Deuterium Isotope Effects in the Metabolism of Drugs and Xenobiotics: Implications for Drug Design

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1 Introduction

The majority of drugs, when administered to humans and animals, are metabolized, often rapidly and extensively (Testa and Jenner, 1976). Metabolism, which usually occurs mostly in the liver but which can also occur in numerous other organs (Fry and Bridges, 1977), e.g., kidney, lungs, skin, and small intestine, has been regarded as a defense mechanism whereby ingested xenobiotics are converted into more polar derivatives that are excreted more readily either directly or after conjugation. However, in the case of drugs, rapid metabolism may limit plasma levels and half-lives and, consequently, efficacy.

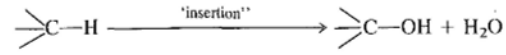
Although some drugs [e.g., cyclophosphamide (Connors *et al.*, 1974)] are activated by metabolism or may be deliberately designed as prodrugs (Bodor, 1981a, 1984), the usual consequence of metabolism is deactivation. Moreover, in addition to being more rapidly excreted, metabolites usually have an affinity for the target (receptor, enzyme, membrane, etc.) lower than that of the parent drug or may have properties which limit access to, and therefore interaction with, the target. Metabolism can also generate products which have a biological activity different from that of the parent drug or which may be toxic and, in some instances, carcinogenic (Jefcoate, 1983).

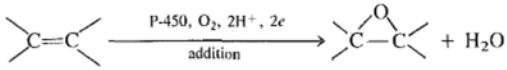
Thus, the metabolism of drugs is usually an adverse process and its importance is often indicated when candidate drugs which show high activity in *in vitro* assays are inactive *in vivo*. The metabolism-directed approach (Jarman and Foster, 1978; Bodor, 1981b, 1984) to drug design is concerned with the rational modification of molecular structure in order to control adverse metabolism and/or confer desirable characteristics. The ideal starting point for a metabolism-directed design study involves a drug in clinical or experimental use with a known target, metabolism profile, and origin of toxicity. Structure–activity studies can then be undertaken aimed at retarding or blocking adverse metabolism while retaining (preferably, increasing) affinity for the target and ensuring that a plasma level and half-life can be achieved practicably which will optimize interaction with the target.

It is in this context that specific deuterium substitution in drugs and the magnitude and consequences of the resulting deuterium isotope effects are now considered.

A wide variety of pathways of drug metabolism have been identified and categorized as phase I and II reactions (Fry and Bridges, 1977), also designated as functionalization and conjugation reactions (Testa and Jenner, 1978). The former category includes reactions whereby functional groups are introduced (e.g., hydroxylation), modified (e.g., aldehyde oxidation and reduction), or exposed (e.g., O-dealkylation) whereas the latter category includes reactions such as glucuronidation and sulfation. It is within the phase I category that metabolism pathways are found which are susceptible to deuterium isotope ef-

fects, namely, those in which a C—H bond is broken, for example, hydroxylation (C—H → C—OH) and dehydrogenation (CH—OH → C=O). Hydroxylation is a frequently encountered pathway of metabolism which is mediated by the cytochrome P-450 enzymes (mono-oxygenases, mixed function oxidases). Treatises on this category of enzyme are now available (e.g., Schenkman and Kupfer, 1982) so that only the salient relevant points need be noted here. The cytochrome P-450s are heme proteins commonly found as clusters of membrane-bound isoenzymes in the endoplasmic reticulum of many types of cell but they are particularly prevalent in liver cells. Some of these isoenzymes are inducible [e.g., by phenobarbital (PB) and methylcholanthrene], each has a characteristic substrate specificity, and each can generate from molecular oxygen an electrophilic species which will react with, and deliver to, the substrate the formal equivalent of atomic oxygen. The structure of this reactive species remains to be defined precisely but it has been termed oxenoid because its reactions are formally analogous to those of carbenes in adding to double bonds and inserting into single bonds. The overall reactions are as follows:





The so-called "oxygen insertion reaction" is by far the most important metabolism pathway considered in this article (see Section 3.4.2 for comments on mechanism).

2 Deuteration of Drugs and Xenobiotics

Deuterium labeling has found widespread application in studies of drug metabolism in such diverse aspects as identification and quantification (coupled with mass spectrometry) of drugs and metabolites in plasma, urine, and *in vitro* systems, determination of pharmacokinetics in general, and in chronic administration, bioavailability studies, mechanism of enzyme and drug action, elucidation of metabolic and biosynthetic pathways, differential metabolism of enantiomers, and isotope effects. Several reviews have appeared in the past decade dealing with deuterium labeling in the general context of applications of stable isotopes in biomedical research, pharmacology, and medicinal chemistry (Knapp and Gaffney, 1972; Knapp *et al.*, 1973; Gregg, 1974; Gaffney *et al.*, 1974; Nelson and Pohl, 1977; Halliday and Lockhart, 1978; Baillie, 1981; Baillie *et al.*, 1982; Haskins, 1982). Each review has a particular emphasis and viewpoint and all are well worth reading. The review by Blake *et al.* (1975) is devoted to

deuterium and contains an excellent survey of the literature prior to 1975 on, *inter alia*, deuterium isotope effects associated with the metabolism and biological activity of drugs and related compounds. The present article is concerned mainly with deuterium isotope effects and is not intended to be comprehensive but illustrative. Apologies are tendered herewith to those authors whose relevant work is not mentioned.

2.1 DEUTERIUM ISOTOPE EFFECTS

In a reaction (chemical or enzymatic) in which cleavage of a C—H bond is rate determining the same reaction of the C—D analog will be retarded. The ratio (K_H/K_D) of the respective rate constants defines the *primary deuterium isotope effect* (DIE). The maximum theoretical DIE has been calculated (Bigeleisen, 1949) as 18 and although values up to 11–12 have been observed experimentally in metabolism studies (see below) most observed DIEs are relatively small (<5). The origin of DIEs relates to the difference in mass between hydrogen and deuterium which results in the zero-point energy (lowest ground state vibrational level) for C—D bonds being 1.2–1.5 kcal/mol lower than that of the C—H bond with a consequent increase in bond stability. Deuterium substitution near to a reaction center can give rise to *secondary isotope effects* which are usually small (1.05–1.25) and not likely to contribute significantly to the DIEs considered in this article.

DIEs in chemical reactions were reviewed by Wiberg (1955), and Wolfsberg (1982) has given a general theoretical analysis. Northrop (1982) has presented a detailed consideration of enzyme-catalyzed reactions in terms of a family of DIEs and emphasized the fact that the observed DIE (^Dv), which relates to the rates of disappearance of substrate and/or appearance of products, can be very much smaller than the intrinsic DIE (^Dk), which is associated with the conversion of the substrate into product(s) within the activated enzyme–substrate(product) complex. The mechanisms of action of various enzymes which can be involved in drug metabolism studies have been clarified on the basis of DIEs, e.g., aldehyde dehydrogenase (Feldman and Weiner, 1972), xanthine oxidase (Edmondson *et al.*, 1973), urocanase (Egan *et al.*, 1981), liver alcohol dehydrogenase (Cook and Cleland, 1981), and dopamine β-monooxygenase (Miller and Klinman, 1982). Deuterium labeling has also been used elegantly to probe the steric requirements of drug–receptor interaction of neuromuscular blocking agents in the norcoralydine series (Stenlake and Dhar, 1978). However, this article is concerned primarily with "observed" DIEs, frequently expressed as K_H/K_D or V^H_{max}/V^D_{max} [^Dv in Northrop's (1982) terminology], which reflect the gross effect of deuterium substitution on the rate and pathways of metabolism of drugs and xenobiotics and on their biological activity. Unless stated otherwise in the sections below, the term DIE connotes the observed deuterium isotope effect.

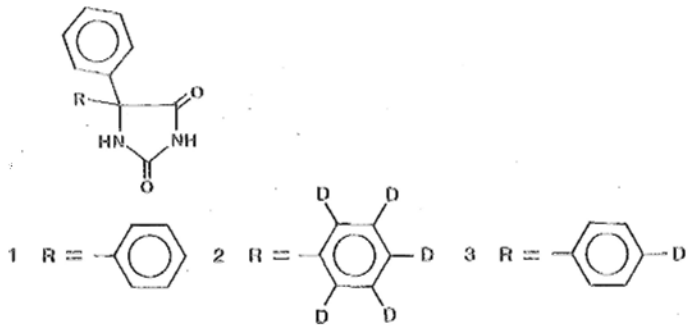
2.2 CONTROL OF METABOLISM BY DEUTERIUM SUBSTITUTION

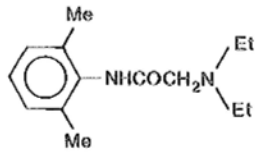
For a DIE of 2 the rate of reaction of the C—D-containing compound will be 50% of that of the C—H analog and for DIEs of >5 the introduction of deuterium will suppress reactivity very substantially. Since DIEs in the range 6–12 associated with metabolic reactions have been reported (see below), interest has been stimulated in the use of this phenomenon to retard certain drug metabolism pathways and to explore the consequences *in vivo*. The attraction of specific deuterium substitution as a parameter in drug design is based on the facts that not only is the replacement of one or a few hydrogens in a drug molecule by deuterium the smallest structural change that can be made but also such a change will have negligible steric consequences or influence on physicochemical properties (providing that the deuterium is not α to nitrogen, see Section 5.1). This is in contrast to the use of groups such as alkyl or fluorine to block metabolism at a particular point in a drug molecule. The introduction of an alkyl group may create new possibilities for metabolism and significantly change lipophilicity and the introduction of fluorine may markedly modify the character of neighboring functional groups or remote ones if there is an intervening conjugated or aromatic system.

Cytochrome P-450-mediated aromatic hydroxylation usually involves initial oxene addition to give an epoxide (arene oxide) which, *inter alia*, can rearrange into a phenol. Although for deuterated aromatic compounds deuterium migration occurs (NIH shift; Daly *et al.*, 1968, 1969), the DIE is negligible for the overall hydroxylation process (e.g., Farmer *et al.*, 1975) when hydroxyl groups are introduced into the o- and p-positions in substituted aromatics. It was inferred that a different mechanism operates for m-hydroxylation *in vitro* and *in vivo* and for which DIEs of 1.3–1.75 have been observed (Tomaszewski *et al.*, 1975).

The biological activity of xenobiotics can sometimes be modified by polydeuteration (see review by Blake *et al.*, 1975) but a remarkable effect of monodeuteration has been reported by Dumont *et al.* (1981). The anticonvulsant potency of diphenylhydantoin (**1**) was enhanced by pentadeuteration of one phenyl group (→ **2**) and even more so by p-deuteration (→**3**). The mechanistic significance of these findings is not clear. p-Hydroxylation of one phenyl group is the main initial metabolism pathway for (**1**) and Hoskins and Farmer (1982) found no significant DIE for p- and m-hydroxylation of d_5-diphenylhydantoin (**2**) by liver microsomes (PB-induced rats) or in humans. Moreover, Moustafa *et al.* (1983) concluded that m- and p-hydroxylation of diphenylhydantoin (**1**) proceeded via the 3,4-epoxide. These findings contrast with those of Tomaszewski *et al.* (1975) noted above and suggest that m-hydroxylation could involve a duality of mechanisms.

Progressive replacement of hydrogen in a drug or another xenobiotic molecule with deuterium will progressively change the lipophilicity and the magnitude of





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this effect can be conveniently assessed by normal (Farmer *et al.*, 1978) and reversed-phase high-pressure liquid chromatography (HPLC) (Tanaka and Thornton, 1976). The shake-flask and HPLC methods were recently compared (El Tayar *et al.*, 1984). The results indicated deuterated compounds to be less lipophilic than the corresponding protium forms by ~0.006/D on the log P_{oct} scale. The effect of deuteration on binding, for example, to microsomal cytochrome P-450, is usually given by the ratio of the Michaelis constants K_m^D/K_m^H. When this ratio is <1 (see Section 5.1), stronger binding of the deuterated compound to the enzyme is indicated. Amines are an exception in that deuteration at the α-carbon will give a K_m^D/K_m^H ratio of >1. For example, deuteration of the NEt_2 moiety of lidocaine (**4**) results (Nelson *et al.*, 1975) in a K_m^D/K_m^H ratio of 1.23 (for rat liver microsomes) for the $N(CD_2CH_3)_2$ analog in contrast to a ratio of 0.92 for the $N(CH_2CD_3)_2$ analog.

2.2.1 *Metabolic Switching*

When a drug is metabolized by two or more alternative pathways a possible consequence of deuteration is "metabolic switching." This term was introduced by Horning *et al.* (1976), who found that the metabolism of antipyrine (**5**), after intraperitoneal (ip) injection into rats and as reflected by the urinary metabolites, was switched from oxidation of the C-3-methyl group (normal major pathway) to N-demethylation (normal minor pathway) on trideuteration of the former group.

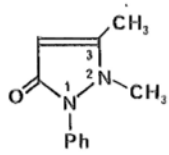
The effect was even more marked *in vitro*. Using the 10,000 *g* supernatant of homogenized rat liver, the ratio of 3-hydroxymethylantipyrine to 4-hydroxyan-

tipyrine from antipyrine was 1.3 and 1.6 when the N-methyl group was trideuterated. However, the ratio changed dramatically to <0.1 when the C-3-methyl group was trideuterated. This low ratio corresponded to a DIE of ~15.

A similar situation was encountered by Gorumaru *et al.* (1981) for the metabolism of aminopyrine (**6**) administered orally to rats. Analysis of the urinary metabolites revealed that trideuteration of the C-3-methyl group switched metabolism to N-demethylation of the C-4-dimethylamino group. No metabolic switching occurred when the N-2-methyl group or the C-4-dimethylamino group was fully deuterated.

Horning *et al.* (1978) showed that for methsuximide (**7**), N-demethylation was suppressed and hydroxylation of the phenyl group was increased when the N-methyl group was trideuterated.

In studies with caffeine (**8**), Horning *et al.* (1976) found that trideuteration of the N-1-methyl group depressed N-demethylation at N-1 and, for the rat and ip administration, 1,3-dimethylxanthine (theophylline) became the major urinary metabolite. Likewise, trideuteration of the N-7-methyl group resulted in 1,7-dimethylxanthine being the major urinary metabolite. The same group (Horning *et al.*, 1979) also found that after ip injection into rats the plasma half-lives of caffeine (**8**) and its derivatives with the N-1-, N-7-, or N-9-methyl groups trideuterated were similar. However, the plasma half-life of the derivative with all these N-methyl groups trideuterated was twice that of caffeine (**8**). These results were taken to indicate that N-demethylation at positions 1, 7, and 9 occurred at the same rate *in vivo* and that replacement of CH_3 by CD_3 switches metabolism to de-N-methylation of an unlabeled methyl group.



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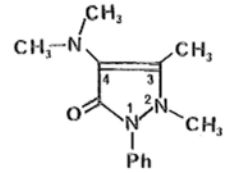

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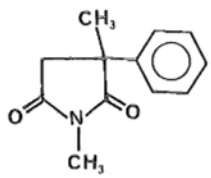

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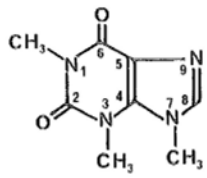

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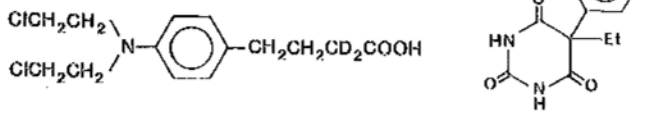
The foregoing results variously illustrate metabolic switching of three types, namely C→N, N→C, and N→N. An example of C→C switching associated with 7-ethylcoumarin is noted in Section 4.1.

The possibility exists, although apparently not yet realized, of using metabolic switching in drug design to deflect metabolism away from a pathway yielding a toxic metabolite to one (or more) leading to innocuous products or away from a pathway leading to inactive metabolites toward one yielding an active metabolite.

2.2.2 D/H Exchange

In *in vitro* and *in vivo* studies of the metabolism of deuterated drugs and other xenobiotics it is essential that the deuterium content of unchanged drug and its metabolites be monitored by mass spectrometry if other techniques are used for quantification. This precaution is essential in order to ensure that D/H exchange does not occur. Where enzymes, receptors, or other macromolecules are involved there is always the possibility of microenvironments in which D/H exchange can be promoted. Thus, following ip administration of α-d_2-chlorambucil (**9**) to rats, monitoring of the drug in the plasma by mass spectrometry revealed that D/H exchange was complete within 30 min even though chemically the deuterium was not intrinsically labile (Farmer *et al.*, 1979). Perel *et al.* (1967) found that, after administration of *p*-deuterophenobarbital (**10**) to dogs, the drug excreted in the urine had undergone 13–26% D/H exchange. Singer and Lijinsky (1979) have noted that, for nitrosamines deuterated α to nitrogen, pronounced biological isotope effects in feeding experiments (see Section 5.4) were observed only for those compounds which were not very susceptible to base-catalyzed D/H exchange. It was not practicable to monitor D/H exchange *in vivo* for these deuterated nitrosamines.



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3 Hydroxylation of Hydrocarbons and Hydrocarbon Moieties

3.1 ALIPHATIC COMPOUNDS

3.1.1 Hydrocarbons

The outcome of microsomal hydroxylation of linear, saturated aliphatic hydrocarbons depends on the chain length and the inducer used. For the homologous

series $CH_3(CH_2)_nCH_3$ when $n = 1$ or 2, two monohydroxy derivatives are possible, three when $n = 3$ or 4, four when $n = 5$ or 6, etc. Also, for hydroxylation at some secondary positions, the possibility of stereoselectivity exists and D- and/or L-alcohols can be formed. The regioselectivity associated with microsomal hydroxylation is illustrated by the results for *n*-hexane ($n = 4$) and *n*-heptane ($n = 5$).

Using liver microsomes (PB-treated rats) the ratio of 1- (ω), 2- (ω−1), and 3-hexanols (ω−2) from *n*-hexane was ~1:11:2 and diols were also formed (1,2, 1,3, and 2,3; ratio ~1.6:0.3:0.7) (Kramer *et al.*, 1974). The ratio of the three hexanols was not changed dramatically when noninduced microsomes were used. Under essentially similar conditions the ratio of 1- (ω), 2- (ω−1), 3- (ω−2), and 4-heptanols (ω−3) from *n*-heptane was ~1:19.5:3.7:1.5 (Frommer *et al.*, 1972). The relative proportions of the four heptanols was not greatly changed when noninduced microsomes were used but with benzpyrene-induced microsomes the ratio became ~1:16.5:13.8:21.4. Thus, for non- and PB-induced microsomes (ω−1)-hydroxylation of linear, saturated aliphatic hydrocarbons preponderates.

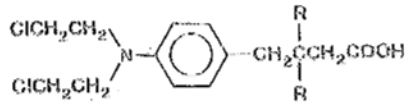
Although the microsomal hydroxylation of cyclohexane and cyclohexane-d_{12} has been studied (see Section 3.4.1) apparently there has been no comparable investigation of linear aliphatic hydrocarbons.

3.1.2 Fatty Acids

The regioselectivity of microsomal hydroxylation of saturated linear fatty acids is dependent on chain length. Thus, for decanoic acid, $CH_3(CH_2)_8COOH$ (Hamberg and Björkhem, 1971), the ratio of 10- (ω) and 9-hydroxylation (ω−1) was >9:<1. Metabolism of the 10-d_3 and 9-d_2 derivatives of decanoic acid revealed a DIE (1.5–2 based on yields of products) only for 9-hydroxylation. The ratio of the D- and L-forms of 9-hydroxydecanoic acid was ~1:3 and this was changed to ~2:1 when 9-d_2-decanoic acid was hydroxylated.

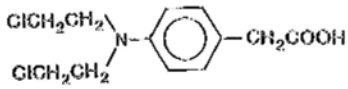
A somewhat different situation was encountered with lauric acid, $CH_3(CH_2)_{10}COOH$ (Björkhem and Hamberg, 1972). The ratio of microsomal 12- (ω) and 11-hydroxylation (ω−1) was ~3:2 but for 11-d_2-lauric acid this ratio changed to >9:<1, reflecting a significant DIE (~2.5 based on yields of products). The ratio of D- and L-forms of the 11-hydroxy derivative was ~3:2, which, apparently, was not affected by deuteration at position 11.

The antitumor alkylating agent chlorambucil (**11**) is metabolized *in vivo* to give, *inter alia,* phenylacetic mustard (**12**) presumably via β-oxidation. This metabolism pathway is probably adverse since the therapeutic index of the metabolite (**12**) is inferior to that of the parent drug (**11**) against, for example, the Walker 256 carcinoma in rats. Moreover, the neurotoxicity associated with high doses of chlorambucil (**11**) could be due to the formation of (**12**). Following ip administration of β-d_2-chlorambucil (**13**) to rats the plasma levels of phe-



11 R = H

13 R = D



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nylacetic mustard (**12**) were lower than those from the parent drug but the therapeutic index was not altered significantly (Farmer *et al.*, 1979).

Reinsch *et al.* (1980) have reported a remarkably high DIE for the reaction of perdeuterobutyryl-CoA with fatty acyl-CoA dehydrogenase:

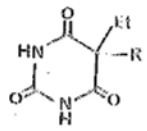
$$RCH_2CH_2COSCoA \rightleftharpoons RCH_2CHCOSCoA \rightleftharpoons RCH{=}CHCOSCoA$$

A DIE of 2 was found for the first step (H^+ abstraction) and a value of 30–50 was found for the second step.

3.1.3 Barbiturates

The effect of specific deuteration of the *n*-butyl group in 5-*n*-butyl-5-ethylbarbituric acid (**14,** butethal) has been explored. Soboren *et al.* (1965) observed that dideuteration at position 3 (→**15**) doubled the sleep time of mice whereas trideuteration at position 4 (→**16**) had no effect. That the modified behavior of **15** reflected a DIE was suggested by the identification of the 3-hydroxy derivative **17** as a microsomal metabolite of butethal. The same group (Tanabe *et al.*, 1969) showed later that dideuteration at position 3 in butethal (→**15**) increased the half-life from 100 to 270 min on incubation with the postmitochondrial supernatant of homogenized liver. They confirmed the 3-hydroxy derivative **17** to be the major metabolite and noted a DIE of ~1.6. Similar results were reported by Mark *et al.* (1971) for 5-ethyl-5-(1-methylbutyl)barbituric acid (**18,** pentobarbital). Thus, dideuteration at position 3 (→**19**) virtually doubled the plasma half-life on administration iv to dogs or ip to mice and delayed the time to peak sedation but prolonged the total sleep time.

The major metabolic route for 5-ethyl-5-phenylbarbituric acid (**20,** phenobarbital) is p-hydroxylation of the phenyl moiety. As would now be expected (Tomaszewski *et al.*, 1975), no DIE was found (Perel *et al.*, 1967) when the p-deutero derivative **21** was administered iv to dogs and rats.



14 R = $CH_2CH_2CH_2CH_3$

15 R = $CH_2CH_2CD_2CH_3$

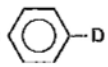
16 R = $CH_2CH_2CH_2CD_3$

17 R = $CH_2CH_2CH(OH)CH_3$

18 R = $CHMeCH_2CH_2CH_3$

19 R = $CHMeCH_2CD_2CH_3$

20 R = 

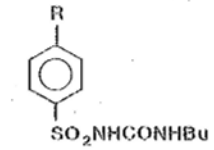
21 R = 

3.2 ARALKYL COMPOUNDS

Unlike O-methyl groups (Section 4.1) the metabolism of aryl methyl groups, presumably via initial hydroxylation, does not appear to be subject to a significant DIE. Thus, for the antidiabetic drug tolbutamide (**22**), the rate of urinary excretion of the acid **23** in humans was not affected by trideuteration of the methyl group (Lemieux *et al.*, 1961) nor was the hypoglycemic activity in male rats (Kimbrough, 1972). In the rat and the rabbit the hydroxymethyl derivative **24** was the major metabolite which was also formed on metabolism with liver microsomes (PB-treated rats) and a small DIE (1.14) was found for the *in vitro* metabolism (Tagg *et al.*, 1967).

McMahon *et al.* (1969) reported an elegant series of metabolism experiments with ethylbenzene (**25**). With liver microsomes (noninduced rats) **25** was hydroxylated to give both enantiomers of 1-phenylethanol (**26**) with an R(+)/S(−) ratio of ~4:1. When microsomes from PB-treated rats were used the stereoselectivity of the hydroxylation was substantially reduced. Using noninduced microsomes a DIE of 1.8 was found on dideuteration of the α-methylene group of **25** but there was no change in the R(+)/S(−) ratio for the metabolite **26.** Metabolism of *S*(+)-α-d_1-ethylbenzene gave ~92% of *R*(+)-1-phenylethanol with 86% retention of the deuterium.

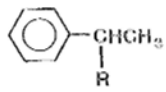
In exploring the mode of action of chloramphenicol (**27**) Kutter and Machleidt (1971) prepared the deutero-α-D-threo derivative **28** and found it to have ~80% of the growth-inhibitory activity of the protium form **27** against *Escherichia coli*.



22 R = CH_3

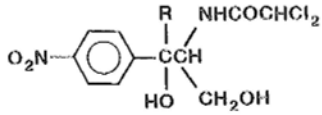
23 R = COOH

24 R = CH_2OH



25 R = H

26 R = OH

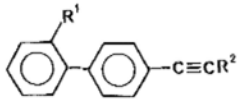


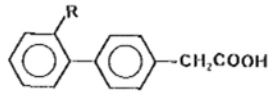
27 R = H

28 R = D

It was concluded that metabolism at the benzylic center in chloramphenicol (**27**) was involved in the expression of biological activity. A DIE of 1.4 for d_1-chloramphenicol (**28**) has been noted (Kutter and Garett, 1970).

Metabolism of 4-ethynylbiphenyl (**29**) and its 2′-fluoro derivative (**30**) with a 9000 *g* supernatant of rat liver homogenate gave the respective phenylacetic acid derivatives **31** and **32.** Although deuteration of the ethynyl group (→**33** and **34**) resulted in DIEs of 1.42 and 1.95, respectively, with almost complete retention





29 $R^1 = R^2 = H$

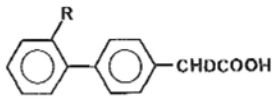
30 $R^1 = F, R^2 = H$

33 $R^1 = H, R^2 = D$

34 $R^1 = F, R^2 = D$

31 R = H

32 R = F



35 R = H

36 R = F

of deuterium in the products (35 and 36) it was not possible to distinguish between the two alternative mechanisms, namely, epoxidation of the triple bond or formation of an ethynyl alcohol (McMahon *et al.*, 1981). Each of these products could rearrange via a 1,2-deuterotropic migration with retention of deuterium to give a ketene (R—CD=C=O) which, on reaction with water, would yield a phenylacetic acid derivative. That prototropic and not biphenyl migration occurs was established by Ortiz de Montellano and Kunze (1981), who showed that $R^{13}CH_2COOH$ was the product formed on microsomal metabolism of [^{13}C]ethynylbiphenyl ($R^{13}C{\equiv}CH$).

3.3 CONTROL OF DRUG LIPOPHILICITY

The variation of the lipophilicity of drugs by variation of the size and branching of alkyl groups attached thereto is a classic maneuver in structure–activity studies associated with drug design. However, as noted above, such groups may be hydroxylated *in vivo* and consequently the efficacy of the drug may be impaired. The DIEs encountered in aliphatic hydroxylation indicate that control of this metabolism pathway *in vivo* is not likely to be achieved effectively by selective or general deuteration.

An alternative approach worth considering is the use of polyfluorinated alkyl groups, e.g., $CF_3(CF_2)_nCH_2$-, where the point of attachment to the drug is not to nitrogen either directly or to a position which is conjugated to nitrogen. Alkyl halides and sulfonates of the type $CF_3(CF_2)_nCH_2X$ are alkylating agents which are readily available via reduction of the corresponding perfluorinated carboxylic acids and the methylene group in $CF_3(CF_2)_nCH_2$- substituents would be expected to be resistant to metabolic attack (see Section 4.1.1), as would perfluorinated alkyl groups. Thus, although perfluorohexane was found to bind to cytochrome P-450 it was not metabolized (Ullrich and Diehl, 1971). Perfluorooctanoic acid, when administered by gavage to rats, was rapidly absorbed but not metabolized (Ophang and Singer, 1980). Moreover, the contribution of such groups to the lipophilicity of a molecule can be calculated readily (Hansch and Leo, 1979). The influence of a CF_3CF_2 group is exemplified by the fact that metabolism of 1,1,1,2,2-pentafluorohexane with liver microsomes (PB-induced rats) gave only the 5-hydroxy derivative, hydroxylation at positions 3 and 4 being completely inhibited (Baker *et al.*, 1984) [cf. the behavior of hexane on hydroxylation (Section 3.4.1)]. Although $CF_3(CF_2)_nCH_2$- groups attached to amino-nitrogen will also be markedly resistant to metabolic N-dealkylation, the basicity of the amine will be greatly reduced (Reifenrath *et al.*, 1980).

3.4 ALICYCLIC COMPOUNDS

3.4.1 Cyclohexane and Its Derivatives

Cyclohexane is rapidly metabolized to a single product, cyclohexanol, by liver microsomes (PB-treated rats) but no DIE was observed for cyclohexane-d_{12}

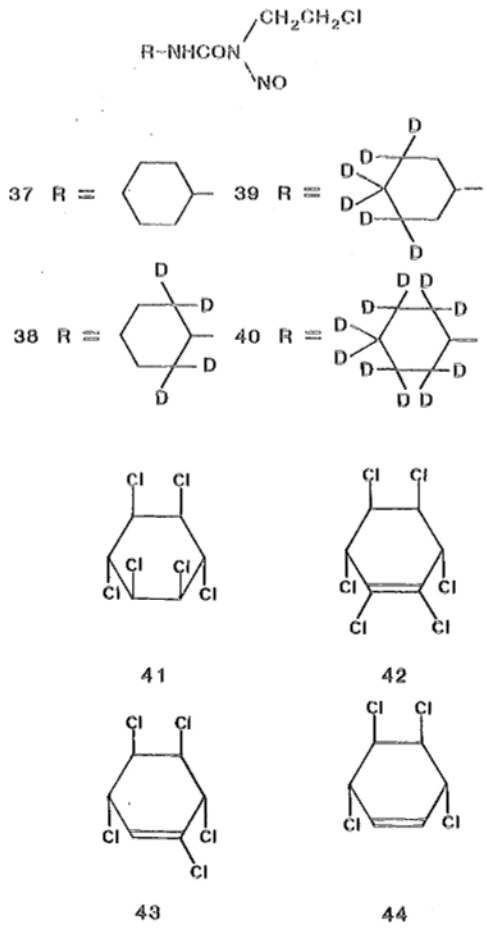
(Ullrich, 1969). The cyclohexyl moiety of the antitumor drug CCNU [**37**, 1-(2-chloroethyl) 3-cyclohexyl-1-nitrosourea] is also rapidly hydroxylated by rat liver microsomes (May *et al.*, 1975) and a small DIE (~1.7) was observed (Farmer *et al.*, 1978) for CCNU-d_{10} (**40**). Metabolic switching of trans-2-hydroxylation of CCNU (a minor metabolism pathway accounting for ~2.3% of the total hydroxylated metabolites) was found for partially deuterated derivatives, namely, away from position 2 in CCNU-d_4 (**38**, ~0.2% of trans-2-hydroxy) and toward position 2 in CCNU-d_6 (**39**, ~17% trans-2-hydroxy). However, there was no significant difference in the activities of CCNU and its d_4- (**38**), d_6- (**39**), and d_{10}- (**40**) derivatives against the TLX-5 lymphoma in mice.

A DIE of ~6.8 was found (Tanaka *et al.*, 1976; see also Portig *et al.*, 1979) for the insecticide lindane (**41**, 1*α*/2*α*/3*β*/4*α*/5*α*/6*β*-hexachlorocyclohexane, "γ-benzenehexachloride") and lindane-d_6 on metabolism by the 105,000 *g* supernatant of homogenized houseflies. The intrinsic activities of lindane and lindane-d_6 were similar but the *in vivo* toxicity of the latter compound was higher because of the slower rate of metabolism.

The metabolism of lindane involves dehydrogenation, dehydrochlorination, and dechlorination, and DIEs would be expected for the first two pathways. Kurihara *et al.* (1980) concluded that, on aerobic metabolism of a 1:1 mixture of lindane (**41**) and lindane-d_6 by rat liver microsomes, the dehydrogenation (**41**→**42**) and dehydrochlorination (**41**→**44**) pathways were associated with DIEs of 10 and ~2.3, respectively. Also, for the microsomal metabolism of the dehydrochlorination product **43**-d_5 the DIEs associated with the disappearance of substrate and appearance of 2,4,6-trichlorophenol were ~5.1 and ~6.7, respectively.

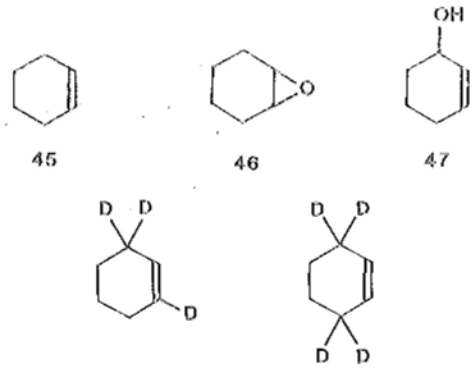
Somewhat lower DIEs were observed *in vivo*. The rat urinary metabolites of lindane are mainly conjugates, namely, mercapturic acids formed by the reaction of glutathione with the first-formed metabolites. Thus, **42, 43,** and **44** (formed by dechlorination of lindane) give rise to tri-, di-, and monochlorophenylmercapturic acids, respectively. Following ip injection of a 1:1 mixture of lindane and lindane-d_6 into rats 5- to 10-fold more of the latter was excreted unchanged and the DIEs associated with the excreted tri- (2,4,5 and 2,3,5), di- (2,4, 2,5, and 3,4), and monochlorophenylmercapturic acids were ~2.7, 2.4–3.5, and ~1.3, respectively.

The above results illustrate the well-known susceptibility to metabolic attack of hydrogen geminal to one or more chlorine substituents (Anders, 1982) and there are now several examples (Burke *et al.*, 1980; Teitelbaum *et al.*, 1981; Marcotte and Robinson, 1982) where fluorine is the geminal substituent although, apparently, no DIE studies have been reported for the latter category. However, it is becoming clear that fluorine substituents can markedly reduce the susceptibility of a vicinal C—H bond to metabolic attack. Thus, in the metabolism of 1,1-difluorocyclohexane with liver microsomes (PB-treated rats) (Baker



et al., 1984) virtually no hydroxylation occurred at position 2 and the rates of 3- and 4-hydroxylation were in the ratio 1:~5.5.

Cyclohexene (**45**) was metabolized by a fully reconstituted cytochrome P-450 system from rabbit liver by alternative pathways to give cyclohexene oxide (**46**) and cyclohex-1-en-3-ol (**47**) in the ratio 1:1.1 (Groves *et al.*, 1980). The ratio changed slightly (→1.5:1) for the d_3-cyclohexene **48** but substantially (→4.8:1) for the d_4-cyclohexene **49.** The relatively large DIE (4.9) calculated for cyclohexene hydroxylation probably reflects metabolic switching (Section 2.2.1). By analogy with aromatic compounds (Tomaszewski *et al.*, 1975) no DIE would be expected for epoxidation of the d_3-cyclohexene **48.**



45 46 47

48 49

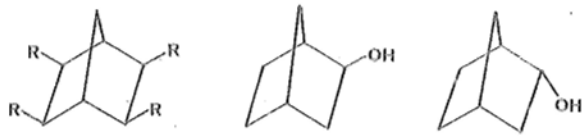
3.4.2 Norbornane and Camphor

Studies of the microsomal metabolism of these hydrocarbons and appropriate deuterated derivatives have helped to clarify the mechanism of hydroxylation mediated by cytochrome P-450.

Using purified rabbit liver microsomal P-450 (LM2, PB induction), Groves *et al.* (1978) showed that, whereas norbornane (**50**) gave a mixture of *exo*- (**52**) and *endo*-2-borneol (**53**) in the ratio 3.4:1, the ratio of alcohols from the exo-d_4-derivative **51** was 0.76:1. The overall yield of alcohols from **50** and **51** and the rates of hydroxylation were similar. Moreover, there was 25% retention of deuterium at the hydroxylated carbon in the exo-alcohol and 91% retention in the endo-alcohol, indicating a significant amount of epimerization during hydroxylation. The DIE for exo-hydrogen abstraction was 11.5 ±1. These findings are indicative of a hydrogen abstraction process giving a carbon radical intermediate. A much larger DIE would be expected in a reaction sequence where C—H bond cleavage is complete before hydroxylation occurs (Miwa *et al.*, 1980) than where an oxenoid species is inserted into a C—H bond and a three-center transition state is involved.

The norbornyl ring system is present in the antihypertensive drug tripamide (**54**) and among the urinary metabolites present after oral administration of the drug to the rat (Horie *et al.*, 1981) was the 8-hydroxy derivative subsequently (Horie *et al.*, 1983) assigned the exo-configuration **55.** However, the DIE (2) found for 8-exo-hydroxylation of the exo-d_2 derivative **56** by rat liver microsomes was much lower than that noted above for norbornane.

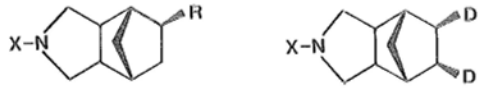
The hydrogen abstraction hypothesis received further support from the results of Gelb *et al.* (1982) for the hydroxylation of camphor (**57**) mediated by cytochrome P-450 from the soil bacterium, *Pseudomonas putida*. This cytochrome P-450 is similar to adrenal mitochondrial steroid 11β-hydroxylase.



50 R = H
51 R = D

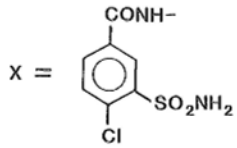
52 (exo)

53 (endo)

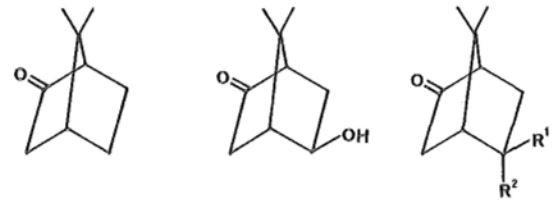


54 R = H
55 R = OH

56



5-*exo*-Hydroxycamphor (**58**) was the sole product and only shall DIEs (^{D}v <1.25) were observed for the hydroxylation of 5-*exo*-d_1- (**59**) and 5-*endo*-d_1-camphor (**60**). The D/H ratios for 5-*exo*-hydroxycamphor from these deuterated



57

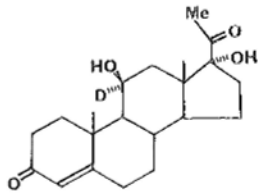
58

59 R^1 = D, R^2 = H
60 R^1 = H, R^2 = D

derivatives were 1.18 and 4.39, respectively, which reflected the preference of the enzyme for 5-exo-H(D) abstraction and also epimerization. It was concluded that, although 5-*exo*-hydroxycamphor was the sole product, both exo- and endo-H(D)-abstraction could occur and that the H(D)-abstracting and oxygen-donating species had different identities. A substantial but masked intramolecular (intrinsic) DIE (^{D}k) was found which was considered to be consistent with a reversible H(D)-abstraction step and/or a highly nonsymmetric transition state.

3.4.3 Steroids

The effect of deuteration on steroid hydroxylations has been widely investigated. In an early study Bollinger and Wendler (1959) found that 11α-deuteration of hydrocortisone (→**61**) reduced the potency in oral glycogen deposition tests in mice and enhanced the potency in the oral systemic granuloma assay in rats. It was concluded that oxidation–reduction at position 11 was involved in determining pharmacological properties. Likewise (Ringold *et al.*, 1961), oxidation to the 3-keto form was considered important for the expression of the androgenicity of 17α-methyl-5α-androstane-3β,17β-diol since 3α-deuteration (→**62**) reduced the androgenic potency in castrated rats.



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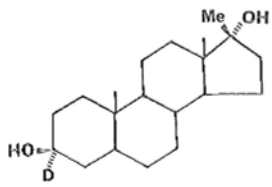

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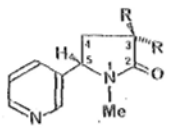
Björkhem (1971), using rat liver microsomes, found no DIE for 7α-hydroxylation of cholesterol and cholestanol and no tritium IE for 6β-hydroxylation of taurochenodeoxycholic acid but a tritium IE of 3.8 was found for 7α-hydroxylation of taurodeoxycholic acid. In further reports (Björkhem, 1972, 1975) no significant DIEs were found for microsomal 6β-hydroxylation of testosterone, 26-hydroxylation of 5β-cholestane-3α,7α,12α-triol, and 12α-hydroxylation of 7α-hydroxy-4-cholestene-3-one but DIEs of 3–4 were found for 16α-hydroxylation of pregnenolone and 24-hydroxylation of 5β-cholestane-3α,7α,12α-triol.

The variation in the magnitude of the DIEs associated with steroid C-hydroxylations is striking and although explicable in terms of current understanding of the mechanism of enzyme reactions (Northrop, 1982) they are certainly not as yet predictable (Björkhem, 1977).

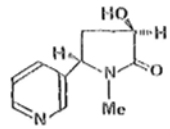
3.4.4 Cotinine

Following iv administration of cotinine (**63**) to the rhesus monkey the metabolites which appear in the urine reflect pyridine N-oxidation, N-demethylation, and 5- and trans-3-hydroxylation (→**64**). When 3-d_2-cotinine (**65**) was administered a DIE of 6.2 was found for the trans-3-hydroxylation pathway (Dagne *et al.*, 1974).



63 R = H

65 R = D



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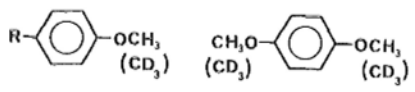
4 Hydroxylation of Carbon α to Oxygen

4.1 O-DEALKYLATION

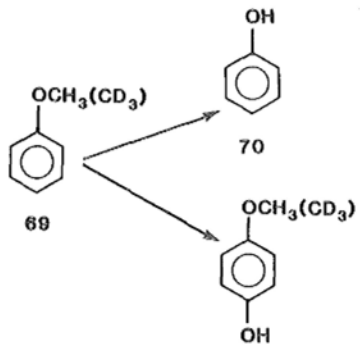
Mitoma *et al.* (1967) were the first to describe a DIE associated with O-demethylation. Using liver microsomes (PB-treated rabbits) they found a DIE of ~2 for *p*-nitroanisole (**66**). They also noted K_m^D/K_m^H to be 0.4, which indicated stronger binding of the deuterated compound to the microsomal protein. DIEs of ~2 were found (Foster *et al.*, 1974) and K_m^D/K_m^H values of <1 for the O-demethylation of *p*-methoxyacetanilide (**67**) and *p*-dimethoxybenzene (**68**) with liver microsomes (PB-treated rats).



66 R = NO_2

67 R = AcNH

68



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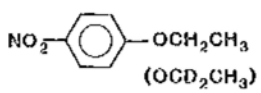
71

The metabolism of anisole (**69**) by rat liver microsomes involves the alternative pathways demethylation (→phenol, **70**) and p-hydroxylation (→4-methoxyphenol, **71**). By comparing the ratio of the yield of phenol to that of 4-methoxyphenol on metabolism of anisole and its CD_3O analog a DIE of ~7.5 was obtained (Lindsay Smith *et al.*, 1982). This relatively large DIE reflects metabolic switching since it was subsequently reported (Lindsay Smith and Sleath, 1983) that trideuteration of the methoxyl group depressed demethylation and enhanced p-hydroxylation although the combined yield of phenol (**70**) and 4-methoxyphenol (**71**) was not affected by the deuteration.

A DIE of 2.7–3.0 was reported by Al-Gailany *et al.* (1975) for the microsomal (PB-treated rats) O-deethylation of *p*-nitrophenetole (**72**). These workers also reported type I binding of **72** to cytochrome P-450.

Garland *et al.* (1976) metabolized phenacetin (**73**, *p*-ethoxyacetanilide) with noninduced rabbit liver microsomes and found a DIE of ~1.6 for the O-deethylation of **73**-α-d_2 but virtually none (~1.03) for **73**-β-d_3, reflecting a very weak secondary isotope effect. The K_m values for **73** and **73**-α-d_2 were closely similar and significantly higher than that for **73**-β-d_3. However, using a perfused rat liver system, Pang *et al.* (1982) found no marked difference in the rate of formation (by O-deethylation) of acetaminophen from phenacetin (**73**) and α-d_2,β-d_3-phenacetin injected simultaneously.

Using purified cytochromes P-450 and P-448 from liver microsomes of PB- and methylcholanthrene-induced rats, respectively, DIEs of ~3.8 and ~2 were obtained (Lu *et al.*, 1984) for the O-deethylation of α-d_2-7-ethylcoumarin (**74**) and the formation of some 6-hydroxy derivative **75** reflected metabolic switching. A DIE of ~6 was found for the O-deethylation of **74** with human liver microsomes. Apparently, this is the first example of the use of human liver microsomes in the study of DIEs and the finding of such a relatively high DIE is of potential importance. In evaluating the scope for using a DIE to influence the



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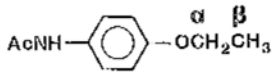

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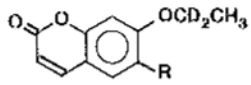


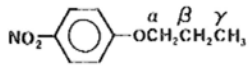
74 R = H

75 R = OH

metabolism of a drug in humans, liver microsomes from a human source should be used since DIEs associated with animal liver microsomes may be misleadingly low.

4.2 HYDROXYLATION OF O-ALKYL GROUPS

The effect of deuterium substitution on hydroxylation at β- and γ-positions in O-alkyl groups has been described by Mitoma *et al.* (1971). *p*-Nitrophenyl propyl ether (**76**) was metabolized by liver microsomes from (PB-treated) rats to give *p*-nitrophenol (**77**), 2-hydroxyl-1-propyl *p*-nitrophenyl ether (**78**) and 3-(*p*-nitrophenoxy)propionic acid (**79**), reflecting α-, β-, and γ-hydroxylation, respectively. The yields were always in the sequence **77**>**78**>**79** but the ratios varied quite markedly with each microsomal preparation. Deuteration severally at the α-, β-, and γ-positions in the propoxy group of **76** markedly suppressed metabolism at the deuterated site. DIEs were not reported but the effect of deuteration was expressed as the ratio of the yields of the appropriate metabolite from the protium and particular deuterium form. Thus, the ratio for the phenol **77** from **76** and **76**-α-d_2 was 0.06 (α-hydroxylation), that for the alcohol **78** from **76** and **76**-β-d_2 was 0.22 (β-hydroxylation), and that for the acid **79** from **76** and **76**-γ-d_3 was 0.24 (initial γ-hydroxylation followed by oxidation).



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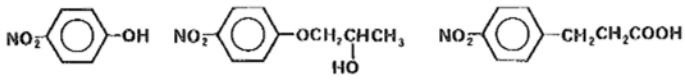
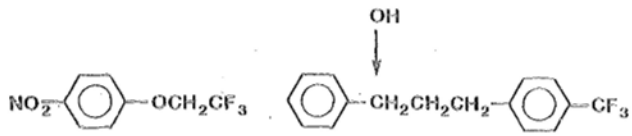


77 **78** **79**

4.3 CONTROL OF METABOLIC O-DEALKYLATION

An approach alternative to α-deuteration for blocking metabolic O-dealkylation is β-fluorination. Thus, under conditions where *p*-nitroanisole (**66**) and *p*-nitrophenetole (**72**) underwent rapid microsomally mediated O-demethylation, *p*-(2,2,2-trifluoroethoxy)nitrobenzene (**80**) was virtually unaffected (Baker *et al.*, 1984). The relay of this type of deactivating effect of a trifluoromethyl group through a benzene ring is nicely illustrated by the observation (Hjelmeland *et al.*, 1977b) that microsomally mediated benzylic hydroxylation of 1-phenyl-3-(*p*-trifluoromethylphenyl)propane (**81**) occurs mainly (>95%) at position 1 (adjacent to phenyl).



80 81

Further examples of the effect of vicinal fluorine substituents on the microsomally mediated hydroxylation of aliphatic and alicyclic hydrocarbons are noted in Sections 3.3 and 3.4.1.

Although S-dealkylation is a known, albeit not widely exemplified, metabolism pathway (e.g., Sarcione and Stutzman, 1960; Mazel *et al.*, 1964; Taylor, 1973) apparently there has been no report of DIEs.

5 Hydroxylation of Carbon α to Nitrogen

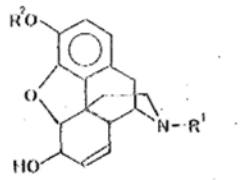
5.1 N-DEALKYLATION

The first report on DIEs associated with N-demethylation was that of Elison *et al.* (1961, 1963) on morphine (**82**). Using rat liver microsomes a DIE of 1.4 was found for the trideutero derivative **83.** It was also noted that **83** was the stronger base (pK_a values: **82,** 8.05; **83,** 8.17) due to the greater inductive effect of the CD_3 group and that the ratio of the Michaelis constants K_m^D/K_m^H was 1.43. A value of this ratio of >1 signifies better binding of the protium form and the situation for N-alkyl derivatives contrasts with that for O-alkyl compounds, where the K_m^D/K_m^H ratio is <1, indicating better binding of the deuterium form (see Section 4.1). As compared with morphine (**82**), the trideutero derivative **83** was a less potent analgesic in mice, and showed less toxicity (higher LD_{50}) toward this species.

A somewhat smaller DIE (1.21–1.28) was found by Thompson and Holtzman (1974) for the N-demethylation of ethylmorphine (**84**) and trideuteromethylnorethylmorphine (**85**) by liver microsomes (noninduced rats).

Arguing that, for O-demethylation, the lower K_m for the deuterium form reinforces the DIE whereas, for N-demethylation, the higher K_m for the deuterium form opposes the DIE, Abdel-Monem (1975) studied the N-demethylation of *N*-methyl-*N*-trideuteromethyl-3-phenylpropylamine (**86**). Using liver 9000 *g* supernatant (noninduced and PB-induced rats), a DIE of 1.31 was found (calculated from the ratio of the products). A somewhat higher DIE (1.45) was found when mouse liver 9000 *g* supernatant was used. The metabolism of **86** has been categorized (Hjelmeland *et al.*, 1977a) as reflecting an intramolecular DIE (see Section 7).

This approach was developed further by Miwa *et al.* (1980) in studies with *N,N*-dimethylphentermine (**87**). No DIE was found for the N,N-di-(trideutero-

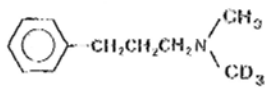


82 $R^1 = CH_3$, $R^2 = H$

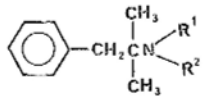
83 $R^1 = CD_3$, $R^2 = H$

84 $R^1 = CH_3$, $R^2 = Et$

85 $R^1 = CD_3$, $R^2 = Et$



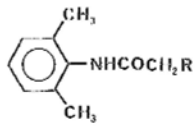
86



87 $R^1 = R^2 = CH_3$

88 $R^1 = R^2 = CD_3$

89 $R^1 = CH_3$, $R^2 = CD_3$



90 $R = N(CH_2CH_3)_2$

91 $R = N(CD_2CH_3)_2$

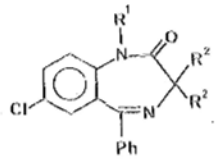
92 $R = N(CH_2CD_3)_2$

methyl) derivative **88** on metabolism with liver microsomes (PB-treated rats). However, a DIE of 1.6–2.0 was found for the N-methyl-N-trideuteromethyl derivative **89**. No significant DIE in the K_m parameters was found and it was concluded that C—H bond cleavage did not contribute to V_{max} and that the NMe groups were free to exchange at the active site of the enzyme. It was also suggested that N-demethylation could involve a transition state different from that (probably free radical, see Section 3.4.2) associated with, for example, O-demethylation.

Nelson *et al.* (1975) investigated the N-deethylation of the anesthetic lidocaine (**90**) using liver microsomes (noninduced rats). For the d_4-derivative **91** (deuterium α to nitrogen) a primary DIE of 1.36 was found and, as expected, a K_m^D/K_m^H ratio of >1 (1.23). This finding accords with those noted above for N-demethylation. However, for the d_6-derivative **92** (deuterium β to nitrogen) a secondary DIE of 1.40 was obtained with, as expected, a K_m^D/K_m^H ratio of <1 (0.92). Secondary DIEs in metabolism studies are rare and a convincing explanation for the above observation remains to be found.

5.2 α-C-HYDROXYLATION

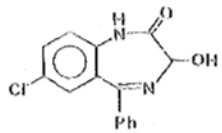
N-Desmethyldiazepam (**94**) is a major metabolite of diazepam (**93**) and is further metabolized by 3-hydroxylation to give oxazepam (**95**), which accumulates in



93 $R^1 = CH_3$, $R^2 = H$

94 $R^1 = R^2 = H$

96 $R^1 = H$, $R^2 = D$



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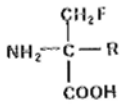


97 R = H

98 R = D

99

100

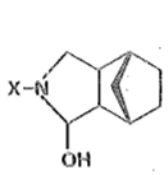
the brain of mice and is responsible for the prolonged anticonvulsant action of the administered drug. Dideuteration at position 3 (→**96**) reduced the duration of the anticonvulsant activity from 20 to 5 hr and in *in vitro* experiments using liver microsomes (PB-induced mice) the extent of 3-hydroxylation was reduced ~7.5-fold (Marcucci *et al.*, 1973).

3-Fluoro-L-alanine (**97**) was designed as a specific antibacterial metabolite and in refining the design (Kollonitsch and Barash, 1976) deuterium was introduced (→**98**) in order to exploit the DIE to retard metabolism *in vivo*. The d_1-derivative **98** had enhanced metabolic stability in animals but the antibacterial activity was the same as that of the protium form.

Callery *et al.* (1980) reported a DIE of ~1.7 for the conversion of Δ^1-pyrroline (**99**) into 2-pyrrolidinone (**100**) by a 10,000 *g* supernatant of rabbit liver homogenate. 2-Pyrrolidinone (**100**) is a metabolite of putrescine (1,4-diaminobutane).

Among the rat urinary metabolites of the antihypertensive drug tripamide (**54**) was the 3-hydroxy derivative (**101**). A DIE of 1.6 was found on metabolism of the d_4-derivative (**102**) by rat liver microsomes (Horie *et al.*, 1981).

A major metabolic detoxification pathway for the antitumor agent 6-mercaptopurine (**103**) is via 8-hydroxy-6-thiopurine (**104**) to thiouric acid (**105**) mediated by xanthine oxidase. Significant DIEs (3.5–3.8) were found (Jarman *et al.*, 1982) for the action of this enzyme *in vitro* on 8-d_1- (**106**) and 2,8-d_2-6-mercaptopurine (**107**) but not for the 2-d_1 derivative (**108**). Following ip administration of the 2,8-d_2 derivative (**107**) to rats 2.2–3.7 times as much unchanged drug was



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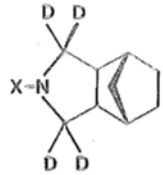

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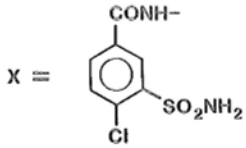
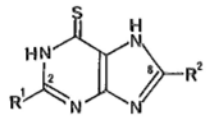
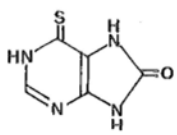
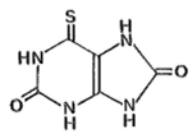


excreted in the urine and 54–70% of thiouric acid in comparison with 6-mercaptopurine (**103**). Although the potency of the 2,8-d_2 derivative (**107**) against the adenocarcinoma Ca755 in mice was increased three- to fivefold the 8-d_1 derivative (**106**) had the same potency as 6-mercaptopurine. These results suggest that oxidation mechanisms other than or additional to that mediated by xanthine oxidase may occur *in vivo*.







103 $R^1 = R^2 = H$

104

105

106 $R^1 = H, R^2 = D$

107 $R^1 = R^2 = D$

108 $R^1 = D, R^2 = H$

5.3 OXIDATIVE DEAMINATION

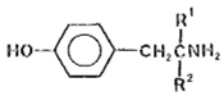
These reactions are mediated by enzymes such as monoamine oxidases and monooxygenases (Miller and Klinman, 1982).

5.3.1 Phenylethylamine Derivatives

Belleau *et al.* (1961), in an early attempt to modify biological properties of a drug by specific deuteration and thereby illuminate the mode of action, examined

the effect of deuteration α to nitrogen in a series of biologically active amines which were substrates for monoamine oxidase.

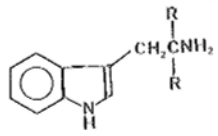
Thus α-dideuteration (→**110**) of the adrenergic *p*-tyramine (**109**) doubled the duration of the effect on arterial pressure and nictitating membrane contractions following iv administration to cats. A similar effect was found on α-dideuteration (→**112**) of tryptamine (**111**) but there was no such effect on α-dideuteration (→**114**) of (−)-norepinephrine (**113**). It was also found that for the enantiomeric monodeutero derivatives (**115**) of tyramine only one (not designated) showed a DIE.



109 $R^1 = R^2 = H$

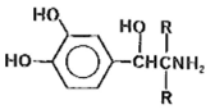
110 $R^1 = R^2 = D$

115 $R^1 = H(D)$, $R^2 = D(H)$



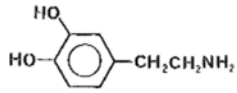
111 R = H

112 R = D



113 R = H

114 R = D



116

Perel *et al.* (1972) found that for dopamine (**116,** 3,4-dihydroxyphenylethylamine), the side chain of which is oxidized by dopamine β-hydroxylase and monoamine oxidase, dideuteration α or β to nitrogen had no effect on the pharmacological activity. No *in vitro* studies were reported.

More recently Yu *et al.* (1981) have reported on the metabolism of *p*-tyramine (**109**) and related amines by monoamine oxidase isolated from rat liver mitochondria. A DIE of ~2.4 was found for the α-dideutero derivative **110** (and also an increased K_m); there was no effect on β-dideuteration. Likewise, α-dideuteration of *m*-tyramine and β-phenylethylamine resulted in DIEs of ~2.2 and ~1.8, respectively, but β-dideuteration had no effect. It was noted without details that β-d_2-*p*-tyramine was less readily β-hydroxylated than the protium form.

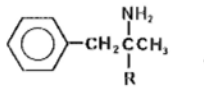
The antidepressant phenelzine ($PhCH_2CH_2NHNH_2$) is a nonspecific inhibitor of monoamine oxidase and is metabolized *in vivo* to phenylacetic acid ($PhCH_2COOH$), presumably via hydroxylation α to nitrogen. Tetradeuteration

($\rightarrow PhCD_2CD_2NHNH_2$) did not change the enzyme inhibition potency *in vitro* (Dyck *et al.*, 1983) but profoundly potentiated the biphasic behavioral stimulation in the rat (Dourish *et al.*, 1983). It seems likely that the central potency was increased because of slowing of peripheral inactivation consequent on a DIE.

5.3.2 *Amphetamines*

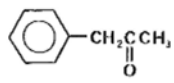
Foreman *et al.* (1969) were the first to report a DIE for the oxidative deamination of amphetamine (**117**, 2-amino-1-phenylpropane) to phenylacetone (**118**). Using a rabbit liver homogenate and (−)-amphetamine a DIE of 1.9 was found for (−)-2-d_1-amphetamine (**119**).

Vree *et al.* (1971) subsequently reported on the fate of several N-substituted amphetamines and their deuterated derivatives in man. They found only a small DIE for (+)-amphetamine. After ingestion of a 1:1 mixture of (+)-amphetamine (**117**) and (+)-2-d_1-amphetamine (**119**) by patients the amphetamine in urine samples collected during the following 48 hr contained a 7–18% excess of deuterium. Similar studies with (+)-*N*-methylamphetamine (**120**) and its trideuteromethyl analog (**121**) and also (+)-*N*,*N*-dimethylamphetamine (**122**) and its *N*,*N*-di(trideuteromethyl) analog (**123**) revealed virtually no DIE. However, for a ~1:1 mixture of (+)-*N*-isopropylamphetamine (**124**) and its (+)-*N*-2′-d_1-isopropyl analog (**125**) a substantial DIE was observed since the deuterium content of the drug excreted in the urine increased from 48 to 80–90%. No such



117 R = H

119 R = D



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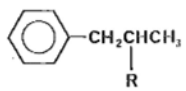
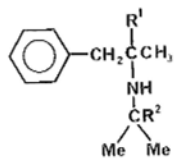


120 R = $HNCH_3$

121 R = $HNCD_3$

122 R = $N(CH_3)_2$

123 R = $N(CD_3)_2$



124 $R^1 = R^2 = H$

125 R^1 = H, R^2 = D

126 $R^1 = R^2$ = D

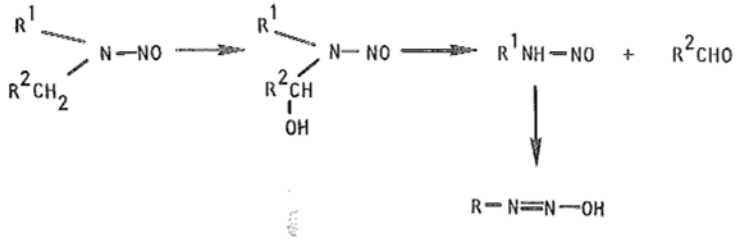
effect was observed for the (−)-analogs. Likewise, for a 1:1 mixture of **124** and (+)-*N*-2′-d_1-isopropyl-2-d_1-amphetamine (**126**) there was a substantial increase in the deuterium content of the excreted drug and there was no such effect for the (−)-analogs.

The members of the (−)-series of amphetamines are metabolized slowly via N-oxidation so that DIEs in the deuterated compounds noted above would not be expected whereas the corresponding (+)-compounds are metabolized by hydroxylation α to nitrogen. In an *in vitro* study, Henderson *et al.* (1974) found that, with liver microsomes (noninduced rats), (+)-*N*-isopropylamphetamine (**124**) was converted into (+)-amphetamine (**117**) and phenylacetone (**118**) and for (+)-*N*-2′-d_1-isopropyl-2-d_1-amphetamine (**126**) a DIE of 1.64 was found but the effect on the balance of the two metabolism pathways was not noted. In contrast to the findings of Foreman *et al.* (1969) for the *in vitro* metabolism of (−)-2-d_1-amphetamine (**119**) Henderson *et al.* (1974) found no DIE for (−)-*N*-2′-d_1-isopropyl-2-d_1-amphetamine (**126**).

5.4 NITROSAMINES

Dialkylnitrosamines and related cyclic compounds, e.g., nitrosomorpholine, are potent carcinogens which can cause the development of tumors in various organs when administered to animals via their drinking water. These compounds are activated by metabolism by membrane-bound (microsomal) and soluble enzymes to yield alkylating species which are believed to be responsible for carcinogenesis (Lai and Arcos, 1980). It is well established that dialkylnitrosamines undergo metabolic hydroxylation α to nitrogen and this process can yield an alkylating species (R—N=N—OH, R—N≡N, or R^+) by the initial sequence shown in Scheme 1. The alkylating species would be susceptible to attack by cellular nucleophiles, including DNA.

Interest in dialkylnitrosamines has been stimulated not only because they are potent carcinogens which could be responsible for some human cancers, but also because variation in the structure of R^1 and R^2 varies the organotrophy. In seeking to define more precisely the mode of action of dialkylnitrosamines the effect of deuterium substitution α to nitrogen has been extensively explored.



Scheme 1

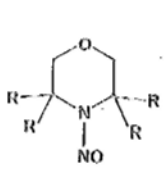
Keefer *et al.* (1973) showed that the administration of di-(trideuteromethyl)nitrosamine [d_6-DMN, $(CD_3)_2N$-NO] at 5 ppm in the drinking water of rats for 30 weeks caused a ninefold lower incidence of liver tumors than did dimethylnitrosamine [DMN, $(CH_3)_2N$-NO]. Dagani and Archer (1976) subsequently reported a DIE of 3.8 for the demethylation of d_6-DMN by liver microsomes (PB-treated rats) and a K_m^D/K_m^H ratio of 0.62, indicating stronger binding of the deuterated compound to the hydroxylating enzyme [the opposite effect is found for amines (Section 5.1)].

There are at least two DMN demethylases in rat liver microsomes which operate at low (<4 m*M*) and high (50–200 m*M*) substrate concentrations (Arcos *et al.*, 1977). Using the S-9 fraction (9000 *g* supernatant) from the homogenized liver of PB-treated Long–Evans rats, Kroeger-Koepke and Michejda (1979) found a DIE (1.82) for the demethylation of d_6-DMN which was much lower than that (3.8) noted above. They also obtained evidence for a soluble demethylase and pointed out that the magnitude of the DIE depended on the cell fraction used (microsomal, postmicrosomal), rat species, inducer, and concentration of substrate and noted that variation in the levels of these enzymes might be partly responsible for the organotrophy of dialkylnitrosamines. A DIE of 5.38 was found for the demethylation of phenyltrideuteromethylnitrosamine [$Ph(CD_3)N$-NO].

Charnley and Archer (1977) reported that, after activation by a liver homogenate (PB-treated rats), nitrosomorpholine (**127**) was five times more mutagenic toward *Salmonella typhimurium* TA1535 than the tetradeuterated analog **128.** A similar finding was reported by Elespura (1978) using a different mutagenesis system (reversion of a nonsense mutation in *E. coli*). It was also noted that d_6-DMN and the octadeuterated derivative of dinitrosopiperazine (**129**) were less mutagenic than the corresponding protium forms.

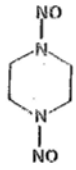
This apparently clear-cut picture of DIEs in dialkylnitrosamine carcinogenesis subsequently became somewhat blurred mainly as a result of the findings of Lijinsky and co-workers. As noted in Section 2.2.2, it is important in determining the magnitude and biological consequences of DIEs to verify that no D/H exchange occurs *in vitro* or *in vivo*. This precaution cannot be taken in animal feeding experiments with dialkylnitrosamines and Singer and Lijinsky (1979) have suggested that biological DIEs are only likely to occur when compounds with deuterium α to nitrogen have a relatively low susceptibility to base-catalyzed D/H exchange.

Lijinsky *et al.* (1980a) found that the d_4-derivative (**131,** D α to nitrogen) of 2,6-dimethylnitrosomorpholine (**130**) was less carcinogenic and the d_2-derivative (**132,** D α to oxygen) was more carcinogenic than the parent compound in causing esophageal cancer in the rat. This result is suggestive of metabolic switching in the d_2-derivative **132** away from hydroxylation α to oxygen, a process known (Section 4) to have a DIE of ~2 and which would not be expected to yield alkylating species.



127 R = H

128 R = D



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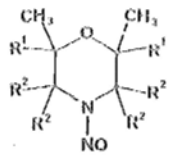
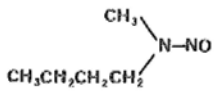


130 $R^1 = R^2 = H$

131 $R^1 = H, R^2 = D$

132 $R^1 = D, R^2 = H$



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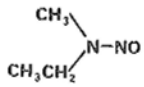

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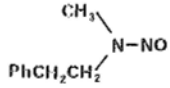

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For *n*-butylmethylnitrosamine (**133**), replacement of the methyl group by CD_3 increased the carcinogenic potency whereas replacement of the n-butyl group by $CH_3CH_2CH_2CD_2$ decreased the potency (Lijinsky *et al.*, 1980b). These findings contrast with those (Lijinsky and Reuber, 1980) for ethylmethylnitrosamine (**134**), which causes esophageal and liver cancer in rats. Replacement of the ethyl group by CH_3CD_2 or CD_3CD_2 enhanced the carcinogenicity in feeding experiments whereas replacement of the methyl group by CD_3 or perdeuteration of the molecule did not significantly change the potency. When the modified ethyl group contained CD_3 there was an increased incidence of esophageal tumors.

Farrelly *et al.* (1982) reported on the metabolism of deuterated *n*-butylmethylnitrosamine (**133,** BMN) and ethylmethylnitrosamine (**134,** EMN) *in vitro* using liver microsomes (noninduced rats). DIEs of 1.9 and ~5, respectively, were found for the debutylation of d_2-BMN ($CH_3CH_2CH_2CD_2$ group) and demethylation of d_3-BMN (CD_3 group). Using the same procedure a DIE of 2.2 was found for both deethylation of d_2-EMN (CH_3CD_2 group) and demethylation of d_3-EMN (CD_3 group). Yet another trend was found for methylphenylethylnitrosamine (**135,** MPN). DIEs of 3.2 and 1.35, respectively, were found for dephenylethylation of d_2-MPN ($PhCH_2CD_2$ group) and demethylation of d_3-MPN (CD_3 group). Marked metabolic switching to demethylation was found for d_2-BMN, d_2-EMN, and d_2-MPN.

The foregoing DIEs for BMN (**133**), EMN (**134**), and MPN (**135**) relate to the low-affinity enzyme (Arcos *et al.*, 1977), which probably contributes little to the overall metabolism at the concentrations of dialkylnitrosamines used in feeding experiments. The carcinogenic potencies of d_2-EMN and d_2-MPN (Lijinsky *et*

al., 1982) were higher than those of the corresponding protium forms but this was not so for d_2-BMN.

As would be expected since the mode of action does not involve metabolic activation, no biological DIEs were observed in rat feeding experiments with nitroso-*N*-methylurethane and its CD_3 analog and nitroso-*N*-ethylurethane and its CD_3CD_2 analog (Lijinsky and Reuber, 1982).

6 Miscellaneous Compounds

6.1 ANESTHETICS

6.1.1 Chloroform

An early study (Krantz *et al.*, 1967) showed that, for dogs and mice, exhaled $CDCl_3$ had undergone no D/H exchange and that $CDCl_3$ was somewhat more potent than $CHCl_3$.

Chloroform causes renal and hepatic damage in humans and animals. That the formation of phosgene from chloroform by the process

$$CHCl_3 \rightarrow [HOCCl_3] \rightarrow COCl_2$$

is probably responsible for this damage is indicated by the reduced hepato- and nephrotoxicity of $CDCl_3$ in rats (Pohl and Krishna, 1978; McCarty *et al.*, 1979; Ahmadizadeh *et al.*, 1981). Using liver microsomes (PB-treated rats), Pohl and Krishna (1978) found a DIE of ~1.9 for $CDCl_3$ (based on the yield of phosgene). Serum pyruvic transaminase levels are a reflection of hepatic damage and after administration of $CDCl_3$ to rats the serum level of this enzyme was 38.3 mU/ml compared to values of 56.3 and 24.8 for $CHCl_3$ and controls, respectively (McCarty *et al.*, 1979).

6.1.2 Methoxyflurane

The metabolism of methoxyflurane (MOF) proceeds by two pathways which reflect hydroxylation at the methyl and dichlorodifluoroethyl groups:

Fluoride ion is released only after hydroxylation of the methyl group. Using liver microsomes (PB-treated rats), Hitt *et al.* (1979) showed that the release of fluoride ion from MOF was reduced from 19.6 to 3.8 nmol F^-/15 min/mg of

protein on tetradeuteration ($\rightarrow CD_3OCF_2CDCl_2$). This corresponds to a DIE of 5.24 (reduced to 1.19 when liver microsomes from noninduced rats were used). The K_m values of MOF and d_4-MOF were similar (13 and 19 μM, respectively).

McCarty *et al.* (1979) noted a 19% *increase* in urinary excretion of fluoride ion in rats after administration of d_1-MOF ($CH_3OCF_2CDCl_2$) and a 29% *decrease* for d_3-MOF [$CD_3OCF_2CHCl_2$; cf. 33% for d_4-MOF ($CD_3OCF_2CDCl_2$)]. These results are suggestive of metabolic switching. However, Baden *et al.* (1982) noted that for rats the serum fluoride levels and renal dysfunction after anesthesia for 2 hr with d_4-MOF were still unacceptable.

6.1.3 Enflurane and Related Compounds

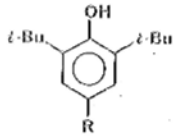
Two metabolism pathways are possible for enflurane (HCF_2OCF_2CFClH) reflecting attack at the two CH centers. Each pathway could lead to the release of fluoride ion but that leading to HCF_2OCF_2COOH has been suggested to be the major route (Cousins and Mazze, 1974). For d_1-enflurane ($HCF_2OCFClD$) there was a 65% decrease in excretion of urinary fluoride (McCarty *et al.*, 1979). An even higher reduction (76%) was found for d_1-difluoroflurane (HCF_2OCF_2CFBrD) and there was a twofold increase in serum bromide. These results accord with expectations if HCF_2OCF_2COOH is the major metabolite. However, a decrease of 29% was found for d_1-difluoromethoxyflurane ($HCF_2OCF_2CCl_2D$) and this finding is not readily explained.

6.1.4 Halothane

The metabolism of halothane ($CF_3CBrClH$) can involve dehydrobromination (via hydroxylation $\rightarrow CF_3COCl$) and dehydrofluorination ($\rightarrow CF_2{=}CBrCl$) to give products that can undergo further reactions. Each of these routes should be subject to a DIE and after administration of d_1-halothane ($CF_3CBrClD$) to rats there was a 15–26% reduction in serum bromide (McCarty *et al.*, 1979).

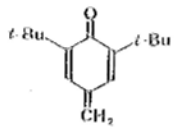
6.2 ANTIOXIDANTS

Butylated hydroxytoluene (**136,** BHT, 2,6-di-*tert*-butyl-4-methylphenol) is a widely used antioxidant. It causes lung damage in mice and the covalent binding to lung tissue is probably mediated by the reactive quinone methide metabolite (**137,** BHT-QM, 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone). Metabolism of BHT (**136**) with 9000 *g* supernatant of homogenized mouse liver (Mizutani *et al.*, 1983) gave BHT-QM (**137**) and BHT-OH (**138,** 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone). Metabolism of a 1:1 mixture of BHT and d_3-BHT (**139,** CD_3 group) resulted in ratios of 0.59 for d_2-BHT-QM/BHT-QM and 1.68 for d_3-BHT-OH/BHT-OH, reflecting metabolic switching.

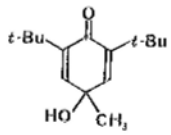


136 R = CH_3

139 R = CD_3



137



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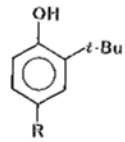


140 R = CH_2CH_3

141 R = CD_2CH_3

142 R = CH_2CD_3

In *in vivo* experiments, it was shown that trideuteration of the methyl group in BHT (→**139**) reduced the pulmonary toxicity. In contrast to the usual practice of studying urinary metabolites, Mizutani *et al.* (1983) investigated the metabolites in lung and liver tissue. Thus, after ip administration of a 1:1 mixture of BHT and d_3-BHT to mice the ratios of d_2-BHT-QM/BHT-QM in lung and liver tissue were 0.66 and 0.85, respectively. The ratios of d_3-BHT-OH/BHT-OH, which had to be obtained by separate administration of d_3-BHT and BHT, were 1.39 and 1.26 for lung and liver tissue, respectively. Hence, the DIE and metabolic switching observed *in vitro* were paralleled *in vivo* and BHT-QM (**137**) is further indicated to be the reactive metabolite of BHT (**136**).

The pulmonary toxicity of 2-*tert*-butyl-4-ethylphenol (**140**) was reduced on replacing the ethyl group with CH_3CD_2 (→**141**) but there was no effect on replacement by CD_3CH_2 (→**142**).

7 Intrinsic and Intramolecular Deuterium Isotope Effects

In contrast to chemical reactions, enzyme-catalyzed reactions display a broad range of DIEs, depending on the reaction conditions. Northrop (1982) has described a family of DIEs associated with the following sequence of events:

$$E + A \rightleftharpoons EA + B \rightleftharpoons EAB \rightleftharpoons {}^*EAB \rightleftharpoons {}^*EPQ \rightleftharpoons EPQ \rightarrow P + EQ \rightarrow Q + E$$

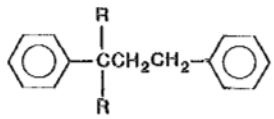
where E is the enzyme, A and B are substrates or substrate and cofactor, P and Q are the products, and * connotes as activated complex. Only two of this family of

DIEs need be considered here, namely, (1) the observed DIE (^{D}v), which is dependent on the entire steady-state distribution of enzyme forms and which is usually determined from the ratio of the rates (k_H/k_D) of the disappearance of the protium and deuterium forms under particular conditions of metabolism, and (2) the intrinsic DIE (^{D}k), which refers only to the *EAB $\rightleftharpoons$ *EPQ component of the above sequence and, in reflecting the full effect of hydride transfer, is analogous to the DIEs in chemical reactions.

The maximal velocity of most enzyme reactions is dependent on several rate-contributing or partially rate-limiting steps (Northrop, 1982). It is therefore not surprising that, for an enzyme such as cytochrome P-450, which operates by a multistep process (Schenkman and Kupfer, 1982), most of the ^{D}v values reported in the literature are lower (range 1–5) than the expected ^{D}k values. The variation in ^{D}v values is also explicable if not predictable.

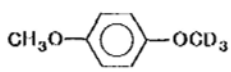
Lu *et al.* (1984) have shown that, for the metabolism (O-deethylation) of 7-ethoxycoumarin (**74**) and its d_2-derivative **75** with purified cytochrome P-450 from liver microsomes (PB-treated rats), the values of ^{D}v and ^{D}k were ~3.8 and ~12, respectively. Such a high ^{D}k value is consistent with a hydrogen abstraction process (see Section 3.4.2). Although the determination of ^{D}k values is important in studies of the mechanism of enzyme action it is the magnitude of the ^{D}v values which determines the scope of specific deuterium substitution for controlling drug metabolism.

The ^{D}v values reported in this article so far may also be classified as intermolecular in that they have usually been determined by monitoring the relative rates of metabolism of, or the appearance of products from, the protium and deuterium forms either separately in parallel reactions or as a mixture (usually 1:1) in a single reaction. Hjelmeland *et al.* (1977a) suggested that, for two alternative sites for metabolic attack in the same molecule which differ only in isotopic substitution, the intramolecular DIE associated with the relative rates of reaction at the protonated and deuterated sites should approximate to the ^{D}k values. They found an intramolecular DIE of 11 ±1 for the benzylic hydroxylation of 1,3-diphenylpropane (**143**) and its 1-d_2-derivative **144** with liver microsomes from noninduced rats which contrasts with, for example, the intermolecular DIE of 1.14 for the benzylic hydroxylation of tolbutamide (**22**) with liver microsomes from PB-treated rats (Tagg *et al.*, 1967).



143 R = H

144 R = D



145

Foster *et al.* (1974) reported an intramolecular DIE of ~10 for the O-demethylation of *p*-(trideuteromethoxy)anisole (**145**) by liver microsomes (PB-treated rats) and an intermolecular DIE of ~2 for *p*-dimethoxybenzene (**68**) and its *p*-di-(trideuteromethoxy) analog. These findings conflict with those of Watanabe *et al.* (1982) using liver microsomes from PB treated rabbits, who reported inter- and intramolecular DIEs of 5.1 and 3.4, respectively. Hitherto, the highest reported intermolecular DIE for microsomally mediated O-demethylation was 2.7 for *p*-nitroanisole (Al-Gailany *et al.*, 1975) (see Section 4.1) and it is not easy to explain why the intermolecular DIE has the higher value in the work reported by Wanatabe *et al.* (1982).

Although no DIE was found for the hydroxylation of perdeutercyclohexane (C_6D_{12}) using liver microsomes (PB-treated rats) (Ullrich, 1969), a large k_H/k_D value (8.6) was found for $C_6D_{11}H$ (Castle and Ullrich, 1980). Presumably, most of the hydroxylation occurred at the carbon carrying the single protium substituent.

Other examples which could reflect an intramolecular DIE involve α-hydroxylation of *S*(+)-α-d_1-ethylbenzene (McMahon *et al.*, 1969) (see Section 3.2) and the N-demethylation of *N*-methyl-*N*-trideuteromethyl-3-phenylpropylamine (**86**) (Abdel-Monem, 1975) and *N*-methyl-*N*-trideuteromethylphentermine (**89**) (Miwa *et al.*, 1980) (Section 5.1).

Although the use of intramolecular competition reactions are of value in determining intrinsic DIEs (^Dk values), there is little scope for their deployment in drug design because of the symmetry requirements. The one type of intramolecular DIE of those so far described which could easily be deployed in drug design, namely, that associated with the replacement of $—N(CH_3)_2$ by $—N(CH_3)CD_3$, has a relatively low magnitude (<2) and would not be expected to cause a marked change in metabolism profile.

8 Conclusions

It is now becoming clear that the scope for using DIEs effectively in drug design to block adverse metabolism or to deflect metabolism away from toxic products (metabolic switching) is very limited although there is little doubt that DIEs will continue to be of value in studies of the mechanism of enzyme action and that specifically deuterated compounds will continue to be used to probe metabolism pathways.

In their excellent review, Blake *et al.* (1975) commented "At the present time, there are no drugs on the market that contain deuterium in the molecule. . . ." A decade later the situation has not changed and some of the reasons are not difficult to identify. For drugs not intended for use in humans or for products such as insecticides the advantage to be gained by specific or general

deuteration in modifying biological activity and/or duration of action must significantly outweigh the additional cost of chemical synthesis. For drugs intended for use in humans there will be a substantial additional cost, namely, that associated with preclinical toxicology and clinical trials. It seems very unlikely that the regulatory authorities associated with the drug industry would regard a deuterated drug designed to have biological activity significantly different from that of the parent protium form as other than a new drug.

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