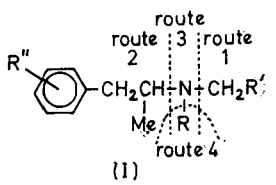
Metabolic oxidation on aliphatic basic nitrogen atoms and their α-carbon atoms—some unifying principles

Among the pathways of metabolism of amines of type (I), metabolic oxidative cleavage from nitrogen at 1 and 4 is usually considered as dealkylation (McMahon 1966), and at 2 as deamination (Axelrod, 1955), while oxidation of the nitrogen atom at 3, when tertiary, is considered as *N*-oxidation (Beckett, Gorrod & Jenner, 1971; Beckett, Mitchard & Shihab, 1971). Some workers consider that C–N cleavage occurs after migration of O from N to α-C atoms (Ziegler & Petit, 1964). Difficulties arise in interpretation because of inability to isolate unstable metabolites.



(I)

R = H or lower alkyl
R' = H, lower alkyl or aryl
R'' = H, CF_3, halogens, etc

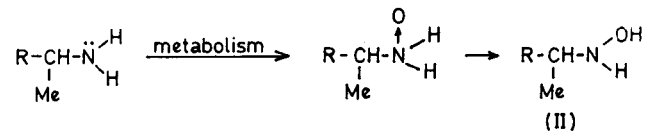
Many 'metabolites' have been identified from compounds of type (I), e.g. for benzylamphetamine (I; R=R''=H; R'=Ph), amphetamine, benzylamine, benzylmethylketone, benzaldehyde, α-methyl-β-phenylethanol, benzylalcohol, *syn*- and *anti*-benzylmethylketoximes, benzaldoxime, benzoic acid, but information is lacking concerning whether these arise by enzymatic action or by chemical changes subsequent to enzyme action.

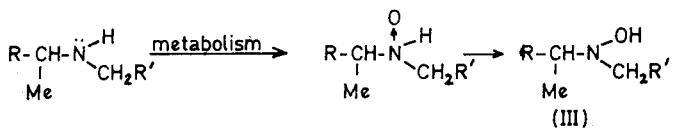
We have observed, for instance, that storage of aqueous solutions following incubation of I, R=R''=H, R'=Ph, with liver microsomal preparations led to large changes in the concentration of some of the 'metabolites' and these changes depended on the conditions of storage, e.g. at pH 12 at 4° for 72 h the concentrations of free oximes and benzaldehyde *increased* by several hundred per cent, the rate of increase being most rapid when the solutions were shaken. The concentrations of amphetamines and benzylamines sometimes increased by as much as 50 per cent on storage under these conditions but these increases were variable, while the concentrations of benzylmethylketone *decreased* and those of benzylalcohol remained constant. At pH 1 the concentrations of benzylmethylketone and benzaldehyde increased by at least 50 per cent, whereas the concentrations of the alcohols remained constant and oximes could not be detected even after storage; increases also occurred in the concentrations of some 'metabolites' when solutions were stored at pH 7·4. Increases were obtained in benzylmethylketoxime content on storage of solutions after incubation of *N*-alkyl amphetamines with liver microsomes; oximes were generated more quickly the smaller the group R' in (I). In acidic solutions, the concentrations of oximes decreased rapidly but the concentrations of benzylmethylketone increased (Beckett, Van Dyk & others, 1971). Organic solvent extracts of the solutions immediately after incubation yielded increasing amounts of oximes on shaking with alkali and increasing amounts of aldehydes on shaking with acid.

Thus, the measurement of the above substances after incubation of the parent compounds with liver microsomes may give misleading information about metabolic dealkylation or deamination of compounds of type (I) because the products of

chemical changes of metabolites* and the amount of chemical conversion is largely dependent upon the time after incubation that the analysis is made and the conditions of storage.

N-Oxidation of tertiary amines has been shown to be a metabolic route substantially inhibited by cysteamine and dithiothreitol in concentrations that have little effect on *N*-demethylation or *C*-oxidation (Beckett & others, 1971c; Gorrod & others, 1971); these inhibitors also substantially reduced the production of those metabolites which, when shaken in alkaline solution, gave oximes as metabonates, but they did not greatly reduce the formation of primary amines from metabolites of the secondary amines (I; R=H). The production of the metabolites which yield oximes from primary amines was also reduced by these inhibitors. Carbon monoxide inhibited the demethylation of methylamphetamine (I; R=R′=R″=H) without affecting the formation of oxime; SKF 525A (2-diethylaminoethyl-2, 2-diphenylvalerate hydrochloride) inhibited demethylation to a much greater extent than oxime formation. We therefore infer that the metabolites yielding oximes as metabonates from primary and secondary amines arise as follows:





Hydroxylamines of type II and III as primary metabolites are converted to ketoximes (R–C(Me)=NOH); III is also converted into traces of aldoximes (R′CH=NOH) as well as aldehydes (R′CHO) as metabonates. The rate of conversion depends on the temperature and alkalinity of the solution, upon the amount of shaking in air and on the presence of catalysts as well as upon the characteristics of group R′. Solutions containing the hydroxylamine metabolites and those containing synthetic hydroxylamines behaved similarly on polarography and by yielding oximes upon shaking in alkaline solution; on g.l.c. some breakdown of hydroxylamines to their corresponding oximes occurred. Amines were not produced when the hydroxylamines were stored in acidic, neutral or alkaline solution, and incubation of oximes and hydroxylamine derived from amphetamine did not yield amphetamine: amine metabonates must therefore have been derived from non-hydroxylamine metabolites.

We therefore conclude that these primary metabolites which yield amines from compounds of type I are produced by oxidation of the carbon α to the nitrogen atom

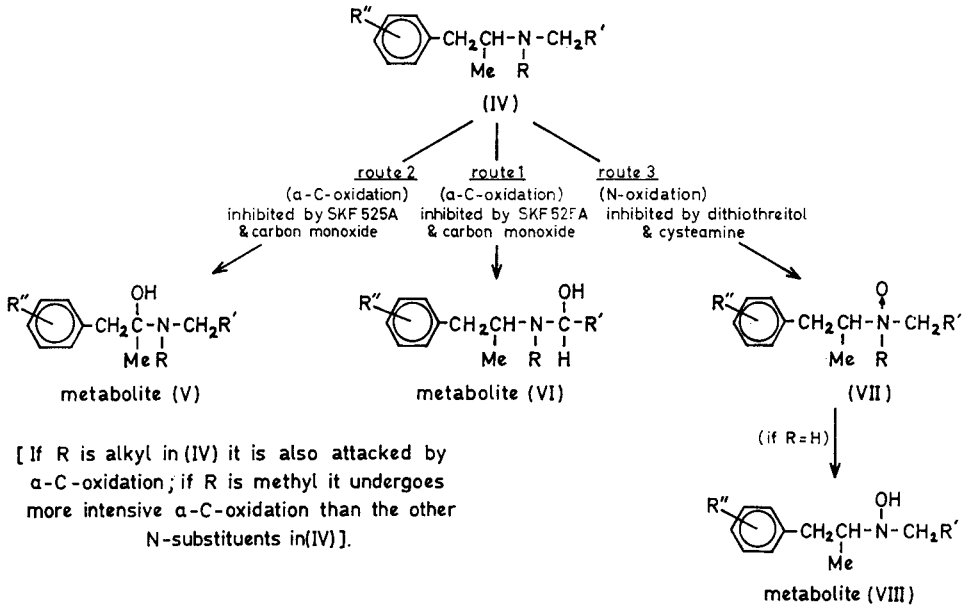
* It is necessary to distinguish between those substances formed directly by enzymatic action and those which result from chemical changes of these substances, especially if the chemical change is the rate controlling step in the formation of the compound which is finally determined.

The term *primary metabolite* is used to define a substance resulting directly from enzyme attack on an *added* substrate or a substrate formed chemically from a substance added to the biological system.

The term *secondary metabolite* will be used to define a substance derived from an enzymatic process utilising a metabolite as substrate.

Metabonate is used to define a substance which is a product of metabolism but is not a metabolite as defined above, i.e. it is formed as a result of non-enzymatic changes other than proton transfer in a metabolite, these changes occurring in the biological system or during isolation or during analytical procedures.

to yield $-N-\underset{\underset{OH}{|}}{C}-$ groups which are not very stable in neutral solution and are converted almost immediately in acidic solution to amines and ketones or aldehydes, depending upon the type of α–C group. If there are no H-atoms on the α–C-atom, this route of metabolism is not possible. Also hydroxy groups on β–C-atoms substantially inhibit the route.



Scheme 1

The metabolic oxidation on the basic nitrogen and its α-carbon atoms in aralkylamines is illustrated in scheme 1; the metabolism of benzphetamine (IV; R=Me, R′=Ph, R″=H) and benzylamphetamine (IV; R=R″=H; R′=Ph) to metabolites which are converted to metabolic products is representative of the general principles.

Incubation of benzphetamine with liver microsomes results in extensive production of benzylamphetamine and formaldehyde but much less production of those primary metabolites that yield amphetamine, benzylamine, benzylmethylketone or benzaldehyde as metabonates, i.e. the N–Me group undergoes α-C-oxidation before the other alkyl substituents. Incubation of benzylamphetamine (IV; R=R″=H; R′=Ph) yields the metabolite (V) which is converted at least 50% to the metabonates, ketone and benzylamine; after incubation, storage of the solution at acidic, neutral or alkaline pH values converts any remaining amounts of (V) to these compounds. In alkaline, but not acidic or neutral solutions, the concentrations of benzylmethylketone are slowly reduced by chemical attack to yield benzaldehyde. Only traces of benzylmethylketone produced from V are reduced to the corresponding secondary alcohol during incubation; this alcohol is regarded as a metabonate since it is not derived metabolically from an *added* substrate.

The metabolite VI, also produced during incubation of IV, suffers substantial breakdown during incubation to amphetamine and benzaldehyde but the latter is immediately reduced almost completely to benzyl alcohol. After incubation, storage of solutions of any remaining VI at acidic, neutral or alkaline pH values, yields amphetamine and benzaldehyde which remain as such, but at alkaline pH values

further amounts of benzaldehyde are produced from chemical oxidation of benzylmethylketone.

The hydroxylamine metabolite VIII is produced during incubation but is not changed significantly to metabonates during incubation. After incubation, storage of the neutral solution produces little conversion of VIII to oximes unless the solution is shaken; if the solution is made alkaline after incubation, and especially if the solution is shaken, there is rapid conversion of VIII to *anti* and *syn* benzylmethylketoximes, with the former predominating, and to small amounts of benzaldoxime. If the solution after incubation is made acidic, VIII yields no oximes.

The chemical conversions of metabolites to metabonates rather than the enzymatic conversion of substrates to metabolites are therefore the rate-controlling steps in the production of metabolic products from benzylamphetamine and substances of type I, and these rate-controlling steps are influenced greatly by changes in the conditions and environment of the metabolites in solution. Consequently, the relative importance of direct nitrogen oxidation and the various α-carbon oxidative metabolic routes can be established only by measurement of the metabolites directly, and, if these are unstable, by measurement of their metabonates only when complete conversion of metabolites to their metabonates has occurred.

Quantitative analysis of amphetamine, benzylamine, benzylmethylketone and ketoximes, benzaldehyde, benzaldoxime, benzyl alcohol and α-methyl-β-phenyl ethanol in solutions immediately after incubation of (+)-benzylamphetamine (IV; R″=R=H, R′=Ph) with rat liver microsomes plus soluble fraction, and at intervals after incubation during storage under different conditions until maxima for the concentrations of these compounds were obtained, indicated that the primary metabolites V, VI and VIII were produced in the ratio 0·5 to 1·1 to 1·0 respectively; about 90% of benzylamphetamine metabolized is accounted for by routes 1, 2 and 3 (scheme 1).

Substitution of the phenyl rings of IV alters the relative importance of oxidative routes 1, 2 and 3, as do changes in group R′ in IV from aryl to alkyl.

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Since preparing this work, two communications have been published that suggest the imine or the α-C–OH hydroxylamine are precursors of oximes in metabolic deamination (see: Hucker, H. B., Michniewicz, B. M. & Rhodes, R. E. 1971. *Biochem. Pharmac.*, **20**, 2123–2128; Parli, C. J., Wang, N. & McMahon, R. E. *Biochem. biophys. Res. Commun.*, **43**, 1204–1208.