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Review Article

Microsomal Dealkylation of Drugs

Substrate Specificity and Mechanism

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ABOUT TWENTY-FIVE years ago Butler and Bush (1) reported the *in vivo* demethylation of dimethylbarbital in the dog. This observation represented the first example of the *N*-demethylation of a synthetic drug. In the intervening years the dealkylation reaction has become recognized as a major pathway of drug metabolism. However, studies at the *in vitro* level have been undertaken only in recent years. The first such study was that of Mueller and Miller at Wisconsin. They found (2) that the demethylation of the carcinogenic dye, *p*-dimethylaminoazobenzene could be effected by an *in vitro* system composed of the liver microsomal fraction, an enzyme in the soluble fraction together with oxygen, NADP, NAD, and hexose phosphate. In the 2 years that followed this discovery, the field developed rapidly and the general characteristics of the reaction became well established.

Among the early studies were those of LaDu *et al.* (3) and of Axelrod (4, 5). These workers demonstrated that the oxidative *N*-dealkylation of amines is catalyzed by the microsomal fraction (derived from the endoplasmic reticulum) from mammalian liver and requires molecular oxygen and NADPH. The requirement for a soluble enzyme, NADP, and hexose phosphate experienced by the earlier workers proved to be a requirement for a NADPH generating system. It was also demonstrated that alkyl groups other than methyl were oxidatively removed by the *N*-dealkylase system.

The over-all oxidative *N*-dealkylation reaction has the following stoichiometry:

$$R_2NCH_2R \xrightarrow{[O]} R_2NH + RCHO$$

A key discovery of the Brodie (6) group was that the *N*-demethylase activity was a member of a family of oxidative enzymes involved in the metabolism of drugs. All of these enzymes are located in the microsomal fraction of mammalian liver and require NADPH and molecular oxygen. In addition to dealkylation these enzymes catalyze aromatic hydroxylation, side chain oxidation, *S*-oxidation of thioethers, *N*-oxidation of tertiary amines, and the oxidation of phosphorothionates. A key property of these enzymes is their remarkable nonspecificity.

A number of excellent reviews of the general field of microsomal enzyme systems are available. Among these, the recent reviews by Gillette (7), Siekevitz (8), and Shuster (9) are particularly informative. In this review we plan to consider recent developments in the field of microsomal dealkylation from the viewpoint of the medicinal chemist interested in structure–activity studies and in the mechanism of the reaction. The review has been divided into three sections:

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TABLE I.—*In Vitro* DEMETHYLATION RATES

Substrate	H/W	μmole HCHO/Gm. Liver/hr. Guinea Pig	Rat	Mouse
I	4970	5.25	5.32	7.45
II	4060	6.31	7.87	7.90
III	406	4.44	4.47	5.80
IV	107	4.35	4.22	3.85
V	47	3.89	2.87	3.40
VI	35	1.75	2.89	3.60

structure–activity studies, the mechanism of the reaction, and a tabulation of important pharmacological agents which undergo *in vivo* dealkylation.

STRUCTURE ACTIVITY CONSIDERATIONS

Known substrates of the microsomal dealkylase system include tertiary amines, secondary amines, *N*-alkyl amides, *N*-alkyl carbamates, *N*-methyl barbituric acids, *N*-methyl sulfonamides, *N*-methyl pyrophosphoramides, *N*-methyl imides, *N*-methyl purine bases, arylalkyl ethers, and aryl methyl thioethers. The characteristics of the dealkylation of each of these classes of compounds are summarized below. A discussion of the chemical nature of reaction intermediates and of the possible mechanism of the reaction is presented in the concluding paragraphs.

Tertiary Amines.—The number of tertiary amines which are known to be dealkylated by the microsomal system is very large. Substrates of such widely varying structures have been found to be active that it is difficult to imagine any single chemical or physical property which could be used to correlate the experimental observations. However, Gaudette and Brodie (10) presented experimental evidence that an important requirement for the substrate is that it be lipid soluble. This proposal was particularly attractive since the endoplasmic reticulum is the principal lipid-bearing structure of the cell. Further, it provided an explanation of why foreign amines were readily acted upon by this system while naturally occurring polar amines, such as methylated amino acids, were not dealkylated.

The question of lipid solubility has been explored in detail by McMahon (11) who studied the relationship between lipid solubility (as measured by distribution between *n*-heptane and pH 7.4 buffer) and the rate of demethylation in both an *in vitro* system and in the intact animal. The compounds investigated were I, *dl*-propoxyphene; II, *dl*-propoxyphene carbinol; III, *p*-chlorophenylpropyldimethylamine; IV, phenylpropyldimethylamine; V, phenylethyldimethylamine; and VI, benzyldimethylamine.

The relative rates of demethylation of these six substrates by microsomes from three species are summarized in Table I. H/W represents the ratio of the equilibrium concentration in heptane to that in pH 7.4 buffer. It is clear from these data that there is a direct correlation of increasing rate with increasing lipid solubility. The two most lipid soluble amines, I and II, however, present an exception. In this case the esterification of the hydroxyl group of II results in a small increase in lipid solubility as expected, but it also results in a somewhat lowered demethylation rate. The introduction of the polar and reactive hydroxyl group produced an effect which overrides simple lipid solubility considerations. Apparently it is important in studies of this sort to select series of compounds which differ from each other by the presence or absence of such relatively inert groups as methylene, phenyl, halogen, etc. The results do suggest that a study of structure–activity relationships in a series of compounds in which the lipid solubility is high and not limiting might be rewarding.

In order for the *in vitro* work described above to be valuable to the medicinal chemist, it was important to correlate it with *in vivo* data in the same species. Radiocarbon labeling served for these studies. The results presented in Table II show an excellent correlation with the *in vitro* results.

With one minor exception the *in vivo* results correlate directly with *in vitro* rates. These studies then demonstrated a relationship between lipid solubility and both *in vitro* and *in vivo* demethylation rates. Also, although the *in vivo* rates of demethylation varied from species to species, the relative order of substrate activity was approximately the same in each species. It is likely that systematic studies of species variation will only be successful when a narrowly defined series of compounds such as these are used.

The structure–activity studies on the effect of lipid solubility were next extended to series of substrates which were totally aliphatic in nature (12). From this study, which will not be reviewed in detail here, it was abundantly clear

TABLE II.—*In Vivo* DEMETHYLATION RATES

Compd.	% Demethylation in 200 min. in— Guinea Pig	Rat	Mouse
I	61	50	47
II	84	55	83
III	58	21	20
IV	55	11	12
V	46	12	11
VI	18	16	9

that there is a direct correlation between lipid solubility and substrate activity. Recently Hansch, Steward, and Iwasa (13) have described an interesting mathematical treatment of the results of the demethylation studies cited above (11, 12) and have concluded that in these series the rate of demethylation could be correlated with lipid solubility and with the pKa of the amine.

There seems little doubt that lipid solubility is an important factor (perhaps the major factor) in controlling the rate of demethylation of tertiary amines. Other factors, probably chemical in nature, are also most certainly involved. To study these factors, however, will require careful and rigorous structure–activity studies. Thus, in a group of randomly selected compounds which are not chemically related, the effect of any single parameter will be obscured [*cf.* Mazel and Henderson (14)].

Secondary Amines.—Although secondary amines were among the earliest substrates to be studied (3, 4, 15), no systematic structure–activity studies have been reported. For example, lipid solubility is undoubtedly an important factor influencing the rate of demethylation of secondary amines, but no data are available at present which bear on this question. However, there are data available which allow comparisons of tertiary and secondary amines as substrates.

For example, a comparison (16) has been made of the kinetics of the demethylation of certain tertiary dimethylamines as compared to the corresponding secondary methylamine. In general it was found that the maximum rate (V_m) of demethylation is greater for tertiary amines. It is believed that the kinetic factor arising from the fact that the tertiary amine has two methyl groups while the secondary amine has but one is at least partly responsible for this observation. Secondary methylamines appear from k_m data to have a greater affinity for enzyme than does the corresponding tertiary dimethylamine. In the secondary amine there is less steric hindrance about the nitrogen which may facilitate binding. In addition, in each of the pairs of amines studied, the electron pair on nitrogen was more readily available for binding (*i.e.*, the secondary amine is the stronger base). Secondary amines are also considerably less responsive to the action of the potent demethylation inhibitor DPEA (17, 18) than are tertiary amines.

The difference in rate of demethylation of tertiary and secondary amines can have interesting pharmacological consequences. For example, McMahon *et al.* (19) found that in both the *in vitro* and *in vivo* systems acetylmethadol was demethylated at a rate considerably greater than was noracetylmethadol, the secondary amine analog. The result of this rate differential was that administration of acetylmethadol led to the accumulation of noracetylmethadol in tissues. Since noracetylmethadol itself is an active analgesic, these data serve to explain the clinical observation that the administration of acetylmethadol to patients leads to accumulated drug effects (20). Another example is that of the antidepressant imipramine. Gillette *et al.* (21) have presented experimental evidence that the delayed onset of action and the antidepressant properties are due to the *in vivo* formation of des-*N*-methyl imipramine.

One important systematic structure–activity study involving the dealkylation of both secondary and tertiary amines has yet to be carried out, *i.e.*, the *N*-dealkylation of amines in which the alkyl group removed is larger than methyl and ethyl. A few examples are known, however. LaDu *et al.* (3) have demonstrated the removal of the *N*-butyl group while Axelrod and Cochin (22) and Leadbeater and Davies (23) have studied the deallylation of nalorphine. The *in vivo* opening of the pyrrolidine ring of nicotine (24, 25), the debutylation of ethoxybutamoxane (26) and chlorethoxybutamoxane (27), and the *N*-deallylation of levallorphan (28) are further examples. The *in vitro* dealkylation of *N*-cyclopropyl-4-phenyl-4-carbethoxypiperidine has also been observed (McMahon, unpublished data).

O-Dealkylation.—In 1948 Huggens, Jensen, and Cleveland (29) reported the *O*-dealkylation of *p*-nitroanisole and *p*-nitrophenetole in both the intact rat and in liver homogenates. In 1955 Axelrod (30) demonstrated that the *O*-demethylation of codeine was closely related to *N*-demethylation by showing the demethylation to be catalyzed by liver microsomes and to require NADPH and oxygen. The products were morphine and formaldehyde. A later study from the same laboratory (31) concerned the effect of ring substitution upon the rate of demethylation of anisole derivatives. It was found that the *p*-substituted analogs were more readily demethylated than the *o*- or *m*-derivatives. The decreasing order of the rate of metabolism for *p*-substituted anisoles was CN, CHO, $NHCOCH_3$, COOH, $CH_2—NH_2$, allyl, NH_2, H. It is difficult at present to correlate these data with the electronic effects produced by the substituents. It is probable that both k_m and $V_{max.}$ are affected by aromatic substitution so

that it would probably be necessary to do a complete kinetic study of a series of substrates.

McMahon *et al.* (32) have studied the rate of dealkylation of a series of *p*-nitrophenylalkoxyethers in order to determine the effect of changing alkyl groups. In general it was found that with increasing chain length of normal alkyl groups, the rate of dealkylation decreases. However, when electron donating groups were introduced adjacent to the methylene, increased rates were observed. For example, allyl, cyanomethyl, and benzyl were more active substrates than *n*-propyl or chloroethyl. The dialkylaminoethoxy grouping which occurs frequently in drugs does not undergo *O*-dealkylation. It is likely that in this case *N*-dealkylation occurs as an alternative pathway. In studying the effect of aromatic substitution on the rate of dealkylation of substituted *p*-nitrophenyl benzyl ethers, a very clear steric effect emerged when groups were placed *ortho* to the methylene group.

In experiments in intact animals, it was found that an excellent correlation exists between the *in vitro* rates and the *in vivo* rates (32).

It is likely that *O*-dealkylation may not be confined to phenolic ethers but that totally aliphatic ethers will be found to be cleaved. In this connection the recent report (33) of the cleavage of glycerol ethers of long chain fatty alcohols is of considerable interest. This reaction requires molecular oxygen and liver microsomes but showed a requirement for a tetrahydropteridine as a cofactor as well as the usual NADPH. These observations suggest a possible relationship between this activity and the phenylalanine hydroxylation which has been the subject of the elegant researches of Kaufman (34).

For information on detailed experimental conditions which govern microsomal *O*-demethylation, the report by Nilsson (35) on the demethylation of the estrogenic isoflavone, biochanin A, should be consulted.

N-Alkylamides and Related Compounds.—The extensive researches by Butler and his associates have shown many *N*-methyl barbiturates and related *N*-methyl heterocyclics to be demethylated in the intact animal. [See Fishman (36) for a review and bibliography of the Butler studies.] Other examples include the demethylation of the herbicide, diphenamid (37), the demethylation of *N*-methyl-α-phenyl-α-ethylglutarimide (38), and the demethylation of diazepam (39, 40) and of chlordiazepoxide (41).

An early report of the *in vitro* demethylation of an amide was that of Hodgson and Casida (42) who investigated the demethylation of *N*-methyl carbamates and demonstrated the reaction to be a typical microsomal oxidation requiring oxygen and NADPH. More recent *in vitro* work (43, 44) has shown the demethylation of *N*-methyl barbiturates, *N'*-methylsulfonylureas, and simple amides to be microsomal *N*-demethylations as well.

Miscellaneous Substrates.—The most interesting recent development has been the demonstration by Mazel and co-workers that the microsomal dealkylases will readily *S*-demethylate various *S*-methyl ethers (45). This discovery serves to emphasize again the remarkable nonspecificity of the microsomal oxygenase systems.

Another interesting report is that of Schreiber and co-workers (46) who found that *N*-methyl sulfonamides are demethylated by the microsomal system, thus confirming earlier (47) *in vivo* observations. Smith, Keasling, and Forist (48) have also described the dealkylation of *N*-alkyl sulfonamides. They observed the *in vivo* removal of methyl, ethyl, allyl, isopropyl, butyl, and pentyl groups from the corresponding *N*-alkyl-*p*-bromobenzenesulfonamide in mice. The dealkylation of sulfonamides is reminiscent of microsomal oxidation of the cholinesterase inhibitor, octamethyl pyrophosphoramide (49). In this case the reaction does not go to completion spontaneously since the intermediate *N*-hydroxymethyl intermediate is reasonably stable (see below).

The observations of Henderson and Mazel (50) on the demethylation of various methylated purine bases should also be mentioned. In view of the role of methylated purines as minor constituents of RNA and DNA, these observations may have special significance (51).

Other Enzymes Which Catalyze Oxidative Dealkylation.—In addition to the liver microsomal system, other enzyme systems occur in nature which can oxidize *N*-methyl groups. There are, for example, several instances in alkaloid biogenesis in plants in which the oxidation of an *N*-methyl group appears to occur. In the Tilden lecture (1963), Battersby (52) speculated that the berberine bridge carbon may have its origin in the *N*-methyl group of a suitably substituted 1-benzyl-*N*-methyl-isoquinoline. It was proposed that in this conversion the initial oxidative step might be *N*-oxidation as had been proposed earlier by Wenkert (53) in connection with other alkaloid biotransformations. Direct attack on the methyl group, either by a heterolytic or a free radical mechanism, was suggested as an alternative mechanism (52). Although it has now been

demonstrated by radiocarbon studies that the *N*-methyl group is indeed the source of the berberine bridge, the oxidation mechanisms involved remain obscure (54, 55).

The discovery of the alkaloid leurocristine (56) among the alkaloids of *Vinca rosea* is also of interest. This alkaloid is identical with vincaleucoblastine except that the *N*-methyl has been replaced by an N—CHO group. This conversion could well have taken place in a two-step process through a hydroxymethyl intermediate in exactly the same way that nicotine is converted to cotinine (25) or tremorine is converted to oxytremorine (57) in the liver. *O*-Demethylation also occurs in plants. The elegant research of Rapoport (58) on the biogenesis of morphine leaves no doubt that thebaine is a precursor of morphine which means that two successive *O*-demethylations must occur on the pathway to the latter alkaloid. The demethylation of nicotine to nornicotine in the intact *N. glutinosa* plant has recently been established by Alworth and Rapoport (59).

Another class of enzymes which catalyze oxidative *N*-dealkylation and for which *N*-oxide intermediates have been suggested are found in liver mitochondria. These enzymes are responsible for the *N*-demethylation of *N*-methyl amino acids, such as *N*,*N*-dimethylglycine, as well as the oxidation of certain polar amines, such as dimethyl tryptamine, and appear to be dehydrogenases which require NAD as cofactor.

Horning and co-workers (60) have suggested that the dealkylations catalyzed by these mitochondrial dehydrogenases proceed *via* *N*-oxide intermediates. This suggestion was based on the observation of these workers and of others that *N*-oxides of certain tertiary amines and of dimethylamino acids rearrange in the presence of iron complexes to form carbinol amines which in turn dissociate to aldehyde and dealkylated amine or amino acid (61–67). Although subsequent studies (68, 69) have shown that the oxidation of dimethyltryptamine does not proceed through an *N*-oxide intermediate, the question of whether *N*-oxides are involved in the demethylation of dimethylamino acids has not been resolved.

MECHANISM OF MICROSOMAL DEALKYLATION

In their early work on the *in vitro* demethylation of dimethylaminoazobenzene, Mueller and Miller (2) presented indirect evidence to support the view that the reaction proceeded through an *N*-hydroxymethyl intermediate:

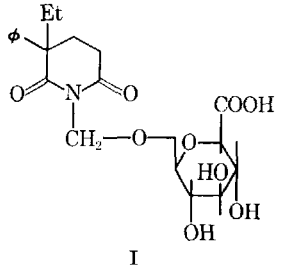
$$R_2NCH_3 \rightarrow [R_2NCH_2OH] \rightarrow R_2NH + HCHO$$

It is now well established that such an intermediate is involved and in certain cases is stable enough to be detected. For example, the active intermediate formed by microsomal oxidation of octamethyl pyrophosphoramide was demonstrated to be *N*-hydroxymethylheptamethyl pyrophosphoramide (49, 70–72).

A particularly relevant observation was that of Keberle *et al.* (38) who found among the metabolites of *N* - methyl -α- phenyl -α- ethylglutarimide the glucuronide of the *N*-hydroxymethyl intermediate (I). Recently McMahon and Sullivan (37) have found an analogous metabolite of the herbicide diphenamid (II). In these cases the hydroxymethyl intermediate has been "trapped" by conversion to a stable derivative, the glucuronide.

The most direct evidence, however, comes from the work of Dorough and Casida (73) who found that the unconjugated *N*-hydroxymethyl derivatives were relatively stable metabolites of α-naphthyl *N*-methylcarbamate and *o*-isopropoxyphenyl *N*-methylcarbamate.

There seems to be little doubt then that the hydroxymethyl compound is indeed an intermediate of *N*-dealkylation. It also seems reasonable to assume that *O*- and *S*- dealkylations proceed through analogous intermediates. There remains, however, the question of how the hydroxymethyl intermediate is formed in the microsomal dealkylation reaction. As a consequence of the work on alkaloid biosynthesis and on the mitochondrial amino acid demethylases discussed, it has been proposed that the *N*-hydroxymethyl intermediate arises from the rearrangement of a tertiary amine *N*-oxide intermediate.



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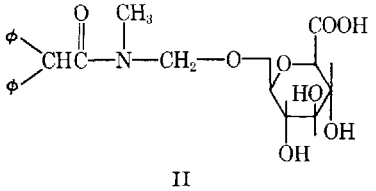

II

TABLE III.—COMPOUND DEALKYLATED IN THE INTACT ANIMAL

Compd.	Classification	Dealkylation Reaction	Species	Ref.
N-Methylbarbital	Sedative	*N*-demethylation	Dog	(1)
N-Methylphenobarbital	Sedative	*N*-demethylation	Dog, human	(106)
Hexobarbital	Sedative	*N*-demethylation	Dog	(107)
Methyl-5-ethyl-5-phenyl hydantoin	Anticonvulsant	*N*-demethylation	Dog, human	(108)
Trimethadione	Anticonvulsant	*N*-demethylation	Dog, human	(109)
Paramethadione	Anticonvulsant	*N*-demethylation	Dog, human	(110)
Caffeine	Stimulant	*N*-demethylation	Rat, dog, human	(111–113)
3-Methyl uric acid	...	*N*-demethylation	Rat, dog	(112)
Morphine	Analgesic	*N*-demethylation	Rat	(114)[a]
Methadone	Analgesic	*N*-demethylation	Dog	[b]
Pethidine	Analgesic	*N*-demethylation	Dog, man	[c]
α-*dl*-Acetylmethadol	Analgesic	*N*-demethylation	Rat	(19)
α-*dl*-nor-Acetylmethadol	Analgesic	*N*-demethylation	Rat	(19)
d-Propoxyphene	Analgesic	*N*-demethylation	Rat, mouse, human	(11, 115)
Ethoheptazine	Analgesic	*N*-demethylation	Rat	(116)
Y-535	Analgesic	*N*-demethylation	Rat	(117)
Dextromethorphan	Antitussive	*N*-demethylation and *O*-demethylation	Dog	(118)
Chlorcyclizine	Antihistamine	*N*-demethylation	Rat	(119)
Cyclizine	Antihistamine	*N*-demethylation	Rat	(119)
Deptropine	Antihistamine	*N*-demethylation	Rat	(120)
d-Methamphetamine	Sympathomimetic	*N*-demethylation	Dog	(121)
Ephedrine	Sympathomimetic	*N*-demethylation	Dog	(122)
Butynamine	Hypertensive	*N*-demethylation	Rat	(123)
Diphenamid	Herbicide	*N*-demethylation	Rat	(37)
Erythromycin	Antibiotic	*N*-demethylation	Rat	(83, 124)
Mepivacaine	Local anesthetic	*N*-demethylation	Rat	(125)
Methixene	Anticholinergic	*N*-demethylation	Rat	(126)
Imipramine	Antidepressant	*N*-demethylation	Rat, rabbit, dog, human, mouse	(31, 127, 128, 129)
Amitriptyline	Antidepressant	*N*-demethylation	Rat, mouse	(130, 131)
Nortriptyline	Antidepressant	*N*-demethylation	Rat	(98)
Thioridazine	Tranquilizer	*N*-demethylation	Rat	(132)
Promazine	Tranquilizer	*N*-demethylation	Dog	(133)
Chlorpromazine	Tranquilizer	*N*-demethylation	Rat, human	(134, 135)
Diazepam	Tranquilizer	*N*-demethylation	Dog, human	(39, 40)
N-Methyl-polythiazide	...	*N*-demethylation	Rat	(47)
Cotinine	...	*N*-demethylation	Dog	(136)

Continued on next page.

Several facts support this point of view. For example, Hanaki and Ishidate (74, 75) have developed a chemical system consisting of ascorbic acid and chelated iron which will demethylate dimethylaminoazobenzene through an intermediate *N*-oxide. Furthermore, studies with the microsomal system have shown that it can indeed form *N*-oxides from certain tertiary amines (76–78). Finally, it has been reported (79) that dimethylaniline *N*-oxide is readily converted to methylaniline and formaldehyde in the presence of liver microsomes. [This report, however, conflicts with an earlier report of Gillette (80) who found dimethylaniline *N*-oxide to be a poor substrate for these systems.]

There are, however, a number of observations which cast doubt on the *N*-oxide mechanism. Although this mechanism would be satisfactory for the dealkylations of tertiary amines, other mechanisms must be evoked to explain the dealkylation of the many other types of compounds which are dealkylated by microsomes. Another difficulty arises from the extensive work of Kiese on the oxidation of aniline derivatives by liver microsomes. He has shown (81) that the k_m value for oxygen in the *N*-oxidation reaction is some 40 times greater than it is for the *N*-dealkylation reaction. This would seem to rule out *N*-oxidation as an intermediate step in *N*-dealkylation. Recently McMahon and Sullivan (82) found that the microsomal demethylation of radiocarbon labeled *l*-propoxyphene in the presence of a pool of cold *l*-propoxyphene *N*-oxide did not result in the incorporation of radioactivity into the oxide pool. This observation also argues against the involvement of an *N*-oxide intermediate in dealkylation. An analogous study with erythromycin and erythromycin-*N*-oxide led Mao and Tardrew (83) to the conclusion that the *N*-oxide was not a reaction intermediate.

Also if *N*-oxide formation occurred as the rate-determining step, an isotope effect such as that seen in the demethylation of trideuteriomorphine (84) would not be expected. In addition the observation (12, 13) that steric interference about the nitrogen atom does not affect the re-

TABLE III.—(*Continued*)

Compd.	Classification	Dealkylation Reaction	Species	Ref.
Dimethylaminoazobenzene	Carcinogen	*N*-demethylation	Rat	(137)
Aminopyrine	Antipyretic	*N*-demethylation	Rat	(138)
N-Ethylbarbital	Sedative	*N*-deethylation	Dog	(139)
Benzquinamide	Neurosedative	*N*-deethylation and *O*-demethylation	Dog, man	(140, 141)
3,5-Diethyl-5-phenylhydantoin	Anticonvulsant	*N*-deethylation	Dog	(142)
Chloroquine	Antimalarial	*N*-deethylation	Man	(143)
Diethylpropion	Ovulation stimulant	*N*-deethylation	Human	(144)
Trifluralin	Herbicide	*N*-depropylation	Rat	(145)
Chlorpropamide	Antidiabetic	*N*-depropylation	Dog	(146)
N-Isopropyl-4-bromobenzene-sulfonamide	Anticonvulsant	*N*-deisopropylation	Mouse	(48)
Diallylmelamine-*N*-oxide	Vasodilator	*N*-deallylation	Dog	(147)
N-Allyl-4-bromobenzene-sulfonamide	Anticonvulsant	*N*-deallylation	Mouse	(48)
N-Dibenzyl-*β*-chloroethylamine	*α*-Adrenergic blocking agent	*N*-dechloroethylation	Dog	(148)
Butamoxane	*α*-Adrenergic blocking agent	*N*-debutylation	Dog	(149)
Ethoxybutamoxane	*α*-Adrenergic blocking agent	*N*-debutylation and *O*-deethylation	Rat, dog	(26)
Chlorethoxybutamoxane	*α*-Adrenergic blocking agent	*N*-debutylation and *O*-decthylation	Rabbit, dog, rat, mouse, guinea pig	(27)
6-Methylthiopurine	Antimetabolite	*S*-demethylation	Man, rat	(150, 151)
Mescaline	Hallucinogen	*O*-demethylation	Man	(152)
Brucine	Convulsant	*O*-demethylation	Rabbit	(153)
Codeine	Analgesic	*O*-demethylation	Rats	(154)
Biochanin A	Estrogen	*O*-demethylation	Rat	(155)
p-Nitroanisole	...	*O*-demethylation	Rat	(156)[d]
Phenacetin	Antipyretic	*O*-deethylation	Rabbit, man	(157, 158)
O-(2-Ethoxy)benzamide	Antipyretic	*O*-deethylation	Man	(159)
Benzyl-*p*-nitrophenyl ether	...	*O*-debenzylation	Rat	(32)[e]

[a] Although the metabolism of morphine has been studied very extensively, there is at present little evidence that *N*-demethylation is a significant pathway. For example, Rapoport (personal communication) has investigated the metabolism of radiomorphine in humans with some care and has found no normorphine among the metabolites. For a complete discussion of the metabolism of morphine and of other analgesics, see Way and Adler (160). [b] Pohland, Sullivan, and Lee, personal communication. [c] For complete bibliography see *Reference 160*. [d] For a discussion of the demethylation of substituted anisoles, see Williams (161). [e] These workers (32) have studied the *in vivo* cleavage of a wide variety of alkyl and arylalkyl-*p*-nitrophenyl ethers.

action rate argues against the *N*-oxide mechanism.

On the basis of data available at present it seems likely that *N*-oxidation and *N*-dealkylation represent alternative pathways for the metabolism of tertiary amines. In this connection the recent reports by Ziegler and co-workers (77, 78) are of interest. They have shown that cholate treatment of microsomes does not destroy the *N*-oxidizing capacity of the microsomes, although such a treatment frequently results in the loss of dealkylase and hydroxylase activity. Thus, the *N*-oxidizing capacity of microsomes may well be a separate system from that required for dealkylation.

Mention should be made of the suggestion of Oae (85, 86) that the demethylation of methionine might proceed through a sulfoxide intermediate (Pummerer reaction). Since methionine is metabolized by methyl transfer rather than methyl oxidation, such a mechanism would not be applicable for its demethylation, but the Oae work does nevertheless raise the question of possible involvement of sulfoxides in microsomal *S*-demethylation.

At the present time the microsomal dealkylation reaction is best understood as a hydroxylation reaction:

$$RXCH_2R' \xrightarrow[\text{oxygen''}]{\text{''Active}} RXCHOHR \rightarrow RXH + O{=}\overset{H}{C}{-}R'$$

X = *O*, *S*, or *N*.

This type of mechanism is attractive for several reasons. For example, it recognizes the close relationship between dealkylation and the microsomal hydroxylation reaction which it so closely resembles. It is also a general mechanism applicable regardless of the nature of X or R. In addition there exists ample analogy in both organic chemistry and biochemistry for the oxidative attack upon a saturated carbon atom.

Although considerable progress has been made in recent years in understanding the oxygen activating system of microsomes, the nature of

the "active oxygen" species that is formed is still obscure. Early investigators, including Udenfriend (87), Mason (88), Hayano (89), and Corey (90) discussed microsomal hydroxylation in terms of heterolytic mechanisms, but more recently free radical intermediates have received more attention. From results with nonenzymatic hydroxylation systems, Breslow and Lukens (91) have suggested hydroxyl radicals as the active hydroxylating species. On the basis of both enzymatic and nonenzymatic hydroxylation studies, Staudinger (92) has suggested the active intermediate to be either the OH or the OOH radical. Evidence for a free radical mechanism has also been presented by Nilsson, Orrenius, and Ernster (93). Finally Gillette (7) reviewed the literature on microsomal oxidation of drugs and found a free radical mechanism to be most consistent with the facts as they are now known.

The interaction of free radicals with *N*-methyl groups has ample analogy in chemical studies (94–96). In fact, Needles and Whitfield (96) have recently shown that dimethylamides are readily demethylated by a free radical mechanism in aqueous solution containing persulfate ion. Further work with systems of this sort would be of considerable value since they appear to be suitable models for the enzymic reaction.

The observation that *N*-dealkylation occurs even more rapidly than does *N*-demethylation (22, 23) is readily understandable in terms of a free radical mechanism, since the intermediate, resonance stabilized, allyl radical, $-\mathrm{N}(\dot{\mathrm{C}}\mathrm{H})-\mathrm{CH}=\mathrm{CH}_2$ would be expected to form readily. For the same reason the ready *O*-dealkylation of *O*-allyl, *O*-benzyl, and *O*-cyanomethyl aryl ethers (32) can be rationalized in terms of a free radical mechanism. It is interesting to note that the radical formed in *S*-demethylation would be substantially stabilized by electron-sharing resonance which is possible in the case of sulfur since it can expand its valence shell to nine electrons by utilizing a vacant 3d orbital (97).

Additional evidence for the free radical mechanism can be found in studies on side chain hydroxylation by microsomes. It is well known that (ω-1)-hydroxylation occurs to a much greater extent than does ω-hydroxylation. This may be due to the greater stability of the $-\dot{\mathrm{C}}\mathrm{H}\mathrm{CH}_3$ radical as compared to $-\mathrm{CH}_2\dot{\mathrm{C}}\mathrm{H}_2$. Also, there are many examples known in which hydroxylation occurs on a benzylic carbon instead of in the aromatic ring itself as might be expected (98–101). The known stability of benzyl radicals serves to explain these observations. In a number of studies of side chain hydroxylation in which a careful analysis of products has been made, it has been found that in addition to the major product smaller amounts of most other possible isomeric alcohols are also observed (102–104). Such nonspecificity is frequently observed in free radical reactions.

Additional support for a free radical mechanism has been recently presented by Hayaishi (105). He has proposed a generalized hypothesis for the action of oxygenases which involves a ternary complex of oxygen, ferrous iron, and substrate which reacts by a concerted one electron transfer to yield oxygenated substrate.

At present then it appears that microsomal dealkylation is best understood as a free radical hydroxylation reaction.

DEALKYLATION IN THE INTACT ANIMAL

In Table III is presented a listing of a variety of compounds that have been demonstrated to undergo dealkylation when administered to the intact animal. The listing is not comprehensive, but an effort has been made to include compounds of various chemical and physiological types.

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Research Articles

Pharmacological Activity of Thalicarpine

By R. A. HAHN*, J. W. NELSON, A. TYE, and J. L. BEAL

The effect of the alkaloid thalicarpine on the cardiovascular system of the anesthetized dog and on several smooth muscle preparations has been studied. In the dog, 2 mg./Kg. produced moderate pressor activity of rather long duration which was sometimes accompanied by a mild tachycardia. This effect does not appear to involve a neural pathway but may be due to a direct action either on the heart or on vascular smooth muscle. Intense, long lasting, noncholinergic hypotension was observed with doses of 10 mg./Kg. Direct depressant effects were seen on several smooth muscle preparations as well as reduction of spasmogenic effects induced by various drugs.

KUPCHAN *et al.* were the first to isolate the alkaloid thalicarpine and to describe its effect on mean arterial blood pressure of the cat (1). They reported that doses up to 5 mg./Kg. caused a transient lowering of blood pressure, while a dose of 10 mg./Kg. caused death. The hypotensive activity, in their opinion, was due to bradycardia, respiratory depression, and a weak adrenergic blocking action.

As part of a continuing study of the genus *Thalictrum* we have observed moderate pressor activity after the administration of 2 mg./Kg. of thalicarpine in the anesthetized dog. The pressor activity was sometimes accompanied by a mild tachycardia. A dose of 10 mg./Kg. was observed to produce an intense depressor response and bradycardia, with some degree of hypotension being observed for 1–3 hr. During this time of prolonged hypotension, no evidence of toxic symptoms was observed. The administration of thalicarpine to the anesthetized cat always produced a transient depressor response.

Reported here are the results of a study of the action of thalicarpine on mean arterial blood pressure of the anesthetized dog and on several smooth muscle preparations.

EXPERIMENTAL

Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital (35 mg./Kg., i.p.). After surgical anesthesia was achieved the trachea was cannulated and bilateral cervical vagotomy performed. The right carotid artery was cannulated and blood pressure recorded *via* a mercury manometer on a kymograph. The right femoral vein was then cannulated with a 3-in. length of polyethylene tubing for the injection of drug solutions. When infusions were administered the left femoral vein was cannulated and the infusion given by means of a Harvard infusion pump. Heart rate was recorded by means of a Sanborn Twin Viso-

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