel.

It is believed that the filing of this document overcomes the date of the references and accordingly it is respectively requested that the application be passed to issue.

For the reasons given hereinabove reconsideration of the rejection of the application is earnestly solicited.

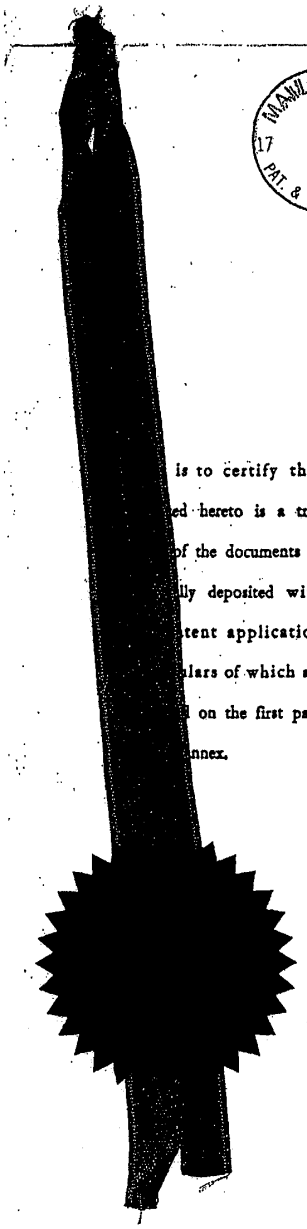
Attorney for applicant

Richard T. Laughlin
Ribis, Graham and Curtin
4 Headquarters Plaza
P.O. Box 1991
Morristown, New Jersey 07960
(201) 292-1700

CERTIFICATE OF MAILING (37 CFR §1.8a)

I hereby certify that this paper (along with along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D. C. 20231.

Date: December 18, 1989 Richard T. Laughlin



SN 320,700
ROSIN
PHIMY CARBAMATE
FILED 03/08/89

STATE OF ISRAEL

זאת לתעודה כי

רצופים בזה העתקים
נכונים של המסמכים
שהופקדו לכתחילה
עם הבקשה לפטנט
לפי הפרטים הרשומים
בעמוד הראשון של
הנספח.

is to certify that

ed hereto is a true
of the documents as
lly deposited with
tent application
lars of which are
on the first page
nnex.

This 26 NOV 1989 חיום

רשם הפטנטים
Registrar of Patents

נתאשר
Certified

ממונה על בתקני פטנטים

חוק הפטנטים, תשכ"ז — 1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
For Office Use

מספר: 74497/1
Number

תאריך: 5 II 1985
Date

תוקדם/נדחה
Ante/Post-dated

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

YISSUM RESEARCH DEVELOPMENT COMPANY
OF THE HEBREW UNIVERSITY OF JERUSALEM
Jerusalem
Inventors: Marta Weinstock Rosin
Zeev Tashma
Michael Chorev

יישום חברה לפתוח המחקר של האוניברסיטת
העברית בירושלים
רחוב ז'בוטינסקי 46, ירושלים
ממציאים: מרתה ויינשטוק רוזין
זאב תא שמע
מיכאל חורב

בעל אמצאה מכח ..... הדין ..... ששמה הוא .....
Owner, by virtue of of an invention the title of which is

(בעברית) תערובות רוקחיות ותולדות פניל קרבאמאט
(Hebrew)

PHARMACEUTICAL COMPOSITIONS AND PHENYL CARBAMATE DERIVATIVES
(באנגלית)
(English)

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. ..... מס'	No. ..... מס'			
dated ..... מיום	dated ..... מיום			
• יפוי כח: כללי / מיוחד — רצוף בזה / עוד יוגש P.O.A.: general / individual-attached / to be filed later-				
filed in case 57024 הוגש בענין				

תמען למסירת מסמכים בישראל
Address for Service in Israel
WOLFF, BREGMAN AND GOLLER
P.O.Box 1352, Jerusalem, Israel

חתימת המבקש
Signature of Applicant
WOLFF, BREGMAN AND GOLLER
By: S. Goller

This 27 יום of 2 בחודש 1985 שנת of the year

לשימוש הלשכה
For Office Use

טו. ס זה, כשהוא מוטבע בחותם לשכת הפטנטים ומושלם בסמר ובתאריך ההגשה, הנו אישור להגשת הבקשה שפרטיה רשומים לעיל.
This from, impressed with the Seal of the Patent Office and indicating the number and date of filing, certifies the filing of the application the particulars of which are set our above.

• מחק את המיותר Delete whatever is inapplicable

PHARMACEUTICAL COMPOSITIONS AND PHENYL CARBAMATE DERIVATIVES

תערובות רוקחיות ותולדות פניל קרבאמאט

The present invention relates to novel phenyl carbamates which are useful as pharmaceutical compositions. The invention further relates to pharmaceutical compositions having anticholinesterase activity.

Acetylcholine is a major neurotransmitter which is found in all parts of the body. Any reduction in its activity, either as a result of neuronal damage, degeneration etc. or as induced by drugs or toxins, causes marked changes in the function of the organism. Acetylcholine itself has an extremely short half life, since it is rapidly hydrolysed at its site of action and in plasma by specific cholinesterase enzymes. Drugs that inhibit acetylcholinesterase, markedly increase and prolong the action of acetylcholine, thereby enhancing cholinergic transmission. Three such agents are used clinically, i.e., physostigmine, a naturally occurring alkaloid, and two synthetic analogues, neostigmine and pyridostigmine. The latter two agents are stongly ionised at physiological pH and therefore are only poorly absorbed from the gastro-intestinal tract, and do not penetrate the central nervous system to any significant extent. Physostigmine is absorbed after oral administration and readily enters the brain. As a therapeutic agent it has several disadvantages. It is chemically unstable and must be prepared in solution with an antioxidant, and protected from light. It has a relatively short half-life (20-40 mins) thereby necessitating frequent administration. The latter is of particular importance when the drug is to be administered chronically. It has a low therapeutic ratio, a value of 4-5 being reported in the majority of studies in laboratory animals, and a small therapeutic window, i.e. small range of dose in which it can be given without the accompaniment of side effects. Although physostigmine is absorbed from the gastro-intestinal tract, this is reported to be irregular and unpredictable, and therefore it is usually preferred to administer the drug parenterally. This is a serious drawback if it is to be used chronically on an outpatient basis.

There are a number of clinical and pathological conditions which are associated with cholinergic under-activity which can be improved by the administration of an anticholinesterase agent. These include reduction in cholinergic transmission induced by a variety of exogenous susbtances acting in the peripheral, or central nervous system. Peripherally acting agents are gallamine, d-tubocurarine and pancuronium, which are used as muscle relaxants. Their action can readily be overcome by an anticholinesterase drug. Drugs which interfere with central cholinergic transmission are numerous, anticholinergic, atropine-like drugs including antiparkinson drugs, tricyclic antidepressants, neuroleptics, opiate analgesics, benzodiazepines and some types of general anaesthetics. So far the only agent that has proved to be of any value in reversing the effects of the latter group of drugs is physostigmine. In all reported cases of drug overdose or lack of recovery when the agent was used peri-operatively, physostigmine is usually administered parenterally, and administration is repeated every 20-30 minutes as required.

Chronic treatment with neuroleptics often results in tardive dyskinesias. The widespread use of agents having anticholinesterase activity for the treatment of schizophrenia makes this side effect an ever increasing possibility. Physostigmine injected intravenously produces a significant but short lived improvement in a proportion of patients.

A number of pathological and degenerative diseases has also been shown to be associated with a reduction or loss of cholinergic transmission. This includes myasthenia gravis and Eaton Lambert syndrome in which there is an interference with neuromuscular transmission.

A selective loss of choline acetyltransferase (the enzyme that synthesises acetylcholine) has been found in specific brain regions of patients with pre-senile dementia of the Alzheimer type. These include the frontal and temporal cortex, hippocampus, amygdala, caudate nucleus substantia innominata. Degeneration of cholinergic neurons in some of these areas appears to be associated with the aphasia, apraxia, agnosia and loss of short term memory that occurs in Alzheimer's disease. A similar type of dementia is also found in patients with Down's syndrome that survive to the age of 40 years and show similar cholinergic deficits. There is also a loss of cholinergic transmission in the caudate nucleus and putamen of patients with Huntington's chorea. Physostigmine injections have also been of some benefit in this condition. Treatment with a centrally acting anticholinesterase should also prove to be beneficial in Friedrich's ataxia.

There are two major classes of potent inhibitors of the enzyme cholinesterase. The first group was modelled primarily on the natural alkaloids physostigmine (a carbamate) and an inhibitor of cholinesterase, and d-tubocurarine, an antagonist of acetylcholine. The second group consists of various organophosphorus compounds, such as diisopropyl-fluorophosphonate, paraxon etc. The vast majority of the compounds of both these series were designed primarily as insecticides. In the first group of carbamate derivatives, almost all of the potent insecticides are monomethyl carbamates lacking a charged nitrogen function. This enables the molecule to penetrate rapidly the insect cuticle and fatty nerve sheath. The dimethyl derivatives are slightly less potent but are

particularly toxic to houseflies and aphids. The monomethyl derivatives tend to be unstable in solution and hydrolyse readily at physiological pH. This greatly limits their biological action in mammals and makes them less suitable as pharmaceutical or therapeutic agents.

The organo-phosphorus group of compounds causes irreversible inhibition of cholinesterase and other serine containing enzymes, which, together with their high relative toxicity, virtually precludes their use in pharmaceutical preparations. The only exception is echothiopate, a quaternary ammonium organo-phosphorus compound, employed in eye drops for the treatment of glaucoma.

The synthetic anticholinesterase agents currently employed as pharmaceuticals all contain a charged nitrogen function and can be broadly classified into 3 groups.

1) Reversible inhibitors which contain a charged nitrogen function attached to an aromatic ring, e.g. edrophonium.

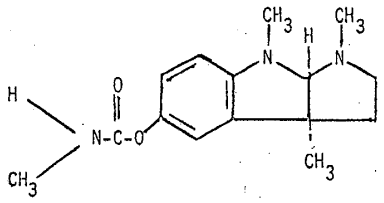
2) Dimethyl carbamates with an aromatic or heterocyclic ring containing a charged nitrogen, neostigmine, pyridostigmine.

3) Bisquaternary structures, e.g. Demacarium, Ambenonium. These agents tend to be more selective inhibitors of acetylcholinesterase than butyrylcholinesterase, compared with the monoquaternary molecules.

The pharmaceutical application of the quaternary anticholinesterase agents is limited because of their poor penetration through cell membranes. They are therefore used for actions outside the central nervous system, and are usually given parenterally, since they are not reliably absorbed from the gastrointestinal tract. Edrophonium, neostigmine and pyridostigmine and the bisquaternary analogues are used in anaesthetic practice for the reversal of the action of muscle relaxants. They are also used for the treatment of myasthenia gravis, and paralytic ileus.

Physostigmine is the only potent anti-cholinesterase agent which has been used clinically to treat conditions in which an elevation of brain acetylcholine activity is desired. These include, Alzheimer's disease, tardive dyskinesias, Down's syndrome and Huntingdon's chorea. Physostigmine is also used to reverse the effects of overdose of anticholinergic agents, anti-Parkinson drugs, benzodiazepines and opiate analgesics.

Physostigmine is a natural alkaloid extracted from calabar beans and the seeds of the vine Physostigma venenosum and has the formula



An object of the present invention is to provide new carbamate derivatives which show greater chemical stability than physostigmine.

Another object of the present invention is to provide new compounds which inhibit acetylcholinesterase in the brain for periods exceeding 3 hours but not more than 12 hours after a single administration.

Another object of the present invention is to provide new compounds which will be completely and reliably absorbed after oral administration.

Another object of the present invention is to provide new compounds which will be relatively less toxic than physostigmine. This means that the therapeutic ratio, defined as

$$\frac{\text{dose to produce therapeutic effect}}{\text{dose to produce mortality in 50\% of animals}}$$

should be significantly higher than those of physostigmine and that the incidence and severity of side effects should be less than those of physostigmine at therapeutic doses.

Another object of the present invention is to provide new compounds which can be given orally or parenterally to treat chronic conditions in which it is desired to raise cholinergic activity in the central nervous system. These include, Alzheimer's disease, Down's syndrome, Huntingdon's chorea, Friedrich's Ataxia.

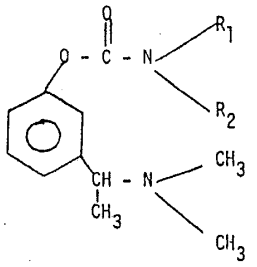
It is also an object of this invention to provide compounds that can be given parenterally at the end of operations, and anaesthetic procedures, to restore wakefulness, respiration and cardiovascular parameters to normal, after the use of anticholinergic, opiates, benzodiazepines, neuroleptics and general anaesthetics, thereby shortening the stay of patients in the recovery room.

It is also an object of this invention to provide compounds that can be given together with narcotic analgesics to patients suffering from severe pain, e.g. traumatic, post-operative, or due to carcinomatosis etc. in order to reduce the side effects (respiratory depression, somnolence, constipation and urinary retention) commonly encountered with narcotics, without impairing their analgesic potency.

It is also an object of this invention to provide compounds that can be given to patients receiving antipsychotic drugs, which have developed tardive dyskinesias, in order to diminish or abolish the latter syndrome, without exascerbating the psychosis.

According to the present invention it has now been surprisingly found that certain novel and known phenyl carbamates also inhibit acetylcholinesterase in the mammalian brain after oral or parenteral administration.

Thus according to the present invention there is now provided a pharmaceutical composition having antichlorinesterase activity comprising a compound of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

R_2 is hydrogen methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor.

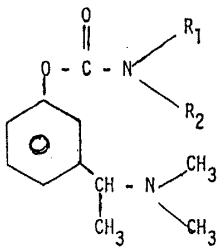
Two compounds of the above formula, i.e., the N-methyl and dimethyl derivatives have previously been described in the literature. The former which is known as Miotine(R) was claimed to be an insecticide and a myopic agent for use in eye drops, and the latter has only been described as an insecticide.

The remaining compounds are believed to be novel and thus the present invention also provides novel phenyl carbamate derivatives of the general formula I

Said article is however an academic work which comes to no conclusions and does not teach or suggest structural changes in the carbamates discussed therein.

In an article by Meltzer, CA-71-111828M there are disclosed two compounds with the code numbers KD 1207 and 1261 which fall under the proviso of the present invention and provides results as to their insecticidal activity. There is also a general statement mentioning that the anticholinesterase activity of alkylphenyl N-methylcarbamates can be improved by introducing a p-dimethylaminomethyl group. There is however no alkyl group in addition to the dialkylaminoalkyl group in the compounds of formula I. Thus said article does not teach the specific compounds of the present invention.

The present invention also provides novel phenyl carbamate derivatives of the general formula I



wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl

R_2 is hydrogen, methyl, ethyl or propyl, or

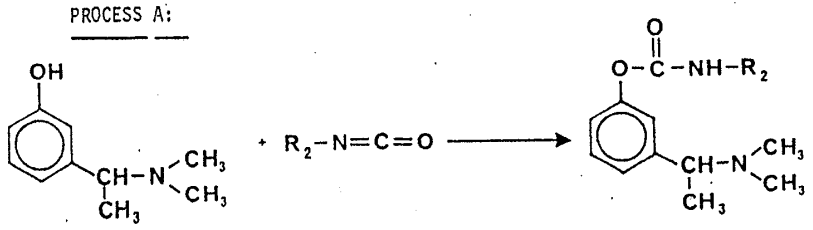
R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical and pharmacologically acceptable salts thereof, provided that when R_2 is methyl, R_1 is neither hydrogen nor methyl.

Preferred compounds of the above formula are N-ethyl-3-[1-(dimethylamino) ethyl]phenyl carbamate, N-propyl-3[1-(Dimethylamino)ethyl]phenyl carbamate, N-allyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, N-ethyl,N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate, N,N-diethyl-3[1-(dimethylamino)ethyl] phenyl carbamate, N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate, ~~N,N-dimethyl-3[1-(dimethylamino)ethyl]phenyl carbamate~~, and 3-[1-(dimethylamino) ethyl] morpholino carbamoyl phenolate.

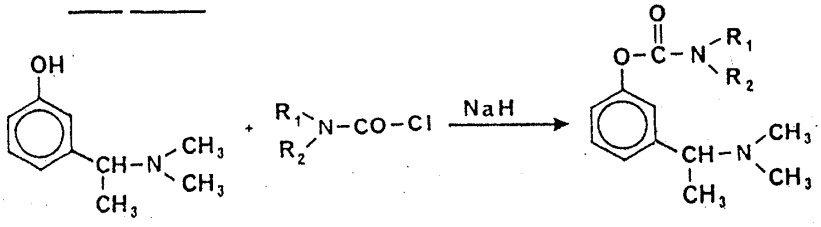
As indicated, the invention also includes the pharmacologically acceptable salts of these compounds such as the acetate, salicylate fumarate, phosphate, sulphate, maleate, succinate, citrate, tartrate, propionate and butyrate salts thereof.

The compounds of the present invention can be prepared by the following processes which processes can be summarized and represented by the following reactions:

PROCESS A:



PROCESS B:



PROCESS A:

A stirred suspension of α-m-Hydroxyphenylethyldimethyl amine in benzene (0.2 -0.3 g/ml) is treated with 2.5-3 fold molar excess of the isocyanate. After stirring for 15-24 hr at ambient temperature the reaction mixture is connected to rotovaporator (20 mm Mg). The residue obtained is dissolved in dry ether (25 ml) and the solution, which is ice cooled, is saturated with dry HCl (g). The formed precipitate (the anticipated carbamate) is filtered off washed with dry ether (25 ml) and dried to constant weight in a desiccator over KOH pellets under high vacuum (0.1 mm Hg).

PROCESS B:

A solution of α-m-hydroxyphenylethyldimethyl amine in dry acetonitrile (0.1-0.5 M) is reacted with 50-70% molar excess of the corresponding carbamoyl chloride in the presence of 200% molar excess of NaH dispersion (50-80% in mineral oil). Reaction mixture is left to stir at ambient temperature for 15-24 hr. Removal of the acetonitrile under reduced pressure (20 mm Hg) is followed by the addition of water (10-25 ml). The pH of the aqueous solution is adjusted to pH = 11 by the addition of the appropriate amount of NaOh 0.1N followed by extraction with ether (3 x 25 ml). The combined organic phases are washed with brine (25 ml) dried over $MgSO_4$ anhydride which is then filtered off. The ice cooled etheral filtrate is saturated with a stream of HCl(g) resulting in the formation of a heavy precipitate (the anticipated carbamate) which is collected by filtration washed with dry ether (20 ml) and dried to constant weight in a desiccator under high vacuum (0.1 mm Hg) over KOH pellets.

The compounds of the invention can be utilized by formulating one or more of them in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. A compound or mixture of compounds of formula (I) or physiologically acceptable salt(s) thereof is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is such that a suitable dosage is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice by dissolving or suspending the active substance in a vehicle such as water for injection. Buffers, preservatives, antioxidants and the like can be incorporated as required.

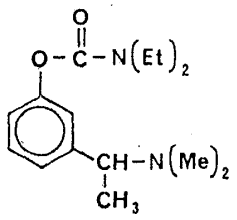
Preferred antioxidants for use with the compounds of the present invention include sodium metabisulphite and ascorbic acid.

While the invention will now be described in connection with certain preferred embodiments in the following examples, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars described are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the invention.

EXAMPLE 1

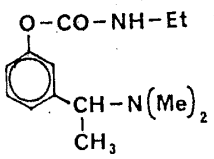
0.5g (3.03 mmole) of α-m-hydroxyphenylethyldimethylamine are dissolved in 15 ml of dry acetonitrile and 0.70g (5.2 mmole) of diethylcarbamylchloride are added to the mixture with stirring. This is followed by NaH 150 mg (50%) of dispersion. The reaction mixture is stirred overnight at 25-30°C. Removal of acetonitrile under reduced pressure is followed by addition of water (10 ml) and adjustment of the pH to 11. The product is extracted in ether, which is washed by brine, dried over $MgSO_4$ and filtered. Upon addition of HCl(g) precipitation occurs immediately, the product is filtered off, washed by dry ether and dried in a desiccator under high vacuum over KOH pellets.

The carbamate is obtained as a white powder 640 mg (80%) mp. 137-138° and identified as N,N-diethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate, having the formula

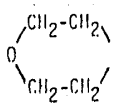


EXAMPLE 2

0.75 g (4.55 mmol) of α-m-Hydroxyphenylethyldimethylamine are suspended in benzene (3 ml) and 0.898 g of ethylisocyanate are added to the mixture with stirring. After stirring 12 hr at room temp. the solvent is removed under reduced pressure. The residue obtained was dissolved in dry ether. Introduction of dry HCl gas into the reaction mixture causes a heavy precipitation. The product is filtered off, washed with ether and dried in a desiccator over KOH pellets. The carbamate is obtained as a white powder 800 mg (75%) mp. 177-179°C and identified as N-ethyl-3[1-(dimethylamino)ethyl]phenyl carbomate having the formula



In a similar manner and following either the procedure of process A or B described hereinbefore, the following compounds were prepared and coded for testing as follows:

Compound	R_1	R_2	Preparation Process
Miotine	H-	CH_3-	A
RA_6	H-	CH_3-CH_2-	A
RA_{15}	H-	CH_3-CH_2-CH_2-	A
RA_{13}	H-	$(CH_3)_2CH$-	A
RA_{14}	H-	CH_2=CH-CH_2-	A
RA_{10}	CH_3	CH_3	B
RA_7	CH_3	CH_3-CH_2-	B
RA_8	CH_3-CH_2-	CH_3-CH_2-	B
RA_{12}	H-	C_6H_{11}-	A
RA_{11}			B

EXAMPLE 3

Tests for anticholinesterase activity in vitro

A solubilized preparation of acetylcholinesterase was prepared from mouse whole brain (minus cerebellum). The brain was homogenized (100 mg/ml) phosphate buffer, pH 8.0), centrifuged, the supernatant discarded, and the pellet mixed with a similar volume as above of buffer pH 8.0 plus 1% Triton; mixed, centrifuged and the supernatant which contained most of the solubilized enzyme, was used for the subsequent determinations of anticholinesterase activity.

The activity of the enzyme (rate of hydrolysis of substrate, acetylthiocholine) was measured using at least 4 different concentrations of substrate, and at least 3 different concentrations of each inhibitor. The enzyme was incubated with inhibitor for periods ranging for 2-180 mins. at 37°C, substrate was then added, and its rate of hydrolysis measured by the spectrophotometric method of Ellman et al. (1961).

The molar concentration of each agent that inhibited the activity of the enzyme by 50% (IC_{50}) at the peak time of activity (15-60 min) was calculated from this data and recorded in Table I hereinafter.

IN VIVO EXPERIMENTS:

a) Assessment of acetylcholinesterase inhibition

The effect of each compound on brain acetylcholinesterase in vivo was measured, after subcutaneous or oral administration to mice. Animals were sacrificed, at different times ranging from 0.25-8 hours after drug administration. The brain was rapidly removed, and the enzyme acetylcholinesterase extracted and solubilized with 0.1% Triton, and its ability to hydrolyse acetylchiocholine assessed as described above (in vitro experiments), in comparison with the enzyme removed from mice injected with normal saline.

b) Assessment of acute toxicity

Mice were given one of at least three different doses of each compound, orally or subcutaneously, a minimum of 10 mice allotted to each dose. The number of animals which died at each dose within 3 hours was determined. From these data, the LD_{50} (dose in mg/kg which was lethal to 50% of the mice) was computed.

This experiment was repeated after the animals had been pretreated with with atropine sulphate, which blocks both peripheral and central muscarinic receptors. The data from these experiments enabled the assessment of the relative degrees of toxicity of the carbamates which result from excessive activation of muscarinic receptors, and from respiratory muscle paralysis, which is insensitive to this blocking agent.

The incidence and degree of side effects was noted for each dose of drug, starting with the lowest that caused any significant (>20%) inhibition of whole brain acetylcholinesterase.

c) Antagonism of the somnolent and respiratory depressant effects of opiates

Different doses of the carbamate compounds were injected intravenously with morphine in rabbits. Respiration rate, arterial blood gas tensions and pH were monitored continuously before and after drug administration for 4-5 hours. In another series of experiments the effect of the anticholinesterase drugs was assessed on the analgesic effect of opiates in rabbits after application of a nociceptive stimulus, i.e. electrical stimulation of the sciatic nerve.

Table 1

In vitro activity on solubilized mouse brain enzyme

Compound	R_1	R_2	IC_{50}(M)	Time of peak activity (mins)
Physostigmine	H	CH_3	$1.1x10^{-8}$	30
Miotine	H	CH_3	$1.3x10^{-8}$	30
RA_6	H	C_2H_5	$4.0x10^{-7}$	120
RA_{15}	H	C_3H_7 n-propyl	$1.1x10^{-7}$	120
RA_{14}	H	C_3H_5 (allyl)	$4.3x10^{-7}$	120
RA_{13}	H	isopropyl	$1.2x10^{-5}$	120
RA_{12}	H	cyclohexyl.	$9.3x10^{-8}$	120
RA_{10}	CH_3	CH_3	$2.7x10^{-8}$	120
RA_7	CH_3	C_2H_5	$3.0x10^{-6}$	120
RA_8	C_2H_5	C_2H_5	$3.5x10^{-5}$	30
RA_{11}	morpholino		$> 2x10^{-5}$	30

Table 2

In vivo activity of compounds on brain acetylcholinesterase in mice

Compound	ED_{50} µmoles/kg sub-cutaneous	% inhib. after 3hrs	ED_{50} µmoles/kg oral	ED_{50} oral / ED_{50} sub-cutaneous
Physostigmine	0.91	0	3.6*	> 4.0
Miotine	1.13	5	2.3	2.0
RA_6	10.6	35	19.1	1.8
RA_{15}	3.1	37	12.0	3.9
RA_{14}	6.1	35	16.2	2.7
RA_{13}	40.0	-	80.0	2.0
RA_{12}	8.7	37	20.8	2.4
RA_{10}	1.04	7	8.3	8.0
RA_7	6.8	41	12.0	1.8
RA_8	56.8	32	56.8	1.0

*Maximum inhibition obtainable 35%. Higher doses caused very marked side effects, fasciculations, tremors, diarrhoea, etc.

Table 3

Relative potency in vitro and in vivo of compounds compared to that of physostigmine

Drug	Relative potency in vitro A	Relative potency in vivo s.c. B	Relative potency in vivo oral C
Physostigmine	100	100	100
Miotine	85	81	156
RA_6	3	9	19
RA_{15}	10	30	30
RA_{14}	3	15	22
RA_{13}	0.1	2	5
RA_{12}	12	9	17
RA_{10}	41	88	43
RA_7	0.4	13	30
RA_8	0.3	2	6

Table 4

Acute toxicity of carbamates in mice

Drug	1) LD_{50} µmoles/kg s.c.	2) LD_{50} µmoles/kg after atropine*	Degree of protection afforded by atropine 2) − 1)	Therapeutic ratio $\frac{LD50\ s.c.}{ED50\ s.c.}$	LD_{50} µmoles/kg oral
Physostigmine	5.09	16.4	3.2	5.6	12.0
Miotine	4.50	10.8	2.4	4.2	5.9
RA6	95.7	255.3	2.7	9.0	206.4
RA15	28.6	139.4	4.9	9.2	111.5
RA14	64.8	141.7	2.2	10.7	161.9
RA13	64.3	278.9	4.3	2.1	95.6
RA12	41.5	145.3	3.5	4.8	134.9
RA10	12.4	70.5	5.7	12.0	91.3
RA7	46.0	500	10.9	6.8	54.0
RA8	>568	-[a]	-	>10.0	-

*Atropine sulphate, 5mg/kg was injected subcutaneously 15 mins before the drugs.

[a]Toxicity of this drug was not tested in the presence of atropine because the LD_{50} had not been reached in the absence of atropine.

The data in Tables 1-3 demonstrate that somewhat larger quantities are required of all the drugs of the RA series than of physostigmine to inhibit the

enzyme acetylcholinesterase. However, a comparison of the data in columns B and C with that in column A in Table 3, shows that compounds RA_6, RA_{15}, RA_{14}, RA_{10}, RA_7 and RA_8 are all 2-75 times relatively more active in vivo compared to physostigmine than one would expect from the in vitro data. This greater in vivo potency is particularly marked when the drugs are administered orally. This relatively greater in vivo activity may be due to:

a) greater chemical stability

b) a slower metabolic degradation or/and excretion

c) a higher lipid solubility, enabling a greater propotion of the drug to gain access to the enzyme in the central nervous system

d) more efficient absorption from gastro-intestinal tract.

For the purposes of their therapeutic application it is of little importance if one needs to give the drug (to human subjects) at a dose of 1-2mg (physostigmine) or 2-50mg that may be required of the compounds of the RA series. What is important is the safety of the drugs and the presence and severity of side effects that may occur at therapeutic doses. A commonly-used measure of drug safety is the therapeutic index - or LD_{50}/ED_{50}

$$\frac{\text{Dose to kill 50\% of animals}}{\text{Dose to cause the desired therapeutic effect}}$$

It is assumed that the therapeutic effect of these anticholinesterase agents results from an elevation of brain cholinergic activity. This in turn, should be related to the degree of inhibition of acetylcholinesterase. For the

purpose of the computation of the denominator of the therapeutic ratio, there is used the dose of drug that inhibits the activity of acetylcholinesterase by 50%. This is based on the observation by Thal et al. (Ann. Neurology 13: 491, 1983) that the maximum improvement in short term memory obtained in a series of patients with Alzheimer's disease was achieved with a dose of physostigmine which blocked the acetylcholinesterase in the cerebro-spinal fluid by 50%. The numerator is the dose found to kill 50% of the animals within 4 hours of a subcutaneous injection.

The therapeutic ratios of compounds RA6,15,14,10 and 8 are all significantly higher than that of physostigmine (see Table 4). This indicates that all these compounds have a wider margin of safety than that of physostigmine. Moreover, these RA compounds do not produce any significant undesirable side effects such as defaecation, lachrymation, fasciculations or tremor at the doses which inhibit the brain enzyme by 50%, while the former 3 side effects are clearly evident when physostigmine is given at the appropriate dose (ED_{50}).

The data in Table 4 show that atropine can afford considerably greater protection against the lethality of the derivatives RA_{10}, RA_{15}, RA_{13} and RA_7. This is particularly important in the treatment of drug overdose since the respiratory muscle paralysis which is not affected by atropine and which is the cause of death induced by excess drug administration in the presence of atropine cannot be satisfactorily reversed by specific antidotes.

The duration of significant brain enzyme inhibition (>30%) induced by physostigmine (ED_{50} dose) is less than 2 hours. Compounds RA_6, RA_{15}, RA_{14}, RA_{12}, RA_7 and RA_8 all act for more than 3 hours at their respective ED_{50} doses and RA_6 still causes significant inhibition (36%) after 7 hours. Since none of these drugs caused noticeable side effects at the ED_{50} doses, an even longer duration of action may be achieved by giving between 50 and 100% larger doses. The longer duration of action is a distinct advantage, particularly if the drugs are to be administered chronically to subjects suffering from neurological and behavioural conditions associated with a deficit in cholinergic transmission in the central nervous system, e.g. Alzheimer's disease, tardive dyskinesias, Huntingdon's chorea, Down's syndrome an Friedrich's ataxia.

The better the absorption of the drug after oral administration the more closely the ED_{50} given by this route resembles that after subcutaneous injection. Table 2 shows that all the drugs of the RA series except RA_{10} are more efficiently absorbed from the gastro-intestinal tract than is physostigmine. The higher oral bioavailability of these compounds is a considerable advantage for their clinical use.

~~RA_{10}~~, RA_6, RA_{14} and RA_{15} produce significant antagonism of the respiratory depressant effects of morphine in rabbits for periods lasting between 3-5 hours depending on the drug and the dose administered. The analgesic activity of morphine is not reduced by the RA compounds. Muscle fasciculations are not evident at the doses of drugs administered. Physostigmine (0.1-0.2mg/kg) antagonizes the

respiratory depressant effect of morphine for 30-60 mins only and fasciculations are marked at the higher dose.

These findings show that the RA compounds may be given together with morphine to obtain adequate analgesia without significant degrees of respiratory depression.

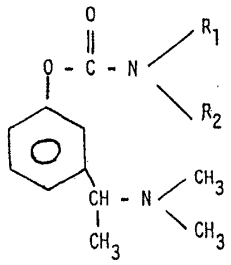
The most preferred compounds of the RA series are RA_6, RA_{15}, RA_{14}, RA_7 and RA_8, all of which produce inhibition of brain acetylcholinesterase after parenteral administration of significantly longer duration than that induced by physostigmine or miotine. These compounds also have a greater safety margin (therapeutic ratio) and show a better bioavailability after oral administration than physostigmine. In addition, the acute toxicity (lethality) induced by RA_7 can be decreased more than 10-fold by the antidote atropine, compared to only a 3-fold increase for physostigmine and miotine.

The next preferred compounds are RA_{12} and RA_{10}. RA_{12} has a longer duration of action and higher oral bioavailability than physostigmine, while RA_{10}, has a much higher margin of safety with and without atropine than either physostigmine or miotine.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative embodiments and examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is, therefore, desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come with the meaning and range of equivalency of the claims are, therefore, intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition having anticholinesterase activity comprising a compound of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl,

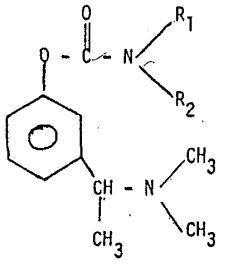
R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical

or a pharmacologically acceptable salt thereof and a physiologically acceptable carrier therefor.

2. A pharmaceutical composition having anticholinesterase activity according to claim 1 wherein R_2 is hydrogen or methyl.

3. A phenylcarbamate of the general formula I



I

wherein

R_1 is hydrogen, lower alkyl, cyclohexyl, allyl or benzyl

R_2 is hydrogen, methyl, ethyl or propyl, or

R_1 and R_2 together with the nitrogen to which they are attached form a morpholino or piperidino radical and pharmacologically acceptable salts thereof, provided that when R_2 is methyl, R_1 is neither hydrogen nor methyl.

4. N-ethyl-3-[1-(dimethylamino)ethyl]phenyl carbamate.

5. N-propyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

6. N-ethyl, N-methyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

7. N,N-diethyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

8. N-cyclohexyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

9. N-allyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

10. N-isopropyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

11. N,N-dimethyl-3[1-(dimethylamino)ethyl]phenyl carbamate.

12. 3-[1-(dimethylamino)ethyl] morpholino carbamoyl phenolate.

13. A phenyl carbamate substantially as hereinbefore described and with reference to the examples.

FOR THE APPLICANT
WOLFF, BREGMAN AND GOLLER

By: S. Goller

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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320,700

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/320,700	03/08/89	ROSIN M	4691022

RICHARD T. LAUGHLIN
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EXAMINER	
SHIPPEN, M	
ART UNIT	PAPER NUMBER
126	23

DATE MAILED: 03/07/90

NOTICE OF ALLOWABILITY

PART I.

1. ☒ This communication is responsive to 11/20/89 + 12/21/89.
2. ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
3. ☒ The allowed claims are 38-41.
4. ☐ The drawings filed on _____ are acceptable.
5. ☒ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has [☒] been received. [_] not been received. [_] been filed in parent application Serial No. _____, filed on _____.
6. ☐ Note the attached Examiner's Amendment.
7. ☐ Note the attached Examiner Interview Summary Record, PTOL-413.
8. ☐ Note the attached Examiner's Statement of Reasons for Allowance.
9. ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
10. ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

1. ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
2. ☐ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
   a. ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. _____. CORRECTION IS REQUIRED.
   b. ☐ The proposed drawing correction filed on _____ has been approved by the examiner. CORRECTION IS REQUIRED.
   c. ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
   d. ☐ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

Attachments:

- _ Examiner's Amendment
- _ Examiner Interview Summary Record, PTOL-413
- _ Reasons for Allowance
- _ Notice of References Cited, PTO-892
- _ Information Disclosure Citation, PTO-1449
- _ Notice of Informal Application, PTO-152
- _ Notice re Patent Drawings, PTO-948
- _ Listing of Bonded Draftsmen
- _ Other

Michael L. Shippen

MICHAEL L. SHIPPEN
PRIMARY EXAMINER
ART UNIT 126

PTOL-37 (REV. 11-88)

USCOMM-DC 89-3615

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NOTICE OF ALLOWANCE AND ISSUE FEE DUE

☑ Note attached communication from the Examiner
☐ This notice is issued in view of applicant's communication filed _____

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
07/320,700	03/08/89	004	SHIPPEN, M 126	03/07/90

First Named Applicant ROSIN, MARTA W.

TITLE OF INVENTION PHENYL CARBAMATES

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	4691022	514-484.000	D51	UTILITY	NO	$620.00	06/07/90

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.

THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.

HOW TO RESPOND TO THIS NOTICE:

I. Review the SMALL ENTITY Status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:
A. If the Status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
B. If the Status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:
A. Pay FEE DUE shown above, or
B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

II. Part B of this notice should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by a charge to deposit account, Part B should be completed and returned. If you are charging the ISSUE FEE to your deposit account, Part C of this notice should also be completed and returned.

III. All communications regarding this application must give series code (or filing date), serial number and batch number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees.

PTOL-85 (REV 12-88)(OMB Clearance is pending)

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15.00 501 B

PART B - ISSUE FEE TRANSMITTAL

MAILING INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE. Blocks 2 through 6 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advanced orders and notification of maintenance fees will be mailed to addressee entered in Block 1 unless you direct otherwise, by: (a) specifying a new correspondence address in Block 3 below; or (b) providing the PTO with a separate "FEE ADDRESS" for maintenance fee notifications with the payment of Issue Fee or thereafter. **See reverse for Certificate of Mailing.**

MAR 2 4 1990 PAT. & TRADEMARK OFF. MAIL ROOM

1. CORRESPONDENCE ADDRESS	2. INVENTOR(S) ADDRESS CHANGE (Complete only if there is a change)
RICHARD T. LAUGHLIN LAUGHLIN, MARKENSOHN, LAGANI & PEGG 129 HEADQUARTERS PLAZA MORRISTOWN, NJ 07960	INVENTOR'S NAME Street Address City, State and ZIP Code CO-INVENTOR'S NAME Street Address City, State and ZIP Code ☐ Check if additional changes are on reverse side

chem

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
07/320,700	03/08/89	004	SHIPPEN, M 126	03/07/90

First Named Applicant: ROSIN, MARTA W.

TITLE OF INVENTION: PHENYL CARBAMATES

	ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1	4691022	514-484.000	D51	UTILITY	NO	$620.00	06/07/90

3. Further correspondence to be mailed to the following:
RICHARD T. LAUGHLIN
RIBIS, GRAHAM, VERDON & CURTIN
4 HEADQUARTERS PLAZA, P.O. BOX 1991
MORRISTOWN, N.J. 07962

4. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed.
1 RIBIS, GRAHAM, VERDON & CURTIN
2 RICHARD T. LAUGHLIN
3

DO NOT USE THIS SPACE

130 JM 06/12/90 07320700 1 142 620.00 CK
130 JM 06/12/90 07320700 1 501 15.00 CK

5. ASSIGNMENT DATA TO BE PRINTED ON THE PATENT (print or type)
(1) NAME OF ASSIGNEE: PROTERRA AG
(2) ADDRESS: (City & State or Country) POSTSTRASSE 9, CH-6300ZUG, SWITZERLAND
(3) STATE OF INCORPORATION, IF ASSIGNEE IS A CORPORATION: SWITZERLAND

A. ☐ This application is NOT assigned.
☐ Assignment previously submitted to the Patent and Trademark Office.
☐ Assignment is being submitted under separate cover. Assignments should be directed to Box ASSIGNMENTS.

PLEASE NOTE: Unless an assignee is identified in Block 5, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

6a. The following fees are enclosed:
☒ Issue Fee ☒ Advanced Order - # of Copies 10 (Minimum of 10)

6b. The following fees should be charged to:
DEPOSIT ACCOUNT NUMBER (Enclose Part C)
☐ Issue Fee ☐ Advanced Order - # of Copies (Minimum of 10)
☐ Any Deficiencies in Enclosed Fees

The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.

(Signature of party in interest of record) (Date) MAY 31, 1990

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

TRANSMIT THIS FORM WITH FEE-CERTIFICATE OF MAILING ON REVERSE

PTOL-85B (REV 12-88)(OMB Clearance is pending)

Certificate of Mailing

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

Box ISSUE FEE
Commissioner of Patents and Trademarks
Washington, D.C. 20231

on MAY 31, 1995
(Date)

RICHARD T. LAUGHLIN
(Name of person making deposit)

(Signature)

5/31/95
(Date)

Note: If this certificate of mailing is used, it can only be used to transmit the Issue Fee. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawings, must have its own certificate of mailing.

120 JM 06\12\90 07320700 01 201 1 12.00 CK
120 JM 06\12\90 07320700 01 1H2 1 250.00 CK

This form is estimated to take 20 minutes to complete. Time will vary depending upon the needs of the individual applicant. Any comments on the amount of time you require to complete this form should be sent to the Office of Management and Organization, Patent and Trademark Office, Washington, D.C. 20231 and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, D.C. 20503.

REVERSE PTOL-85B (REV 12-94)(OMB Clearance is pending)

PTO UTILITY GRANT
Paper Number 10

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Harry F. Manbeck, Jr.

Commissioner of Patents and Trademarks

Attest Melvinia Darry

PTO-1584

FORM PTO-875 (REV 1-86) — U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE

PATENT APPLICATION FEE DETERMINATION RECORD

SERIAL NO	FILING DATE
320700	03/08/89

APPLICANT (FIRST NAMED): Rosen, Marta W. et al

1/1 for filing date only

CLAIMS AS FILED - PART I

FOR:	NO. FILED	NO. EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
BASIC FEE	[redacted]	[redacted]	[redacted]	$170	OR	[redacted]	$340
TOTAL CLAIMS	1 - 20 =	*	X6 =	$	OR	X12 =	$
INDEP. CLAIMS	1 - 3 =	*	X17 =	$	OR	X34 =	$
☐ MULTIPLE DEPENDENT CLAIM PRESENT			X85 =	$	OR	X110 =	$
			TOTAL	$	OR	TOTAL	$340

* If the difference in col. 1 is less than zero, enter "0" in col. 2

CLAIMS AS AMENDED - PART II

	(1)		(2)	(3)	SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
AMENDMENT A	[redacted]	CLAIMS REMAINING AFTER AMENDMENT	[redacted]	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA				
TOTAL	*	MINUS	**	=	$		OR	- 10 =	$
INDEP	*	MINUS	***	=	- 15 =	$		- 30 =	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					- 50 =	$		- 100 =	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

					SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
AMENDMENT B	[redacted]	CLAIMS REMAINING AFTER AMENDMENT	[redacted]	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA				
TOTAL	*	MINUS	**	=	- 8 =	$	OR	- 10 =	$
INDEP.	*	MINUS	***	=	- 15 =	$		- 30 =	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					- 50 =	$		- 100 =	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

					SMALL ENTITY RATE	SMALL ENTITY ADDIT. FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY ADDIT. FEE
AMENDMENT C	[redacted]	CLAIMS REMAINING AFTER AMENDMENT	[redacted]	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA				
TOTAL	*	MINUS	**	=	- 5 =	$	OR	10 =	$
INDEP	*	MINUS	***	=	- 15 =	$		30 =	$
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					- 50 =	$		- 100 =	$
					TOTAL ADDIT. FEE	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest No. Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest No. Previously Paid For" (Total or Indep.) is the highest number found in the appropriate box in Col. 1.

PTO-1130 (REV. 8-87)

PALM III APPLICATION FILE DATA CODING SHEET

U.S. DEPARTMENT OF COMMERCE–PATENT & TM OFFICE

PREPARED BY CHH/PMM/ DATE 05/02/89

FORMAT NO. 2 Serial No. 07/320700

TYPE APPL	FILING DATE Month	Day	Year	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWINGS	ASGT?	TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE RECEIVED	SECURITY CASE?	FOREIGN LICENSE?
2	03	08	89		126	560	0	N	1	1	0	240	N	Y

FORMAT NO. 3 ATTORNEY DOCKET NUMBER (12 spaces): 4691D22

Atty.'s Reg. Nos. ☒ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 4 Applicant's Name & Address ☒ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 5 Title of Invention ☒ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 6&7 Correspondence Address ☒ APPL. PAPERS ☐ CODING SHEET

FORMAT NO. 8

	RECORD	CONTINUITY CODE	PARENT APPLICATION SERIAL NUMBER	PARENT FILING DATE Month Day Year	STATUS CODE	PARENT PATENT NUMBER
	801	02	07/185451	04/25/88	3	
	802	12	06/835466	03/03/86	3	
	803		0			
	804		0			
	805		0			
	806		0			
	807		0			
	808		0			
☐ MORE ON SUPPLEMENTAL CODING SHEET	809		0			
	810		0			

FORMAT NO. 9

	RECORD	COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FILING DATE Month Day Year
	901	ILX	74497	05/05/85
	902			
FOREIGN PRIORITY CLAIMED? ☑ YES ☐ NO	903			
	904			
	905			
	906			
☐ APPLICATION PAPERS	907			
	908			
	909			
☐ MORE ON SUPPLEMENTAL CODING SHEET	910			

L+R1

STAPLE AREA

☆ U.S. GOVERNMENT PRINTING OFFICE: 1989-236-812

PATENT NUMBER

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
514	484

APPLICATION SERIAL NUMBER

320,700

APPLICANT'S NAME (PLEASE PRINT)

Rosin, et al.

IF REISSUE, ORIGINAL PATENT NUMBER

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
514	~~231~~	330	487	490
544	162			
546	226			
560	32	115	136	
514	237.5			

INTERNATIONAL CLASSIFICATION (INT. CL. 4)

C	07	C		125/067
A	61	K		31/27

GROUP ART UNIT: 126

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

Michael L. Shippen

PTO 270 (10-84)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

[redacted]

SEARCHED

Class	Sub.	Date	Exmr.
560	115	9/89	[illegible]
	163		
514	484		
	490		
	487		
560	136		
	update	3/90	[illegible]

SEARCH NOTES

	Date	Exmr.
SN 835,466 filed	4/89	[illegible]

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
560	115	3/90	[illegible]
	136		
514	484		
	490		
	487		

Staple Issue Slip Here

INDEX OF CLAIMS

Claim Final	Claim Original	Date
	1	3/40
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Claim Final	Claim Original	Date
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SYMBOLS

✓	Rejected
=	Allowed
- (Through Numeral)	Canceled
+	Restricted
N	Non-elected
I	Interference
A	Appeal
O	Objected

07 320700 /

Briefed Topic 21/08

APPROVED FOR LICENSE ☐

INITIALS _____

[redacted]

CONTENTS

Entered or Counted			Received or Mailed
14	A. Application papers.		Mar. 8, 1989
15	B. Letter re: Specification included		5/9/89
16	C. Petition ... Filing Date		5/24/89
17	K. Decision On Petition		7-19-89
18	L. Amdt E		July 31, 1989
19	M. Amdt G		July 31, 1989
20	N. R 3 mons.		9-26-89
21	O. Rep Letter		Nov. 20, 1989
22	P. Priority Papers		Dec. 21, 1989
23	Q. Ltr Advs		March 7, 1990
24	R. PTO GRANT AUG 1 4 1990		
25	S.		
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RECEIVED 1989 GROUP 120