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The structure of nitrones† derived from amphetamines

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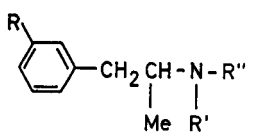
The nitrones obtained by mercuric oxide oxidation of *N*-hydroxy-*N*-n-propylamphetamine and *N*-ethyl-*N*-hydroxyamphetamine have been identified. The major *in vitro* metabolic product of fenfluramine is shown by a nuclear magnetic resonance study to be α-methyl-*N*-[1-(*m*-trifluoromethylbenzyl)ethyl]nitrone and not the isomer as claimed previously (Beckett, Coutts & Ogunbona, 1973a).

The major *in vitro* metabolic product of fenfluramine (Ia), an oxygenated product, was deduced to be a nitrone because of its facile reduction to *N*-hydroxyfenfluramine (Ib) by means of lithium aluminium hydride, and the ease with which it was regenerated from *N*-hydroxyfenfluramine by oxidation with yellow mercuric oxide (Beckett, Coutts & Ogunbona, 1973a). Clearly the nitrone possessed either structure IIa or IIIa but since the former, i.e. *N*-ethyl-α-methyl-α-(*m*-trifluoromethylphenyl)nitrone, can theoretically tautomerize to a conjugated system (IV), it was considered to be more stable than IIIa and was the structure assigned to the metabolic product. Furthermore, an examination of the mass spectrum of the metabolic product showed that the molecular ion expelled a methyl radical. This observation was also considered more in keeping with structures IIa or IV than with IIIa in view of the fact that this radical had been deduced to originate from the *N*-ethyl side-chain in IIa, since the related nitrone prepared from *N*-hydroxy-*N*-propylamphetamine (Ic) was found to expel an ethyl radical.



I

	R	*R'*	*R''*		*R*	*R'*	*R''*
(a)	CF_3	H	Et	(e)	H	OH	nPr
(b)	CF_3	OH	Et	(f)	H	OH	nBu
(c)	H	OH	Me	(g)	CF_3	OH	nPr
(d)	H	OH	Et	(h)	CF_3	OH	nBu

However, further studies by us on other nitrones prepared from various amphetamines (Beckett, Coutts & Ogunbona, 1973b), and additional mass spectral evidence,

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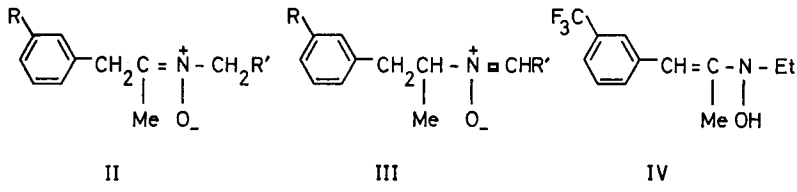
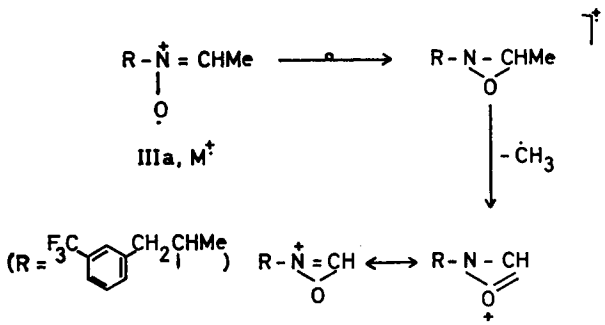
† The compounds are named as nitrones (Hamer & Macaluso, 1964) rather than the alternative naming as derivatives of amine or imine *N*-oxides.



II III IV

	(a)	(b)	(c)	(d)
R	CF_3	H	H	H
R′	Me	Ph	Me	Et

now lead us to conclude that structures IIa and IIIa cannot be differentiated by means of mass spectral data, especially since Neiman, Zhukova & others (1970) have presented evidence that nitrones form oxazirane structures in the mass spectrometer. A mechanism for the expulsion of a methyl radical from the molecular ion of IIIa then becomes feasible (Scheme 1).



Scheme 1

MATERIALS AND METHODS

Gas chromatographic analyses were performed on a Perkin-Elmer Model F-30 instrument with a flame-ionization detector. A glass column, length 2·0 m, o.d. 0·25 inch containing 7½% Carbowax 20M on Chromosorb W, acid-washed and DCMS-treated, was employed at an oven temp. of 160°, injection and manifold temp. 200°, and at the following flow rates or pressures: N_2 90 ml min^{-1}, H_2 20 lb $inch^{-2}$, air 25 lb $inch^{-2}$. Nmr spectra were recorded in $CDCl_3$ by Mr. G. R. McDonough on a Hitachi-Perkin-Elmer R-24 or R-32 instrument using TMS as internal standard.

The refractometer employed was a Hilger Abbé instrument and the ultraviolet spectrum was recorded on a Unicam Model SP800 spectrophotometer.

α-Methyl-N-[1-(*m-trifluoromethylbenzyl*)*ethyl*]*nitrone* (*IIIa*). *N*-Hydroxyfenfluramine acid oxalate (60 mg) was dissolved in water (200 ml) and excess yellow mercuric oxide (60 mg) was added. The suspension was stirred (magnetic stirrer) for 30 min at room temperature (20°), then filtered. The solid remaining was washed with water (2 × 10 ml) and the washings combined with the filtrate. The aqueous solution was extracted with ether (4 × 40 ml), the combined ether extract was dried (anhyd. K_2CO_3) then evaporated to give a yellow oil (48 mg) $n_D^{23} = 1{\cdot}490$, λmax (ethanol): 210 (3·87), 232 (3·65), 263 (3·04), 270 (3·01), 285 sh (2·83) nm (log ε). A g.c. trace of an ether solution of a portion of the oil showed one major peak [retention time (Rt) = 20·3

min], and traces of oxime (Rt = 18·0 min). Nmr (in $CDCl_3$): δ 1·51 (d, 3H, J = 6·5, CH_2CHCH_3); 1·85 (d, 3H, J = 6·5, $=CHCH_3$); 2·50–3·60 (m, 2H, CH_2); 3·70–4·20 (m, 1H, CH_2CHCH_3); 6·43 (q, 1H, J = 6·5, $=CHCH_3$); 7·42 (s, broad base, 4H, C_6H_4). Irradiation at δ 1·85 caused the δ 6·43 quartet to collapse to a singlet; irradiation at δ 1·51 caused the δ 3·70–4·20 multiplet to collapse to a doublet of doublets, δ 4·02 (J = 5) and δ 3·92 (J = 4·5). (Calcd for $C_{12}H_{14}F_3NO$: C, 58·78; H, 5·75; N, 5·71. Found: C, 58·47; H, 5·86; N, 5·58.)

An ethereal solution of the nitrone was prepared and portions of this solution equivalent to 2 mg of nitrone were added to six tubes each containing 2 ml dil. hydrochloric acid (pH 2). The ether was removed by bubbling nitrogen through each solution. The tubes were shaken for different periods of time (0, 5, 10, 15, 20 and 60 min). At the end of each period, the pH of the solution was quickly adjusted to 7·0 ± 0·5 with ammonia and each solution was extracted with ether (3 ml). The ether extracts were concentrated to 50 μl and 3 μl samples were examined gas-chromatographically. The quantities of products in each solution, as indicated by relative peak areas, are listed in the table.

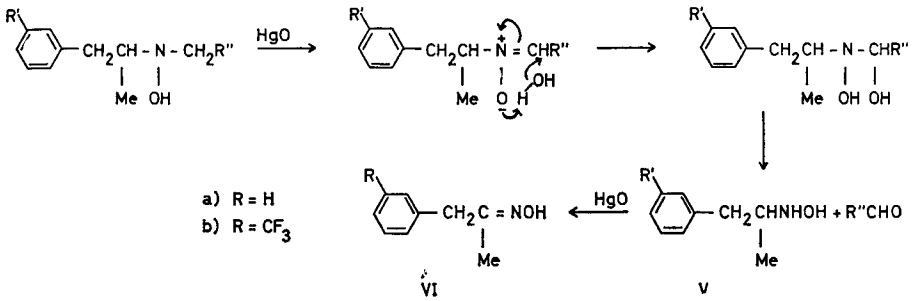
N-(*1-Benzylethyl*)-α-*ethylnitrone* (IIId) was prepared from *N*-hydroxy-*N*-n-propylamphetamine as a yellow oil in the manner described for nitrone IIIa. A g.c. examination of a solution of the oil showed that it contained two products; the major one (90%) was the nitrone (IIId), Rt = 30·5 min, and the minor product (10%) the oxime (VIa), Rt = 21·3 min. The nmr spectrum (in $CDCl_3$): δ 0·89 (t, 3H, J = 6·5, CH_2CH_3); 1·46 (d, 3H, J = 6·5, $CHCH_3$); 2·00–3·55 (m, 4H, CH_2 groups); 3·60–4·36 (m, 1H, $CHCH_3$); 6·27 (t, 1H, J = 6·5, N = C*H*); 7·14 (s, 5H, C_6H_5). Additional small singlets at δ 1·77 and 3·50 were shown, by comparison with an nmr spectrum of authentic material, to derive from the CH_3 and CH_2 groups of the phenyl-2-propanone oxime (VIa) contaminant.

N-(*1-Benzylethyl*)-α-*methylnitrone* (IIIc) was prepared from *N*-ethyl-*N*-hydroxyamphetamine as a yellow oil, containing a small amount of oxime (VIa) contaminant, in the manner described for IIIa. Nmr (in $CDCl_3$): δ 1·46 (d, 3H, J = 6·5, CH_2CHCH_3); 1·85 (d, 3H, J = 6·5, N=$CHCH_3$); 2·40–3·55 (m, 2H, CH_2); 3·60–4·30 (m, 1H, CH_2CH); 6·39 (q, 1H, J = 6·5, N = C*H*); 7·16 (s, 5H, C_6H_5). Rt = 27·6 min.

RESULTS AND DISCUSSION

Chemical and physical evidence has now been accumulated in favour of structure IIIa for the nitrone produced metabolically from *N*-hydroxyfenfluramine. We have found that aqueous solutions of all the *N*-alkyl-*N*-hydroxyamphetamines (Ib—Ih) are readily oxidized to nitrones in the presence of yellow mercuric oxide (Thesing & Mayer, 1957), but if sodium hydroxide is added to the solution, the nitrone is converted mainly to the corresponding oxime (VI). This nitrone and oxime formation was monitored by means of gas chromatography and mass spectrometry. The formation of both products can best be rationalized as illustrated in Scheme 2. This interpretation indicated the necessity of obtaining pure samples of nitrones in quantities sufficient for hydrolysis studies and nmr examination in order to decide which of structures II and III was the correct one for the nitrone oxidation products of *N*-hydroxyamphetamines.

The action of *m*-chloroperbenzoic acid on *N*-benzylamphetamine yields a solid nitrone (Beckett, Coutts & Ogunbona, 1973c), for which structure IIIb was expected

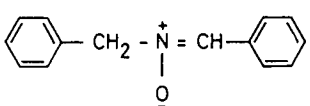


Scheme 2. Mercuric oxide oxidation of secondary hydroxylamines

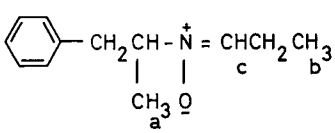
since it represented a system in which the nitrone double bond was in conjugation with the α-phenyl group. An examination of the nuclear magnetic resonance (nmr) spectrum of this nitrone confirmed the validity of structure IIIb. In particular, the methyl signal was a 3-proton doublet (δ 1·55, $J = 6{\cdot}5$ Hz) and the methine signal (= C*H*Ph) came to resonance as a 1-proton singlet far downfield (δ 7·04). The latter chemical shift is consistent with a proton in the environment depicted in structure IIIb; the methine proton signal of *N*-benzyl-α-phenylnitrone (VII) resonates at a similar downfield position (Beckett & others, 1973c).

A pure sample of the nitrone previously identified as IIa (Beckett & others, 1973a) was obtained by mercuric oxide oxidation of *N*-hydroxyfenfluramine in a quantity sufficient for analysis ($C_{12}H_{14}F_3NO$) and nmr examination. It gave a single peak when examined by gas chromatography. Its nmr spectrum confirmed conclusively that it was α-methyl-*N*-[1-(*m*-trifluoromethylbenzyl)ethyl]nitrone (IIIa) and not IIa as previously claimed, since both methyl groups appeared as doublet signals (δ 1·85, $J = 6{\cdot}5$ Hz; δ 1·51, $J = 6{\cdot}5$ Hz) and the methine proton (N=C*H*) was a quartet ($J =$ 6·5 Hz) which came to resonance in a downfield position (δ 6·43), comparable in chemical shift to that of the methine signal in IIIb. Spin-spin decoupling, by irradiating the δ 1·85 signal, caused this quartet to collapse to a sharp singlet indicating that the δ 1·85 and δ 6·43 protons were spin-coupled.

Final confirmation of the validity of the nitrone structure IIIa was obtained from a hydrolysis study. When the nitrone was hydrolysed in aqueous acid and aliquots were extracted and examined gas chromatographically, the major initial product of hydrolysis was *N*-hydroxynorfenfluramine (Vb) rather than ethylhydroxylamine as required by IIa. As time progressed, Vb was oxidized aerially to the oxime (VIb) (Table 1).



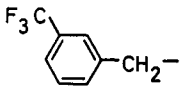
VII



IIId

Table 1. *Acid hydrolysis of nitrone IIIa.*

Tube	Shaking time (min)	Peak areas of hydrolytic products relative to unchanged nitrone considered as 100[1,2]		
		RCMe=NOH	RCHMe | NHOH	RCOMe
1	0	5	0	0
2	5	12	31	4
3	10	13	25	4
4	15	13	30	6
5	20	32	52	7
6	60	24	68	10

[1] R in formulae = 

[2] Rt of compounds, from left to right were 18·0, 13·2 and 2·7 min respectively at a column temp. of 160°. Rt of nitrone was 20.3 min.

Two other nitrones have been prepared in quantities sufficient for nmr examination. Mercuric oxide oxidation of *N*-hydroxy-*N*-n-propylamphetamine (Ie) gave the expected nitrone, the nmr spectrum of which confirmed its identity as *N*-(1-benzylethyl)-α-ethylnitrone (IIId) since the proton signal of one methyl group (a) appeared as a doublet, that of the other methyl group (b) as a triplet, and that of the methine group (c) as a triplet with appropriate chemical shift and coupling constant values.

A nitrone was also prepared by mercuric oxide oxidation of *N*-ethyl-*N*-hydroxyamphetamine (Id). Its nmr spectrum was similar to that of nitrone IIIa, i.e. both methyl groups again appeared as doublets (δ 1·85, $J = 6{\cdot}5$ Hz; δ 1·46, $J = 6{\cdot}5$ Hz), and the methine proton (N=C*H*) as a quartet (δ 6·39; $J = 6{\cdot}5$ Hz), thus establishing identity as *N*-(1-benzylethyl)-α-methylnitrone (IIIc).

None of the four nmr spectra (IIIa–IIId) was contaminated with signals which could be ascribed to the presence of isomeric nitrones (IIa–IId). This indicates that nitrones of general structure III are the major and probably the exclusive initial products of mild oxidation of *N*-alkyl-*N*-hydroxyamphetamines.

This study has shown that nitrones of general structure III are much more stable than suggested by earlier (Exner, 1951) literature claims.

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