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METABOLISM *IN VITRO* OF *N*-METHYLAMPHETAMINE WITH RAT LIVER HOMOGENATES*

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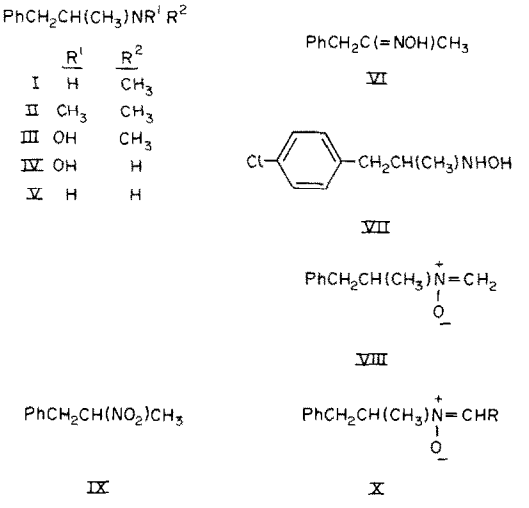
Abstract—Metabolism *in vitro* of *N*-methylamphetamine (NMA) by means of a 10,000 *g* supernatant of rat liver fortified with the usual co-factors has shown that metabolism occurs only to a small (< 10 per cent) but reproducible extent. The major metabolic products isolated were amphetamine (*ca.* 4 per cent) and 1-phenyl-2-propanone oxime (*ca.* 1 per cent); smaller amounts (*ca.* 0.2 per cent) of *N*-hydroxy-*N*-methylamphetamine (N-hydroxy-NMA) were also detected. The yield of the oxime increased to *ca.* 3 per cent when incubation mixtures were basified prior to extraction. This indicated that a chemically unstable precursor of the oxime was present in the incubation mixtures. This intermediate, which could not be detected by gas chromatography, is tentatively identified as *N*-[(1-methyl-2-phenyl)ethyl]methanimine *N*-oxide (a nitrone). A synthetic sample of this nitrone possessed chemical and physical properties comparable to those of the suspected precursor. The nitrone may be formed by oxidation of N-hydroxy-NMA.

Despite the fact that *N*-methylamphetamine (NMA; I) has been a drug of abuse for some years, surprisingly few metabolism studies have been carried out on this compound. It is known that NMA and its metabolites are excreted mainly in the urine of man and various animal species. In dog and man, the major metabolite *in vivo* is amphetamine [1–3], but the former also excretes significant amounts of free and conjugated *p*-hydroxyamphetamine [1]. In rat, aromatic hydroxylation is the major metabolic route; *p*-hydroxy-NMA, *p*-hydroxyamphetamine and *p*-hydroxy-norephedrine were all excreted in appreciable amounts in the urine as well as unchanged NMA and some amphetamine [4]. In contrast, no aromatic hydroxylation occurred in the guinea pig which metabolizes NMA mainly to benzoic acid, norephedrine and amphetamine [4]. No *N*-oxygenated metabolites were observed in any of the investigations cited above.

In 1972, Beckett and Al-Sarraj [5] reported an interesting observation concerning the metabolism *in vivo* of *N*,*N*-dimethylamphetamine (II) in rabbits. Various products were found to be present in the urine in unspecified amounts, including two *N*-hydroxy compounds, *N*-hydroxy-*N*-methylamphetamine (III) and *N*-hydroxyamphetamine (IV). Presumably, II was dealkylated to I which was then oxidized to III, and, also, the sequence II → I → V → IV operated to produce the *N*-hydroxyamphetamine. Other *N*-oxygenated metabolites *in vivo* of I were *N*,*N*-dimethylamphetamine *N*-oxide and *syn*- and *anti*-1-phenyl-2-propanone oximes (VI). Since the appearance of this report of metabolic formation *in vivo* of primary and secondary hydroxylamines, other investigators [6] have shown that the analog *N*-hydroxy-4-chloroamphetamine (VII) is readily reduced *in vivo* in rats to 4-chloroamphetamine. This apparent difference in the stability of hydroxylamines *in vivo* prompted us to investigate the metabolic formation and stability of *N*-oxygenated products of NMA in a system *in vitro* in which subsequent metabolic reduction of any hydroxylamines back to methylamphetamine or amphetamine would be minimized. Previous metabolic studies *in vitro* on NMA [7, 8] have been brief preliminary accounts lacking supportive data which claimed that NMA was oxidized to the hydroxylamine (III).



MATERIALS AND METHODS

Instrumentation. Nuclear magnetic resonance (NMR) spectra were recorded using a Varian A-60D spectrometer with deuterochloroform as solvent and

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TMS as the internal standard. Gas chromatographic (GC) analyses were performed on a Perkin-Elmer model F-11 gas chromatograph equipped with flame ionization detectors, using helium as carrier gas at a flow rate of 60 ml/min. Combined gas chromatography/mass spectrometry (GC-MS) was performed on a Hewlett-Packard 5710A gas chromatograph coupled to a Hewlett-Packard 5980A mass spectrometer at an ionization potential of 70 eV, an ion source temperature of 200°, and an emission current of 0.35 mA. The same columns were used for GC-MS as those employed in the quantitative analysis experiments described below. The MS of the synthetic nitrone (VIII) was obtained by the direct probe method on an A.E.I. MS-12 mass spectrometer at an ionization potential of 70 eV and a source temperature of 150°.

Reagents and animals. Glucose 6-phosphate (G-6-P) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were obtained from the Sigma Chemical Co.; phenylacetone (1-phenyl-2-propanone) and 1-phenyl-2-propanol were purchased from Aldrich Chemical Co. and Fluka AG Chemische Fabrik respectively. (+)-Amphetamine and (+)-*N*-methylamphetamine were kindly supplied by the Health Protection Branch, Health and Welfare, Canada, and *N*-hydroxy-*N*-methylamphetamine [8], 2-nitro-1-phenylpropane [9] and 1-phenyl-2-propanone oxime [10] were synthesized by literature methods. Other chemicals were of reagent grade. Male Wistar rats (250–450 g) were used throughout. They were housed in wire suspension cages and allowed free access to food and water.

Preparation of liver microsomal fraction. Animals were fasted overnight and sacrificed by cervical dislocation. Livers were removed and immediately placed into ice-cold 1.15% KCl. Subsequent operations were performed at 4°. A 25% (w/v) homogenate corresponding to 250 mg wet weight liver ml^{-1} in isotonic KCl was prepared in a Virtis blender operated at medium speed for 0.5 min. The microsomal supernatant was obtained by centrifugation of the liver homogenate at 10,000 *g* for 20 min.

Incubation. Two ml of the 10,000 *g* supernatant was added to 25-ml incubation flasks containing G-6-P (20 μmoles), $NADP^+$ (4.4 μmoles), $MgCl_2$ (20 μmoles), substrate (10 μmoles) and sufficient 0.1 M phosphate, pH 7.4, to make 6 ml. Samples were then incubated for 60 min at 37° in a Dubnoff shaking metabolic incubator (120 oscillations min^{-1}) under atmosphere. At the end of the incubation period, the flasks were immersed in ice, prior to addition of the appropriate internal standard, and extracted three times with freshly distilled ether, either at pH 7.4 or at pH 12–13. The ether extracts were concentrated at 45° on a water bath prior to analysis.

Gas chromatography. The Perkin-Elmer model F-11 chromatograph was used. Column 1 was 1.8-m glass tubing 4 mm i.d., packed with 7.5% Carbowax 20M on acid-washed, DMCS-treated chromosorb W, 80–100 mesh, and was operated at an oven temperature of 170°. Column 2 was 1.8-m glass tubing 4 mm i.d., packed with 10% Apiezon L and 10% KOH on the same solid support as column 1. It was used with an oven temperature of 150°. Other operating conditions for both columns were: injection port, 210°; manifold, 210°; helium, 60 ml min^{-1}; air 25 lb in^{-2}; hydrogen 20 lb in^{-2}. Peak areas were determined with an electronic peak integrator (Hewlett Packard model 3380A), using *p*-chloropropiophenone or *p*-chlorophentermine as internal standard.

Mass spectrometry. Relevant ions, m/e (per cent relative abundance), in the mass spectra of metabolic products A–E, G and I (see Figs. 2 and 3) were as follows: A: 135 (M^+, 0.1), 134 (0.3), 120 (2), 91 (14), 77 (3), 65 (8), 44 (100); B: 149 (M^+, 0.05), 148 (0.2), 134 (1.5), 91 (10), 77 (2), 65 (5), 58 (100); C: 134 (M^+, 57), 119 (8), 91 (100), 77 (10), 65 (26), 43 (16); D: 136 (M^+, 6), 121 (4), 103 (5), 92 (100), 91 (61), 77 (7), 65 (12), 45 (13); E: 165 (M^+, 4), 149 (2), 133 (3), 119 (19), 118 (51), 117 (21), 91 (100), 77 (13), 65 (15); G: M^+ absent, 118 (10), 117 (15), 91 (51), 77 (14), 74 (100), 58 (88), 56 (31); and I: 149 (M^+, 46); 132 (19), 131 (33), 130 (24), 117 (22), 116 (42), 91 (100), 77 (13), 65 (28), 58 (22).

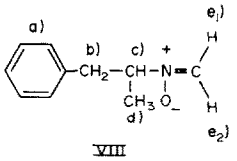
Quantitative analysis. Measured quantities of substrate I and authentic samples of metabolites III, V and VI were added in varying amounts (0.01 to 0.1 μmole of III; 0.01 to 1.0 μmole of V and VI; 1.0 to 10.0 μmoles of I) to separate portions of an aged 10,000 *g* supernatant fraction (pH: 7.4 for III and VI; 12.0 for I and V), followed by an appropriate constant quantity of the reference compound (*p*-chloropropiophenone for III and VI; *p*-chlorophentermine for I and V). Repeated extractions with ether showed that three extractions were sufficient to partition all six compounds into the organic phase. Each ether extract was concentrated and gas-chromatographed. For compounds III and IV, column 1 was used. Retention times (T_r in min) of appropriate compounds, when oven temperature was 170°, were: *p*-chloropropiophenone, 3.72; N-hydroxy-NMA, 6.50; and 1-phenyl-2-propanone oxime, 10.11. For compounds I and V, column 2 was used (oven temperature 150°), and T_r values of the relevant compounds were: amphetamine, 5.50; NMA, 7.19; and *p*-chlorophentermine, 19.20. Peak areas were measured using the electronic integrator, and calibration curves were constructed for the substrate (ratio of peak areas I: reference compounds vs the weight of I added), and similar curves were drawn for each metabolite. All calibration curves were linear over the concentration ranges encountered in the study.

Portions of the metabolism extracts were then treated in a similar manner and the quantities of compounds I, III, V and VI in these metabolism extracts were calculated from the calibration curves.

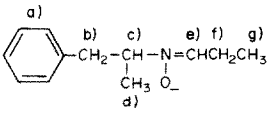
Thin-layer chromatography (T.L.C.). Chromatograms were run on glass plates (20 × 20 cm) spread to 5.0 mm with a slurry of silica gel G-PF254 (Brinkman) and activated at 100° for 1 hr. The solvent system used was chloroform–methanol (8:2). Spots were detected by short-wave u.v. light (254 nm), iodine vapor, or ammoniacal silver nitrate spray. The R_f values of significant compounds under these conditions were: I, 0.07; III, 0.52; V, 0.08; VI, 0.65; and VIII 0.58.

Preparation of N-[(1-*methyl*-2-*phenyl*)*ethyl*]*methanimine* N-*oxide* (*VIII*). To a dried chloroform solution of *N*-hydroxy-*N*-methylamphetamine base (10 mg), liberated in the usual manner from the oxalate salt [8], was added yellow mercuric oxide (25 mg) and the suspension was shaken for 1 hr during which time

Table 1. NMR signals and chemical shifts of nitrones VIII and XI*



VIII



XI

NMR signals		Chemical shifts (δ)	NMR signals		Chemical shifts (δ)
(a)	(5, *s*)	7.22	(a)	(5, *s*)	7.14
(b)	(2, *m*)	2.00–3.50	(b and f)	(4, *m*)	2.00–3.55
(c)	(1, *m*)	3.60–4.40	(c)	(1, *m*)	3.60–4.36
(d)	(3, *d*, $J = 6.5$)	1.51	(d)	(3, *d*, $J = 6.5$)	1.46
(e_1, e_2)	(2, *d* of *d*, $J = 7.7$)	6.06	(e)	(1, *t*, $J = 6.5$)	6.27
			(g)	(3, *t*, $J = 6.5$)	0.89

* NMR signals are enclosed in parentheses: integral, multiplicity and, where appropriate, coupling constant (in Hz). Chemical shifts are expressed in δ units.

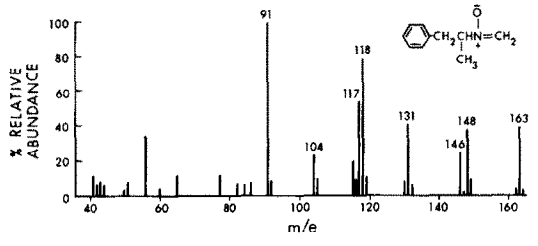


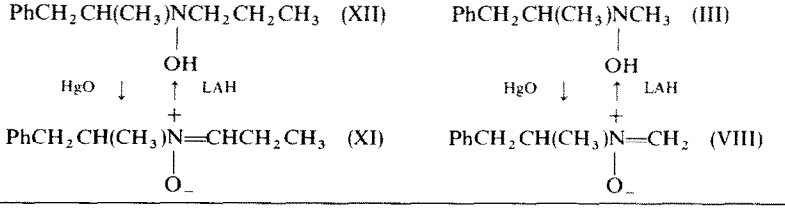
Fig. 1. Mass spectrum of synthetic *N*-[(1-methyl-2-phenyl)-ethyl]methanimine *N*-oxide (VIII).

a black color (Hg) developed. The suspension was centrifuged and the chloroform supernatant was evaporated to give the title compound as a yellow oil which was shown to be virtually pure VIII by its T.L.C. behavior ($R_f = 0.58$), its NMR spectrum (Table 1) and its MS (Fig. 1). The oil was hygroscopic and a satisfactory elemental analysis could not be obtained.

Interconversion of N-*hydroxy*-N-*alkylamphetamines and related nitrones* (*Table* 2). A portion (2 μl) of a solution of *N*-hydroxy-*N*-methylamphetamine (10 mg) in chloroform (3 ml) was gas-chromatographed on column 1 (column temp. 170°) and the integral of the peak, $R_f = 6.9$ min, recorded. Yellow mercuric oxide (25 mg) was added and stirring was continued for 30 min. The suspension was centrifuged and the supernatant was diluted to 3 ml with chloroform. A portion (2 μl) of this solution was gas-chromatographed as before. Finally, lithium aluminum hydride (25 mg) was added to the chloroform solution and it was stirred for 1 hr, treated with water (1 drop), and filtered through glass wool. The solid was washed with chloroform and the filtrate and washings were combined and concentrated to 3 ml. This solution (2 μl) was again gas-chromatographed as before.

A sample of *N*-hydroxy-*N*-propylamphetamine was also treated in exactly the same manner and the GC peaks of the *N*-hydroxy compound (XII) and the related nitrone (XI) monitored and integrated. Results are given in Table 2. A mixture of *syn*- and *anti*-nitrone (XI) might be expected using this preparative method, but NMR data (Table 1) suggests that only one of these geometric isomers was obtained.

Table 2. Gas chromatographic monitoring of hydroxylamine nitrone interconversions



	GC integral counts			
	XII ($T_r = 8.7$)*	XI ($T_r = 15.9$)	III ($T_r = 6.9$)	VIII ($T_r = -$)†
(a) Initial solutions	107,459		122,264	
(b) After HgO treatment	3,511	116,638	1,522	
(c) Solution (b) after LAH reduction	48,484	66,811	37,165	

* Retention times in min on column 1.
† Not detected.

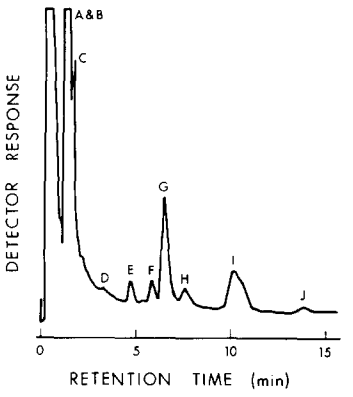


Fig. 2. GC separation on column 1 of metabolites *in vitro* (rat) of *N*-methylamphetamine (pH 7.4 extract). Identity of peaks: A, amphetamine (V) [11]; B, NMA (I) [11]; C, 1-phenyl-2-propanone [11]; D, 1-phenyl-2-propanol [11]; E, 2-nitro-1-phenylpropane (IX) [9]; G, N-hydroxy-NMA (III) [11, 12]; I, *syn*- and *anti*-1-phenyl-2-propanone oxime (VI) [11]; and F, H and J, unidentified.

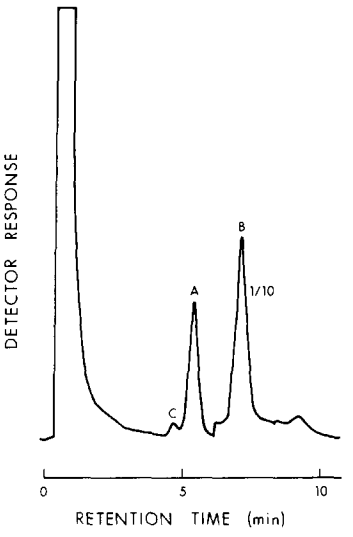


Fig. 3. GC separation of amphetamine (A), *N*-methylamphetamine (B), and 1-phenyl-2-propanone (C) on column 2 (pH 12 extract of metabolism mixture).

RESULTS AND DISCUSSION

Metabolism *in vitro* of NMA was performed at pH 7.4 using the 10,000 *g* supernatant of homogenized rat liver fortified with the usual cofactors (glucose 6-phosphate, $NADP^+$, Mg^{2+}) but omitting nicotinamide. Four replicate experiments were done. A concentrated diethyl ether extract of each cooled metabolism mixture was gas-chromatographed on a Carbowax 20M column and shown to contain a number of metabolites (Fig. 2). Components A–C did not separate on this column but these three compounds were readily separated for quantitation on an alternative GC column (Apiezon L-KOH) (Fig. 3). A mass spectrum of each GC peak was recorded and interpreted. Chemical structures were confirmed by comparing the GC/MS behavior of each metabolic product A–E, G and I with that of the authentic reference compound, and with literature data [12]. The t.l.c. R_f values were also compared. In this way, the metabolic products were identified as shown in Table 3.

To determine quantitatively the amounts of the more abundant metabolites formed, calibration curves were constructed for NMA and each of the metabolites A, G and I, using authentic samples of each, and with *p*-chloropropiophenone or *p*-chlorophentermine as the internal standard. The total amount of metabolism in each of four replicate experiments was low, but reasonably reproducible (Table 3), after incubating 10 μmoles NMA with 2 ml of 10,000 *g* supernatant of rat liver for 60 min. A summation of the amounts of metabolites formed plus recovered NMA (Table 3) did not account for all the NMA available for metabolism, which suggested that at least one additional metabolite was being formed but was not being detected with the analytical system in use. This suggestion was confirmed when metabolism mixtures which had been incubated for 1 hr at 37 at pH 7.4 were basified (pH 12.0) and heated at 37 for an additional 1 hr. This treatment resulted in the disappearance of *N*-hydroxy-NMA (III) from the extract and a more than equivalent increase in the amount of ketoxime (VI) formed.

Rates of formation of metabolites III, V and VI were studied by determining quantitatively the amounts of these three compounds formed after 15, 30, 45 and 60 min. These reproducible data are illustrated in Fig. 4 and indicated that (a) the rate of production of amphetamine was linear over the period examined, (b) the detectable amount of *N*-hydroxy-

Table 3. Products of the incubation of *N*-methylamphetamine with 10,000 *g* supernatant of homogenized rat liver*

	pH 7.4 extract† (%)	pH 12.0 extract‡ (%)
Recovered methylamphetamine (I)	90.5 ± 1.5	90.0 ± 2.8
Amphetamine (V)	4.0 ± 0.5	3.3 ± 0.7
Ketoxime (VI)	0.9 ± 0.2	2.9 ± 0.4
N-hydroxy-*N*-methylamphetamine (III)	0.2 ± 0.05	0.0
Minor detected metabolites§	~0.2	~0.2

* Data represent the amounts of metabolites after 60-min incubations of 10 μmoles of substrate with 2 ml supernatant (equivalent to 0.5 g liver containing 18.8 mg protein/g). Protein content was determined by a reported [13, 14] method.

† Extraction immediately after cooling metabolism mixture (four replicates).

‡ Extraction after heating metabolism mixture at pH 12.0 and at 37° (four replicates).

§ Based on peak area measurements only.

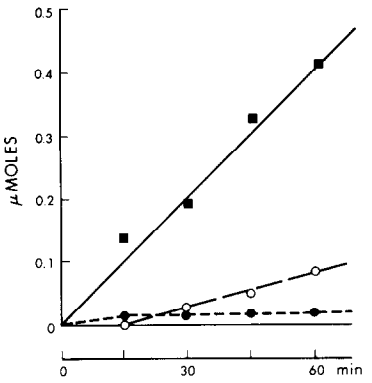
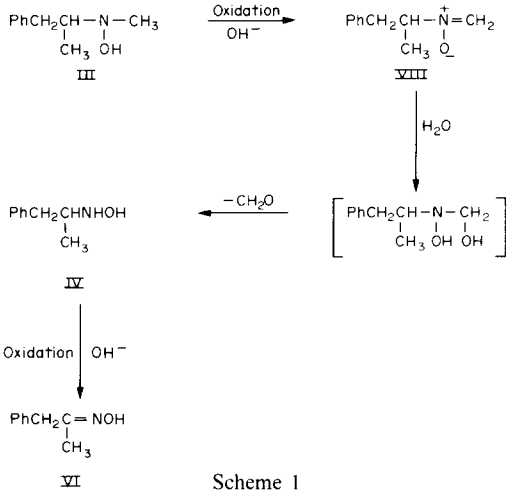


Fig. 4. Metabolism *in vitro* (rat) of *N*-methylamphetamine (10 μmoles)—quantitative estimation of metabolites. Key: (■—■) $PhCH_2CH(CH_3)NH_2$; (○—○) $PhCH_2C(CH_3)$=NOH; and (●—●) $PhCH_2CH(CH_3)N(OH)CH_3$.



Scheme 1

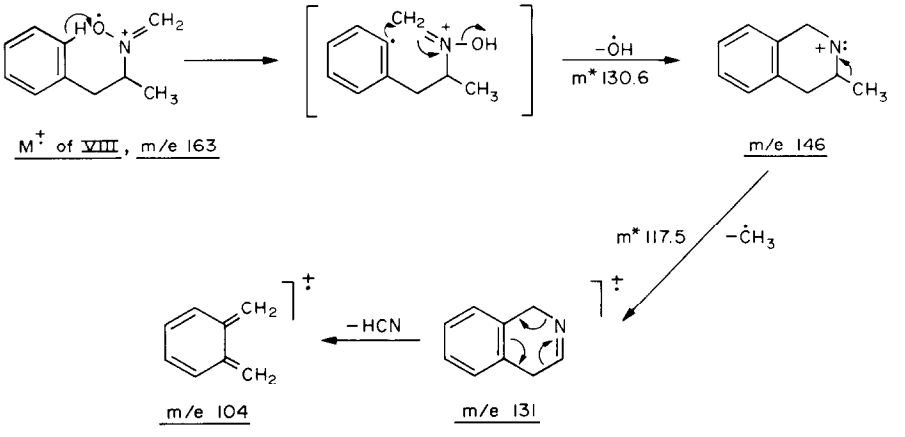
NMA formed levelled off rapidly, and (c) a time delay occurred before the ketoxime (VI) was detected.

A possible conclusion from the experiments just described was that some hitherto undetected water-soluble metabolite was being formed which was slowly converted to the ketoxime when the incubation mixture was maintained at pH 7.4. This chemical conversion of the unknown metabolite to ketoxime was more rapid when the incubation mixture was adjusted to pH 12.0 prior to extraction. The reaction sequence (Scheme 1) suggests a possible explanation, in which the nitrone (VIII) was the undetected metabolite and a key intermediate in the formation of the ketoxime (VI) from the metabolically formed *N*-hydroxy NMA (III). The action of alkali is known [15] to rapidly oxidize the primary hydroxylamine (IV) mainly to the oxime (VI).

Literature evidence [16, 17] suggests that a nitrone of structure VIII would be a very reactive water-soluble compound capable of rapid hydrolysis. A previous study by us [8] had failed to produce any VIII by a method which was capable of producing homologous nitrones (X). In addition, nitrones of general structure X (R = alkyl or aryl) were detectable by the GC procedures used in the present study which failed to detect VIII. Despite the apparent elusiveness of the nitrone (VIII), further attempts were made to prepare it, and it was obtained in good yield as a yellow oil by mild oxidation (yellow HgO) of *N*-hydroxy-NMA (cf. Ref. 8) in chloroform. The authenticity of structure VIII for this nitrone was confirmed by interpretation of its MS and NMR spectra which are discussed below, and by its facile reduction back to *N*-hydroxy-NMA by means of lithium aluminum hydride [17].

The MS of VIII (Fig. 1) was obtained by the direct probe method. It contained a strong molecular ion (m/e 163) and diagnostic ions of m/e 148 $(M-CH_3)^+$, 146 $(M-OH)^+$, 131 $(M-OH-CH_3)^+$, m/e 118, 117, 104 and 91. The structures of most of these ions are readily deduced from our previous studies on related nitrones [12, 18]. The MS also contained two metastable ions of m/e 130.6 and 117.5 which substantiated the fragmentation sequence m/e 163 → m/e 146 → m/e 131, i.e. the successive loss of OH· and CH_3· from the molecular ion. A fragmentation sequence to explain the formation of fragments m/e 146, 131 and 104 is suggested in Scheme 2. It can be concluded that the MS of the product of mild oxidation of *N*-hydroxy-NMA is consistent with the structure proposed for the nitrone (VIII).

Interpretation of the NMR spectrum of the syn-



Scheme 2

thetic nitrone suspected to be VIII confirmed the authenticity of this structure. It gave signals (Table 1) which could be attributed to five ring protons (a), two methylene protons (b), the methine proton (c), three methyl protons (d) and the two additional methine protons (e and f). The chemical shifts of these protons were comparable to the shifts of equivalent protons in the previously described [18] higher homolog (XI, Table 1). Some properties of the synthetic nitrone VIII were determined. It was confirmed that it could not be detected with the GC system used in Fig. 2, nor could it be detected on alternative GC columns. However, it could be detected by t.l.c. and it had an R_f value identical to one of the products of the metabolism *in vitro* of substrate I. The synthetic nitrone VIII was slowly and incompletely reduced in chloroform with lithium aluminum hydride to *N*-hydroxy-NMA (III), an observation which prompted the following experiments. Authentic samples of *N*-hydroxy-NMA and *N*-hydroxy-*N*-(*n*-propyl)amphetamine (*N*-hydroxy-NPA, XII) were dissolved in chloroform and gas-chromatographed, and an integral count was recorded for each peak. The chloroform solutions were then treated with yellow mercuric oxide to oxidize III and XII to the corresponding nitrones (VIII and XI, respectively) and a GC trace was again recorded. Only the latter compound (XI) was detected. The chloroform solutions were then treated with lithium aluminum hydride. A GC examination showed that a partial reduction back to the *N*-hydroxy compounds (III and XII) had occurred. These results are summarized in Table 2, and confirm that *N*-hydroxy-NMA is readily oxidized to a product which cannot be detected by GC and may be the nitrone (VIII).

The ether extract of the metabolism mixture *in vitro* which gave rise to the GC trace shown in Fig. 2 was then evaporated and reduced in chloroform solution with lithium aluminum hydride as described for the reduction of the synthetic nitrones (VIII and XI). A GC examination of the final chloroform solution showed that the relative intensity of peak G had increased to more than three times its original value, indicating that the ether extract of the metabolism mixture contained a compound which was readily reduced by lithium aluminum hydride to *N*-hydroxy-NMA.

In summary, metabolism *in vitro* of NMA in a fortified 10,000 *g* supernatant of homogenized rat liver yields, in addition to the products identified in Fig. 2, another product which has a t.l.c. R_f value identical to the nitrone (VIII) and which, like the nitrone (VIII), can be reduced with lithium aluminum hydride to *N*-hydroxy-NMA (III), and oxidized by the action of alkali to 1-phenyl-2-propanone oxime (VI). The nitrone (VIII), therefore, is a likely candidate as an unstable intermediate in the conversion of N-hydroxy-NMA to the ketoxime (VI) in rat liver homogenates. However, verification of this nitrone as an intermediate metabolite must await further experimentation.

Confirmation that *N*-hydroxy-NMA was produced from NMA by an enzymatic process in the rat liver preparations, and not by a chemical process, was required. The metabolism *in vitro* of the substrate (I) was repeated in duplicate at pH 7.4 exactly as previously described except that a heat-denatured (by boiling for 2 min) 10,000 *g* supernatant of homogenized rat liver, fortified with the usual cofactors, was used. Examination of concentrated diethyl ether extracts of each metabolism mixture by gas chromatography revealed that no *N*-hydroxy-NMA (III) was produced. In addition, neither of the other two *N*-oxygenated metabolites (VI and IX) was detected.

It was also necessary to establish whether formation of the oxime (VI) as outlined in Scheme 1 involved an enzymatic process or whether it was formed by chemical oxidation of *N*-hydroxy-NMA during the metabolism reaction. *N*-Hydroxy-NMA, therefore, was incubated under conditions identical to those used in the metabolism experiments just described except that the incubation mixture contained microsomal supernatant but no cofactors, or heat-denatured microsomal supernatant with cofactors. When the concentrated ether extract of each of these metabolism mixtures was examined by GC, the oxime (VI) was detected. This suggested that the conversion of III to VI occurred non-enzymatically.

Small quantities of 1-phenyl-2-nitropropane (IX) were isolated when NMA was metabolized *in vitro* with 10,000 *g* supernatant of homogenized rat liver containing cofactors. A separate study [9] has confirmed that this nitro compound is formed as a result of an enzymatic oxidation of the oxime (VI).

It is concluded that NMA is metabolically *N*-oxidized *in vitro* in rat to *N*-hydroxy-NMA (III), the oxime (VI) and an unstable compound tentatively identified as the nitrone (VIII). Since the last of these products cannot be detected on GC, it is not possible to determine quantitatively the amount of metabolic *N*-oxidation occurring by summation of the amounts of III, VI and VIII formed. The oxime, however, is stable in dilute sodium hydroxide solutions and both III and VIII can be converted to VI by the reaction sequence depicted in Scheme 1. Thus, the extent of *N*-oxidation *in vitro* of NMA by means of 10,000 *g* rat liver supernatant can be determined by treating the metabolism mixture with alkali and measuring the oxime produced in the manner just described. It is recognized, however, that this quantitative method will underestimate the amount of metabolic *N*-oxidation, since it is known [9] that a portion of the metabolically produced oxime will metabolize further to the nitro compound (IX). In addition, we have observed that although the oxime (VI) is stable in dilute aqueous sodium hydroxide, it decomposes slowly when placed in a dilute solution of sodium hydroxide containing 10,000 *g* supernatant to products which have not yet been identified. The nitro compound (IX) also decomposes under these conditions [9].

This study permits the conclusion that NMA (III) is metabolized *in vitro* only to a small extent (< 10 per cent) in the rat, and that the major metabolites are formed by dealkylation to amphetamine (V) and by *N*-oxidation to a variety of products including *N*-hydroxy-NMA (III), the oxime (VI), the nitro compound (IX) and a product which is unstable in alkaline solution and is tentatively identified as the nitrone (VIII).

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