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LETTER TO THE EDITOR

Metabolic incorporation of oxygen into primary and secondary aliphatic amines and the consequences in carbon-nitrogen bond cleavage

Recently we proposed a mechanism for the metabolic *N*-oxidation of the primary aliphatic amines phentermine and chlorphentermine to their hydroxylamine- and *C*-nitro-compounds; a complex of an anion (oxygen radical/flavoprotein) with the nitrogen radical cation which gave both the nitroso and the hydroxylamine compounds by separate routes was implicated (Beckett & Bélanger, 1974). These amines have quaternary *C*-atoms attached to the basic centre.

We now extend the above scheme to delineate general pathways for the metabolism of primary (1°) aliphatic amines to yield the *N*-oxygenated products, i.e. oximes, hydroxylamines and *C*-nitroso compounds as well as the deaminated products, ketones or aldehydes. Also the extended scheme explains the metabolism of secondary (2°) aliphatic amines to yield the *N*-oxygenated products, i.e. 2° and 1° hydroxylamines and nitrones as well as the dealkylated and deaminated products. Our conclusions of a common metabolic *N*-oxidative pathway for 1° and 2° aliphatic amines, involving enzymes different from those responsible for *N*-oxidation of tertiary (3°) aliphatic amines, have been based on the following results from our laboratories and recently published information from others.

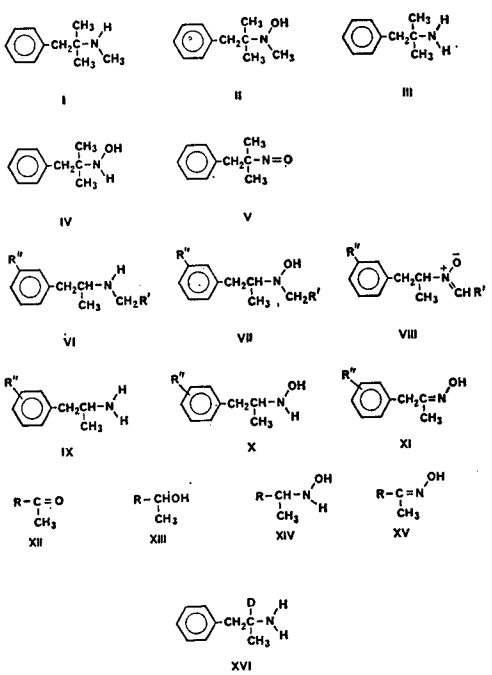


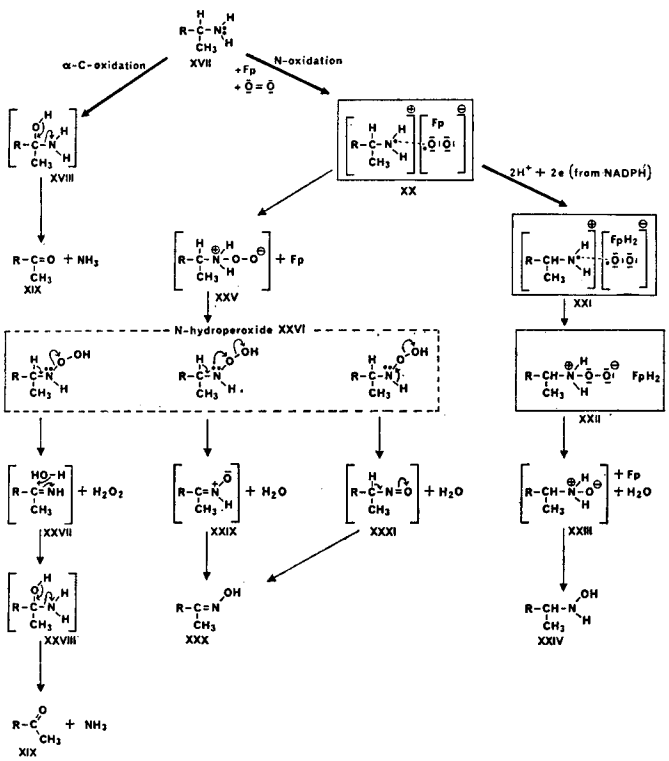
FIG. 1. Structures of some primary and secondary aliphatic amines and their metabolic products.

1. Incubation of phentermine (III) with hepatic microsomes gave much more of the nitroso compound (V) than of the primary hydroxylamine (IV) (Beckett & Bélanger 1974), while incubation of mephentermine (I) gave no nitroso compound (V) but even, more of the primary hydroxylamine (IV) than from phentermine; also the 2° hydroxylamine (II) and the 1° amine (III) were formed from mephentermine.

2. Incubation of *N*-alkylamphetamines (VI, R″=H) or *N*-alkylnorfenfluramines (VI, R″=CF_3) with hepatic microsomal fractions gave the 2° hydroxylamines (VII, R″=H or CF_3) and the nitrones (VIII, R″=H or CF_3) (Beckett, Coutts & Ogunbona, 1973; Ogunbona, 1973); the ratio of the nitrones to the 2° hydroxylamines *increased* as the N-alkyl chain was progressively increased. Incubation of these 2° hydroxylamines also gave nitrones, but the rate of formation of nitrone *decreased* as the *N*-alkyl chain was increased. Thus nitrones (VIII) were not derived solely from the metabolism of the 2° hydroxylamines (VII) produced when the 2° amines (VI) were being metabolized.

3. *In vitro* metabolism of various 1° amines of the "amphetamine type" (IX) gave the 1° hydroxylamines (X) and the oximes (XI); the ratio of these metabolic products varied with the animal species used. Although *chemical* change from (X) to (XI) can occur, the conditions used in the metabolic studies and in the subsequent analyses were chosen to preclude such chemical changes (Chissick, 1973).

4. When, in the presence of $^{18}O_2$, (+)-amphetamine and β,β-difluoroamphetamine were incubated with microsomal fractions from rabbit liver, and 2,3-dichloro-α-



Scheme 1 for the metabolic α-*C*-oxidation of 'amphetamines' to give deamination (O from air) and of metabolic *N*-oxidation to a complex which results in subsequent changes under neutral aqueous conditions to give deamination (O from H_2O) and oxime (O from air) and primary hydroxylamine (O from air). R = $ArCH_2$, Fp = flavoprotein. → = metabolic routes → = chemical changes.

methyl benzylamine with microsomal fractions of rat liver, only 30–45% of the $^{18}O_2$ was incorporated into the ketones (XII) (or 2° alcohols, XIII) formed, whereas incorporation into the hydroxylamines (XIV) and oximes (XV) formed was virtually complete. The incomplete incorporation of $^{18}O_2$ into the ketone (or alcohol) indicates that 60 to 70% of the ketone is derived by a hydrolytic mechanism not involving the oxime (XV) which is known not to be metabolized to any great extent to the ketone (XII) (Parli & McMahon, 1973).

When (±)-amphetamine and (±)-amphetamine-C_2D (XVI) were incubated with rabbit hepatic microsomes the replacement of C_2H by C_2D (deuterium) caused a decrease in the rates of formation ($\mu g\ g^{-1}$ of liver $30\ min^{-1}$) of the ketone (XII) from 1·8 to about 0·4, and of the oxime (XV) from 4·1 to 1·5, but increased the formation of hydroxylamine (XIV) from 2·6 to 6·9; the total of the oxidation products was not affected (Parli & McMahon, 1973).

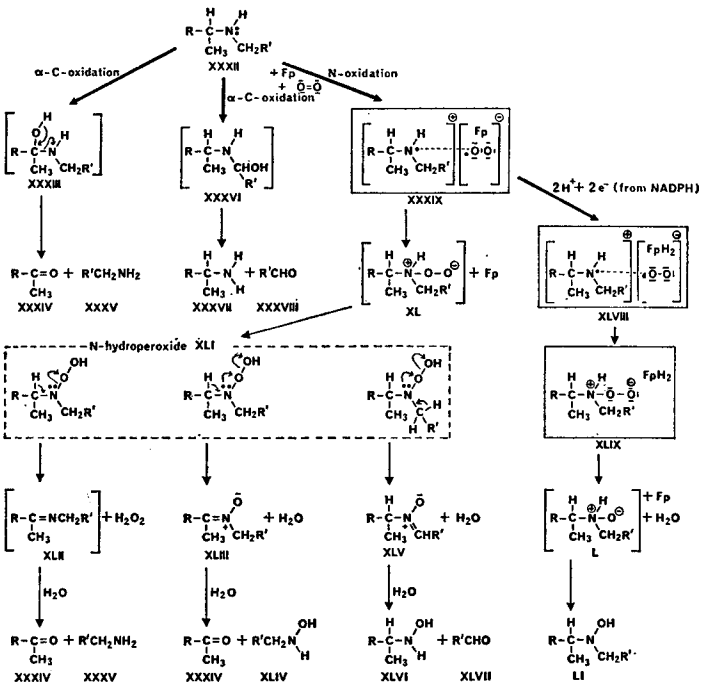
5. The incubation with hepatic microsomal fractions of mixtures of one 1° aliphatic amine with another 1° aliphatic amine, e.g. phentermine with chlorphentermine, norephedrine or "mexiletene" [1-(2,6-dimethyl)phenoxy-2-aminopropane] or with another 2° aliphatic amine, e.g. dibenzylamine, (+)- and (−)-fenfluramine, in equimolar proportions showed that there was mutual interference with the *N*-oxidation pathway; various combinations of compounds gave similar results (Beckett unpublished). Thus there appears to be a common metabolic route for the *N*-oxidation of aliphatic amines of diverse structures.

The results of *N*- and *C*-metabolic oxidation of 1° aliphatic amines possessing at least one H-atom on the α-C-atom may be rationalized by Scheme 1. Metabolic α-*C*-oxidation of (XVII) yields the unstable alkanolamine (XVIII) which readily eliminates ammonia to give the ketone (XIX); by this route the oxygen of the ketone is obtained from the air and thus virtually 100% ^{18}O incorporation will occur in the ketone if $^{18}O_2$ is used.

The free radical ion complex (XX) is similar to that described for phentermine (Beckett & Bélanger, 1974). The complex should be more stable than that for phentermine because it suffers less steric inhibition, i.e. Me changed to H and thus the route to the reduced complex (XXI), and therefore to the hydroxylamine (XXIV), should be emphasized at the expense of dissociation to the *N*-hydroperoxide (XXVI).

The *N*-hydroperoxide (XXVI) will decompose readily in aqueous neutral solution by three different routes Scheme 1. One route will yield the imine (XXVII) with hydrogen peroxide also being formed, the former immediately hydrolysing to the ketone (XIX). The ketone produced by this route derives its oxygen from the water, i.e. ^{16}O and would not contain ^{18}O from the air containing $^{18}O_2$. A second route is via the unstable XXIX which will rearrange immediately to the oxime (XXX). A third route will yield the nitroso compound (XXXI) and, unlike the case with phentermine (Beckett & Bélanger, 1974), an α-hydrogen is available and so rearrangement to the oxime (XXX) will occur. In some "amphetamines" however, if the nitroso compound (XXXI) can readily dimerize, it is possible to isolate the nitroso dimer from the incubation media. The oxygen in the oxime (XXX) produced from either of the routes from (XXVI) is from the air and will contain virtually 100% ^{18}O if $^{18}O_2$ is used. In this scheme, the hydroxylamines (XXIV), e.g. *N*-hydroxyamphetamines and the oximes (XXX), are both derived from the same metabolic precursors (XX).

The replacement of the α-C-H atom by the α-C–D atom will slow down the rate of α-C-oxidation of XVII to XVIII in relation to that of *N*-oxidation to (XX) because of the increased difficulty of cleaving the C–D bond; thus the contribution to ketone production of the α-C-oxidation route will decrease. The decreased importance of the oxime formation (see Parli & McMahon, 1973) (and of ketone production via *N*-oxidation) upon replacing C–H by C–D indicates greater stability of the complex (XX)



Scheme 2 for the metabolic α-*C*-oxidation of *N*-alkylamphetamines to give deamination (O from air) and dealkylation, and of the metabolic *N*-oxidation to a complex which results in subsequent changes under neutral aqueous conditions to give deamination (O from H_2O), dealkylation and hydroxylamine (O from air) and nitrone (O from air) formation. R = $ArCH_2$, Fp = flavoprotein R′ = H, alkyl or aralkyl. → = metabolic routes, → = chemical changes.

or improved reduction of this complex so that the contribution of the route XXI→XXII →XXIII→hydroxylamine (XXIV) is increased at the expense of the hydroperoxide (XXVI) route.

The results of metabolic oxidation on the nitrogen and α-carbon atoms in *N*-alkylaliphatic amines, e.g. *N*-alkylamphetamines (XXXII), can be rationalized as shown in Scheme 2. One route of α-*C*-oxidation will yield the alkanolamine (XXXIII) which will be converted under neutral aqueous conditions to the ketone (XXXIV) and the 1° amine (XXXV); the other route of α-*C*-oxidation will yield XXXVI which will rapidly eliminate the aldehyde (XXXVIII) to give the 1° amine (XXXVII). The ketone and the aldehyde produced by these routes derive their oxygen from the air and will contain virtually 100% ^{18}O if $^{18}O_2$ is used.

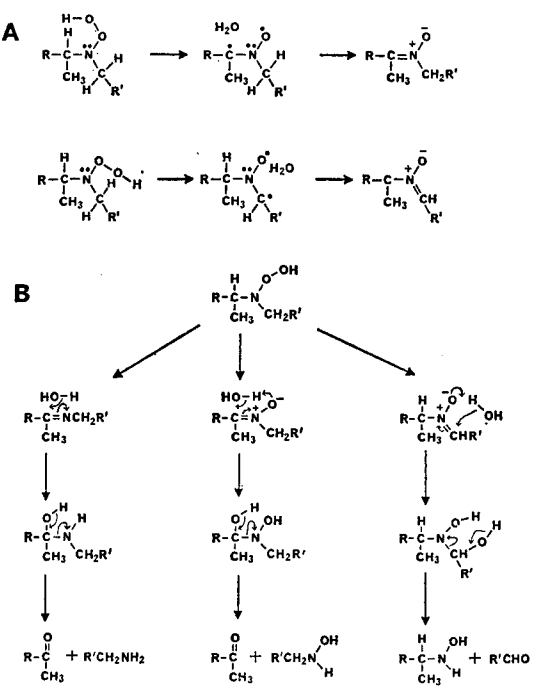
N-Oxidation will give the free radical ion complex (XXXIX) which can be reduced to the reduced complex (XLVIII) which proceeds via XLIX and L to the 2° hydroxylamine (LI); such hydroxylamines are reasonably stable. In addition, dissociation of the complex XXXIX will yield via XL the unstable *N*-hydroperoxide (XLI) which can decompose in neutral aqueous solution in three different ways. One route will give hydrogen peroxide plus the unstable *N*-alkylimine (XLII) which will be readily hydrolysed to the ketone (XXXIV) and the 1° amine (XXXV); the ketone formed via this route derives its oxygen from the water i.e. ^{16}O and will not contain ^{18}O from $^{18}O_2$ in air. The amine (XXXV) is obviously produced by both α-*C*-oxidation via XXXIII and *N*-oxidation via XLI and XLII. The *N*-hydroperoxide XLI can also eliminate H_2O_2 to give an imine with its double bond towards group R′; subsequent hydrolysis

will give the primary amine XXXVII and the aldehyde XXXVIII i.e. the compounds also resulting from α-C-oxidation of XXXII to give XXXVI.

The *N*-hydroperoxide (XLI) can also decompose to the nitrone (XLIII)*; investigations (Beckett & Morgan, unpublished findings) have shown that nitrones of this structure are readily converted at neutral pH to the ketone (XXXIV) and the 1° hydroxylamine (XLIV); the ketone (XXXIV) will have negligible incorporation but the hydroxylamine (XLIV) about 100% incorporation of ^{18}O from air containing $^{18}O_2$.

The third route leads to the nitrones (XLV) which are reasonably stable under neutral aqueous conditions if R′ is other than H; stability of the nitrone increases with the size of the alkyl group R′. If R′=H then hydrolysis will occur in water to yield the 1° hydroxylamine (XLVI) and the aldehyde (XLVII); the oxygen of the hydroxylamine will be from the air but that of the aldehyde will be from water. The probable mechanism of hydrolysis of the imine (XLII) and the two nitrones (XLIII and XLV) to show the origin of the oxygen atom is shown in Scheme 3B. It is probable that nitrones are formed not by ionic mechanisms but by homolytic fission of the O–O bond and the α-C–H bond in the hydroperoxide with elimination of water to give the biradical; electron redistribution gives the nitrones (Scheme 3A).

Increasing the size of R′ would be expected to reduce the stability of the complex (XXXIX) and thus increase the formation of the nitrone (XLV) at the expense of the



Scheme 3A. Free radical mechanism of formation of nitrones from *N*-hydroperoxide intermediates.

3B. Routes and mechanisms for the chemical changes under neutral aqueous conditions of the *N*-hydroperoxide derived from the metabolic *N*-oxidation complex of *N*-alkylamphetamines.

* It is probable that nitrones are formed not by ionic mechanisms but by homolytic fission of the O-O bond and the α-C–H bond in the hydroperoxide with elimination of water to give the biradical; electron redistribution gives the nitrones (Scheme 3A).

hydroxylamine (LI); results agree with this deduction. Upon incubation of the 2° amine (XXXII, R′=H), the 1° hydroxylamine (XLVI) will be formed primarily from the *N*-hydroperoxide (XLI) and also later during metabolism by *N*-oxidation of the 1° amine (XXXVII) produced by *N*-dealkylation of XXXII; results with *N*-methyl-"amphetamines" and *N*-methylphentermine (mephentermine) are thus explicable.

The above schemes for the rationalizing of the observed products of metabolic oxidation of 1° and 2° aliphatic amines emphasise the role of a few key unstable metabolites and the variety of chemical changes which follow under normal conditions of metabolic studies.

N-Oxidation of aliphatic tertiary amines is concluded to involve liver enzyme systems differing from those responsible for the reactions described. The evidence is (Beckett, unpublished):

1. The presence of 3° aliphatic amines, e.g. phendimetrazine or nicotine or (+)- and (−)-dimethylamphetamine during incubation, did not interfere with the metabolic *N*-oxidation of the 1° or 2° aliphatic amines used in the studies reported.

2. The metabolic *N*-oxidation of 3° aliphatic amines is not inhibited significantly by the presence of *p*-chloromercuribenzoic acid under conditions which inhibit completely the *N*-oxidation of the above 1° and 2° amines.

3. Hepatic microsomes from guinea-pigs metabolize 3° aliphatic amines better than they do the above 1° and 2° aliphatic amines, but the reverse is true for hepatic microsomes from the rabbit.

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