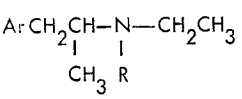
N-ethyl-α-methyl-α-(m-trifluoromethylbenzyl)nitrone—the major *in vitro* metabolic product of fenfluramine in guinea-pig liver microsomal fractions

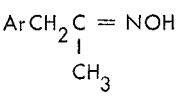
Many tertiary amines are metabolically oxidized *in vitro* and *in vivo* in various animal species to their *N*-oxides (Bickel, 1969; Beckett 1971). Primary amines including amphetamine (Beckett & Al-Sarraj, unpublished observations) and *p*-methoxyamphetamine (Beckett & Midha, unpublished observations) and secondary amines (Beckett, Van Dyk & others, 1971) also undergo metabolic *N*-oxidation to yield hydroxylamines. The hydroxylamines from primary amines are unstable, oxidation converting them to their corresponding oximes.

The anorexigenic secondary amine, fenfluramine (1a), is a derivative of ethylamphetamine. Its *in vivo* metabolism in man has been reported (Beckett & Brookes, 1967; Bruce & Maynard, 1968) but the presence of the *N*-hydroxylamine was not demonstrated. The *in vitro* metabolism of this amine by means of guinea-pig liver homogenates (10 000 *g* supernatant) is now described and a novel product, i.e. a nitrone, has been identified as being a major product of metabolism.

When an aqueous solution, pH 7·4, of fenfluramine was metabolized for 30 min with the 10 000 *g* supernatant of homogenized guinea-pig liver fortified with the necessary co-factors, approximately 60% of the drug was recovered unchanged. An ether extract of the metabolism mixture (pH 7·4) was chromatographed on a 2-metre



I (a) R = H
(b) R = OH



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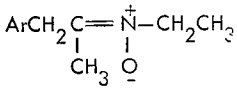

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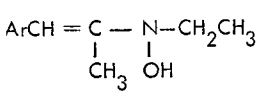

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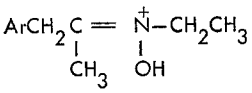

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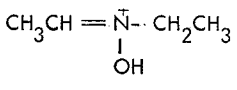

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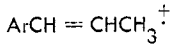

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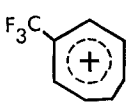

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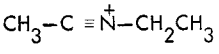

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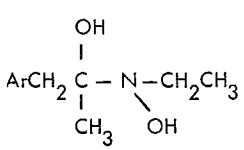

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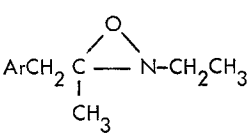

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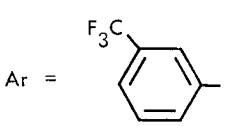

neutral glass column (Carbowax 7½% on Chromosorb W; column temperature 160°; N_2 flow rate 90 ml min^{-1}) and eight products in addition to unchanged fenfluramine were detected. Three of these products are now identified.

The major product (Compound C) had the longest retention time (20·3 min) and represented between 60 and 70% of the total metabolites. Product A (~3%) had a retention time of 10·1 min and product B (~7%) had a retention time of 18·0 min. Compound B was identified as (*m*-trifluoromethylbenzyl)methylketoxime (II) by comparing its thin-layer chromatographic (t.l.c.), gas-liquid chromatographic (g.l.c.) and mass spectral behaviour with those of an authentic sample of II. Compound A had a t.l.c. R_F value of 0·4 on silica gel [solvent chloroform: acetone (9 : 2)]. R_F values of the oxime (II), compound C and fenfluramine in the same system were 0·8, 0·2 and 0·0 respectively. The t.l.c. spot, R_F 0·4 (Product A), turned black immediately when sprayed with ammoniacal silver nitrate solution and red with triphenyltetrazolium chloride solution. When the black spot was extracted with ether and the extract gas-chromatographed under the conditions described above, it was established that compound A had been oxidized by this treatment to compound C and the oxime (II). Compound A was also eluted with ether from unsprayed t.l.c. plates and reduced with titanous chloride to yield the parent amine, fenfluramine. When treated with 20% sodium hydroxide solution for 2 h, compound A was converted almost entirely to compound C, together with a small amount of the oxime (II); prolonged treatment gave the oxime as the sole product.

A correlation of the properties with those observed for the hydroxylamine metabolites of other primary amines (Beckett & Al-Sarraj, unpublished observations) indicated that product A was *N*-hydroxyfenfluramine (Ib) while compound C was a simple oxidation product of Ib.

Eluates from the g.l.c. column were fed directly into a mass spectrometer and the mass spectra of compounds A, B and C were recorded. The mass spectrum of metabolite A was consistent with this product being *N*-hydroxyfenfluramine (Ib). Two fragment ions in the spectrum were of particular diagnostic value, an $[M - 1]^+$ ion (5% rel. abund.) located at *m/e* 246 (i.e. V), and the base peak in the spectrum of *m/e* 88 (i.e. VI).

The mass spectrum of product B was identical to that of an authentic sample of the oxime (II).

The mass spectrum of compound C possessed diagnostic peaks at *m/e* 245 (3%) $[M^+]$, 230 (3%) $[M - 15]^+$, 186 (84%), 159 (100%) and 70 (63%). The last three ions are identified as VII–IX respectively. All five ions are readily derived from the nitrone structure (III) or its tautomer (IV).

N-Hydroxyfenfluramine (oxalate m.p. 152°) was synthesized from fenfluramine by the action of *m*-chloroperbenzoic acid. This synthetic product was identical in behaviour to product A on g.l.c. and t.l.c., and in its ready conversion to the nitrone (III).

It is concluded, therefore, that the major *in vitro* metabolic product of fenfluramine in the guinea-pig is *N*-ethyl-α-methyl-α-(*m*-trifluoromethylbenzyl)nitrone (III). Similar results are obtained using the 10 000 *g* supernatant from homogenized rat liver. The properties described above for compound C are consistent with a nitrone structure (see Hamer & Macaluso, 1964). Nitrones are known to be reduced by means of lithium aluminium hydride to hydroxylamines (Exner, 1955). When a sample of product C eluted from t.l.c. plates was so treated, the product was identified as *N*-hydroxyfenfluramine by its g.l.c. and t.l.c. behaviour.

The possibility that product C was 2-ethyl-3-methyl-3-(*m*-trifluoromethylbenzyl)-oxazirane (XI, i.e. an isomer of III) was considered but rejected in view of the fact that known oxaziranes do not give secondary hydroxylamines when reduced with

lithium aluminium hydride, and do not yield primary hydroxylamines or oximes when reacted with alkali (Emmons, 1957).

We classify the hydroxylamine Ib as a metabolite. It is possible that the nitrone (III) arises from N and α-C metabolic oxidation of Ia to form the dihydroxy compound (X) (i.e. a metabolite) which dehydrates to form the nitrone or alternatively the hydroxylamine metabolite Ib undergoes chemical oxidation on the α-C atom followed by dehydration. We thus classify the major product of metabolism, i.e. the nitrone III, as a metabonate.

Preliminary *in vitro* metabolism studies on other secondary amines derived from amphetamine indicate that both hydroxylamines and nitrones are also formed in these cases.

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