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Metabolic *N*- and α-*C*-oxidation of norephedrine by rabbit liver microsomal fractions and synthesis of the metabolic products

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The products of metabolism of norephedrine by 10 000 *g* fractions of rabbit liver microsomes have been shown to be norephedrine hydroxylamine, 1-hydroxy-2-oxo-1-phenylpropane oxime, 1-hydroxy-2-oxo-1-phenylpropane, 1,2-dihydroxy-1-phenylpropane (*erythro*). *N*-Oxidation was at least as important as α-*C*-oxidation. The synthesis and properties of the metabolic products are described.

Norephedrine is known to be metabolized *in vivo* extensively in rabbits (Sinsheimer, Dring & Williams, 1973), less in rats, little in dogs (Axelrod, 1953) and only to a very slight extent in man (Beckett & Wilkinson, 1965). Although the main route is aromatic hydroxylation in the rat, in the rabbit, according to Sinsheimer & others (1973), deamination and degradation of the side chain is the main route to produce a keto alcohol and a glycol which are conjugated.

However, in many compounds possessing primary (and secondary) aliphatic amino groups, *N*-oxidation to form labile hydroxylamines that further metabolize or are converted to oximes which subsequently lose their nitrogen centres to form ketones or aldehydes, is a very important metabolic route (Beckett, 1971; Beckett, Van Dyk & others, 1971; Beckett & Al-Sarraj, 1972a, 1973; Beckett, 1973; Beckett, Coutts & Ogunbona, 1973).

Furthermore, the *N*-oxidation metabolic route is known to be more important *in vitro* in rabbits than in rats (Beckett & Al-Sarraj, 1972b). Thus the reported deamination products in rabbits (Sinsheimer & others, 1973) could have arisen from *N*-oxidation as well as α-carbon oxidation. Incubations with liver microsomal fractions of rabbits were therefore made to attempt to isolate and characterize the potential products of metabolic *N*-oxidation of norephedrine.

MATERIALS AND METHODS

Compounds and reagents. (±)-Norephedrine hydrochloride (Ralph Emanuel); L-norephedrine sulphate (K & K Labs. Inc., U.S.A.); 3-chloroperbenzoic acid (Koch Light); *erythro* 1,2-dihydroxy-1-phenylpropane was kindly supplied by Dr. J. Caldwell; (+)- and (±)-1-hydroxy-2-oxo-1-phenylpropane oxime and 1-hydroxy-2-oxo-1-phenylpropane were synthesized. Analar ether was freshly distilled.

Syntheses

(±)-Oxime (IV) from (±)-norephedrine. (±)-Norephedrine (extracted from a basic solution of (±)-norephedrine hydrochloride, 400 mg) was oxidized in chloroform using

3-chloroperbenzoic acid in a ratio of 1:2·5. The chloroform was evaporated under vacuum, the residue taken up in ether and washed with 5% potassium carbonate to remove excess oxidant and liberated 3-chlorobenzoic acid. The oxime (IV) was separated from other oxidation products by preparative t.l.c. (system B, p. 947), the oxime being located with 1% cupric chloride. The oxime was eluted from silica gel with ether and recrystallized from chloroform as 30 mg (10% yield) of a white solid; m.p. 116° (uncorrected, cf. 112° Kaji & Nagashima, 1956); nmr δ 1·72 (s, 3, CH_3) 5·23 (s, 1, CH) 7·36 (s, 5, Ar), (cf. Newman & Angier, 1970, spectrum in $(CD_3)_2CO$); infrared (KCl) 1045 (m) 3320 (s, b OH), 1675 (w, C = N) cm^{-1}, (cf. Newman & Angier, 1970, OH bonded in Nujol); mass spectrum (solid probe) *m/e* 79(100) 77(76) 107(61) 51(45) 118(29) . . . . . 136(4) 148(3) molecular ion 165(3).

(+)-Oxime (IV) from L*-norephedrine.* The method was as for (±)-oxime (IV), but using L-norephedrine sulphate. The oxime was isolated as an oil in the quantities used which did not readily crystallize. Nmr, infrared (film) and g.l.c.-m.s. are the same as above; optical rotatory dispersion $[+99°]_{588\ nm}$ $[+970°]_{323\ nm}$ $[+2424°]_{270\ nm}$.

Ketone (III) from oxime (IV). 20 mg of oxime (IV) was shaken with 3 ml 2N hydrochloric acid for 1 h, and the product extracted with ether. Evaporation of the solvent gave a colourless oil in quantitative yield, as determined by g.l.c. system B (p. 947); nmr δ 2·08 (s, 3, CH_3) 5·09 (s, 1, CH) 7·36 (s, 5, Ar), (cf. Newman & Angier, 1970); infrared (film) 3450 (s, b OH) 1730 (s, c = O) cm^{-1} (cf. Newman & Angier, 1970, C=O stretch); g.l.c. m.s. *m/e* 77(100) 79(98) 107(84) 105(79) 43(48) 51(38) . . . 135(3) molecular ion 150(1).

Alcohols (Ia and IIa) by reduction of ketone (III). 15 mg of ketone (III) was quantitatively reduced in sodium-dried ether at room temperature (20°) for 30 min using an excess of $LiAlH_4$ (50 mg total). Excess $LiAlH_4$ was destroyed with water and the resulting alcohols were extracted with ether. Evaporation of the ether yielded an oil; nmr δ (*E* = *erythro T* = *threo*), 1·09 (d, $^{E+T}CH_3$) *ca* 4·0 (m, $^{E+T}CH_b$) 4·38 (d, $^{T}CH_a$) 4·68 (d, $^{E}CH_a$) 7·34 (s, ^{E+T}Ar) $^{E}J_{ab}$ = 4·2 Hz $^{T}J_{ab}$ = 7·0 Hz, (cf. Schmidt, 1968, spectra in $(CD_3)_2CO$); infrared (film) 3420 (s, b OH) cm^{-1} carbonyl absorption absent, (cf. Foltz & Witkop, 1957, in broad agreement with *erythro* spectrum); g.l.c.-m.s., *m/e* 79(100) 108(80) 107(78) 77(67) 105(36) 51(26. . . . 134(3) no molecular ion. Nmr and g.l.c. -m.s. spectra are in agreement with those obtained from the authentic *erythro* alcohol (Ia).

Hydroxylamines (Ic and IIc) from reduction of (+)-oxime (IV). 30 mg of (+)-oxime (IV) was reduced in dry ether at room temperature (20°) with an excess of $LiAlH_4$ (100 mg) for 30 min. After excess $LiAlH_4$ had been destroyed with water, the resulting products were extracted with ether. The extract was concentrated under a stream of nitrogen and then applied to t.l.c. system B (p. 947). The hydroxylamine area (R_F 0·02–0·08, located with Tollen's reagent and ultraviolet light λ 254 nm) was scraped off, extracted with ether, and run with t.l.c. system C (p. 947). The hydroxylamine area (R_F 0·45–0·55) was then extracted with ether and concentrated under a stream of nitrogen; nmr *erythro* (Ic) δ 0·84 (d, CH_3) *ca* 3·3 (m, CH) 5·12 (d, CH) 7·31 (s, Ar) J_{ab} = 3·0 Hz, *threo* (IIc) δ 0·95 (d, CH_3) *ca* 3·1 (m, CH) 4·59 (d, CH) 7·33 (s, Ar) J_{ab} = 8·7 Hz; mass spectrum (solid probe) of t.l.c. extract, *m/e* 60 (100) 44(36) 42(36) 77(30) 79(25) 51(19) 43(19)105(15) 107(15). . . . 149(4) molecular ion 167($<$1).

Metabolism

Incubation and extraction procedure. Liver microsomal preparations (10 000 *g*) of New Zealand White male rabbits (animal weight approximately 3 kg) were used. 5 μmol of the substrate was incubated at 37° for 30 min with 10 000 *g* fortified microsomal preparations (2 ml = 0·5 g liver) (see Beckett, Mitchard & Shihab, 1971). The pooled incubation mixtures of ten flasks were extracted with freshly distilled ether at pH 7·4.

Examination of metabolic extract. The concentrated ethereal extract of the metabolic mixture was examined by t.l.c. system A and g.l.c. system A (p. 947). Areas (a) R_F 0·00–0·30, (b) R_F 0·45–0·55 and (c) R_F 0·55–1·00 (unsprayed) were extracted from the t.l.c. plates with methanol and examined separately on g.l.c. system A. Area (b) was sprayed with ammoniacal silver nitrate, left for 12 h and the black spot extracted with ether, a portion of which was examined on g.l.c. system A and t.l.c. system A. The remainder of the ethereal extract was reduced with an excess of $LiAlH_4$ (50 mg) in dry ether at room temperature (20°) for 30 min. After decomposition of excess $LiAlH_4$ with water, the mixture was extracted with ether and the pooled ether extracts concentrated under a stream of nitrogen and examined by g.l.c. system A and t.l.c. system A. A mass spectrum (solid probe) was obtained of a methanolic extract of an unsprayed area (b). An ethereal extract of area (c) was also reduced with $LiAlH_4$ for 12 h as described for area (b), the final ethereal extract being examined by g.l.c. system A.

Instrumentation

Gas liquid chromatography. Instrument: Perkin-Elmer F11 with flame ionization detector and Hitachi Perkin-Elmer 159 recorder. Conditions: *System A;* glass column, 1 metre, $\frac{1}{4}''$ o.d., 7·5% Carbowax 20 M on acid washed DMCS treated Chromosorb W 80–100 mesh, oven temperature 165°, air 1·4 kg cm^{-2}; H_2 1·4 kg cm^{-2}; $N_2$2·1 kg cm^{-2} (104 ml min^{-1}). *System B;* glass column, 1 metre, $\frac{1}{4}''$ o.d. 2% Carbowax 20 M on acid washed DMCS treated Chromosorb G 100–120 mesh, oven temperature 120°, air 1·4 kg cm^{-2}; H_2 2·1 kg cm^{-2}; N_2 1·8 kg cm^{-2} (176 ml min^{-1}). Both systems were HMDS treated *in situ.*

Thin layer chromatography. System A; Silica gel G (Merck) and water (1:2) spread to 0·5 mm on 20 × 20 cm glass plates and dried at 110° for 1 h. The solvent was chloroform (4) : methanol (1) run to a height of about 15 cm. *System B;* Silica gel HF_{254} (Merck) prepared as above to a thickness of 0·25 mm. The solvent was benzene–ethyl acetate–water (18 : 6 : 0·5). *System C;* was as for system B except the solvent was chloroform –methanol (4:1). Spray reagents used were:—1. Ammoniacal silver nitrate (Tollen's reagent). 2. Triphenyltetrazolium chloride reagent (T.T.C.). 3. Cupric chloride 1% aqueous. 4. Iodine vapour. 5. Dragendorff's reagent.

Combined gas liquid chromatography/mass spectrometry. This was performed on a Perkin Elmer model 270 instrument using a glass column, 1 metre, $\frac{1}{4}''$ o.d. packed with 2% OV17 on Gas-Chrom Q 100–120 mesh, acid washed and DMCS treated. The carrier gas was helium (0·7 kg cm^{-2}); an oven temperature of 110–120°, and an ionizing potential of 70 eV.

Mass spectra (solid probe). Direct inlet spectra were recorded on an A.E.I. MS–9 (probe temperature 160°, ionizing potential 70 eV) or a Perkin-Elmer model 270 mass spectrometer (probe temperature 50–100°, ionizing potential 70 eV).

Nuclear magnetic resonance spectra. Spectra were recorded on a Perkin-Elmer model R32 spectrometer (with lock). All spectra were run in deuterated chloroform with tetramethylsilane as internal standard.

Infrared spectra. Spectra were run on a Unicam SP1000 infrared spectrophotometer. Notations used: s = strong, m = medium, w = weak and b = broad.

Optical rotary dispersion. Spectra were recorded in absolute ethanol on a Bellingham Stanley/Bendix-Ericsson Polaromatic 62 equipped with a 250 W Supersil xenon lamp with constant nitrogen purging at room temperature.

RESULTS AND DISCUSSION

Metabolism

Examination of the incubation mixture extract on g.l.c. system A indicated the presence of the *erythro* alcohol (1a), norephedrine (1b), norephedrine hydroxylamine (1c), the ketone (III) and a small amount of oxime (IV) by reference to authentic samples (see later, see Table 1 for retention times). Some of the oxime (IV) could have come from breakdown of the hydroxylamine (Ic) during g.l.c. analysis (Beckett & Al-Sarraj, 1973). T.l.c. system A further indicated the presence of amine (Ib), R_F *ca* 0·20 (spray 5, Table 1), and hydroxylamine (Ic), R_F 0·50 (sprays 1 and 2), with additional spots observed at R_F values corresponding to those of the alcohol (Ia) and the ketone (III) (iodine vapour, Table 1). Methanolic extracts of the t.l.c. areas (a), (b) and (c) (unsprayed) were examined on g.l.c. system A; area (a) contained

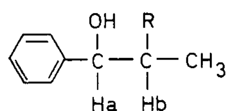
Table 1. *T.l.c. and g.l.c. or nor- and nor-ψ-ephedrine and some of its metabolites and metabonates.*

	T.l.c. R_F values				G.l.c. Rt (min)	
	System			Spray	System	
	A	B	C	colour	A	B
Norephedrine (Ib)	0·17	*ca* 0·01 (Ib and IIb)	0·14	4/brown	4·1	1·1
Norpseudoephedrine (IIb)	0·22		0·20	5/red	3·9	1·1
Norpseudoephedrine hydroxylamine (IIc)	0·49	*ca* 0·05 (IIc and Ic)	0·43	1/black 2/pink 4/brown (IIc and Ic)	13·6	3·4
Norephedrine hydroxylamine (Ic)	0·52		0·44		21·3	5·2
erythro Alcohol (Ia)	0·62	0·13	0·52	4/brown	7·9	2·5
Oxime (IV)	0·61	0·20	0·51	3/green 4/brown	44·8	11·0
Ketone (III)	0·77	0·42	0·69	4/brown	2·5	0·7
Reduced ketone (*erythro* and *threo* alcohols) (Ia and IIa)		0·13	0·52	4/brown	7·9 + 7·2	2·5

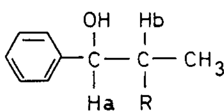
Sprays: (1) Tollen's. (2) T.T.C. (3) 1% cupric chloride. (4) Iodine vapour. (5) Dragendorff's.
No colour is given with a particular spray if not stated.

norephedrine (Ib) and area (b) contained norephedrine hydroxylamine (Ic) and a small amount of oxime (IV). The mass spectrum (solid probe) of t.l.c. area (b) gave an *m/e* 60 ion as the base peak, characteristic of many aralkyl hydroxylamines (Beckett & others, 1973). Twelve h after spraying area (b) with ammoniacal silver nitrate, all the hydroxylamine (Ic) had been converted to oxime (IV) (g.l.c. system A, t.l.c. system A); subsequent partial reduction of the oxime (IV) with $LiAlH_4$ formed norephedrine (Ib), norpseudoephedrine (IIb), norephedrine hydroxylamine (Ic) and norpseudoephedrine hydroxylamine (IIc), plus some unchanged oxime (g.l.c. system A and t.l.c. system A) and an unidentified peak on g.l.c. at Rt 17 min also cf. reduction of synthetic oxime (IV) p. 950. Area (c) contained the *erythro* alcohol (Ia), ketone (III) and a trace of oxime (IV) (g.l.c. system A). Products from the reduction of the ethereal extract of t.l.c. area (c), as examined by g.l.c. system A, were *erythro* alcohol (Ia), *threo* alcohol (IIa) [IIa and some Ia coming from the reduction of the ketone (III), cf. reduction of synthetic III], plus traces of hydroxylamine (Ic and IIc) and amine (Ib and IIb) [Ic, IIc Ib and IIb coming from the reduction of the oxime (IV), cf. reduction of synthetic IV p. 950].

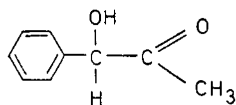
Our preliminary results indicate that metabolic oxidation of the nitrogen atom to yield norephedrine hydroxylamine (Ic) plus oxime (IV) is at least as important as α-carbon oxidation yielding the ketone (III) and its reduction product Ia.



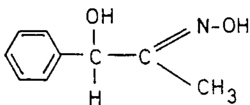
I (*erythro*)



II (*threo*)



III



IV

Ia R=OH 1,2-dihdroxy-1-phenylpropane (erythro)
Ib R=NH_2 Norephedrine
Ic R=NHOH 1-Hydroxy-2-hydroxylamino-1-phenyl propane (erythro): Norephedrine hydroxylamine
IIa R=OH 1,2-dihydroxy-1-phenylpropane (*threo*)
IIb R=NH_2 Norpseudoephedrine
IIc R=NHOH As 1c but *threo*: Norpseudoephedrine hydroxylamine
III 1-Hydroxy-2-oxo-1-phenylpropane
IV 1-Hydroxy-2-oxo-1-phenylpropane oxime

Synthesis and structures of compounds

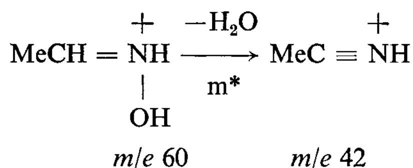
Oximes (IV) and ketone (III). The oxime (IV) was synthesized from norephedrine (Ib) by oxidation with 3-chloroperbenzoic acid and subsequent separation from other products by thin-layer chromatography. Although the preparation gives a fairly poor yield of oxime (IV) (*ca* 10%) it has the advantage of being an essentially one-step reaction from readily available amine (Ib) rather than the conventional preparation of oximes (IV) from their corresponding ketones (e.g. Vogel). The oxime (IV) was characterized by various physical methods and by comparison with published data.

The ketone (III) was prepared quantitatively from the oxime (IV) by dilute, aqueous acid hydrolysis (Patai, 1966; Sidgwick, 1966), completion of the reaction being checked on g.l.c. system B and t.l.c. system B.

Alcohols (Ia and IIa). Reduction of the ketone (III) produced two diastereomeric alcohols (Ia and IIa). Nmr spectra show that the ratio of *erythro* (Ia) to *threo* (IIa) isomers was approximately 5:1. The coupling constants and chemical shifts are in

agreement with literature (Schmidt, 1968) taking into account solvent effects, and for the *erythro* alcohol (Ia) were identical with those of authentic material. G.l.c. system A (but not system B) separated the two isomers, and it was estimated that the amount of *erythro* alcohol (Ia) produced on reduction of the ketone (III) was five times that of the *threo* isomer (IIa). A g.l.c.-mass spectrum of the mixed isomers is the same as that of the authentic *erythro* isomer (Ia). Infrared data of the mixed isomers agree with literature spectra (Foltz & Witkop, 1957).

Hydroxylamines (Ic and IIc). Reduction of either (+)- or (±)-oxime (IV) produced a mixture of two diastereoisomeric hydroxylamines (Ic and IIc) and on further reduction a pair of diastereoisomeric amines (Ib and IIb). The hydroxylamines were separable from the amines on t.l.c. systems A and C (Table 1). An extract of the hydroxylamine area from t.l.c. system C gave three main peaks on g.l.c. system A i.e. norephedrine hydroxylamine (Ic), norpseudoephedrine hydroxylamine (IIc), and oxime (IV) (as a breakdown product, see Beckett & Al-Sarraj, 1973); and usually three main peaks on g.l.c. system B (Table 1), i.e. norephedrine hydroxylamine (Ic), norpseudoephedrine hydroxylamine (IIc) and an unidentified peak (Rt 7·1 min cf. Ic 5·0 min). Both systems showed there to be about three times as much *erythro* (Ic) as *threo* (IIc) isomer. Nmr of the extract showed a spectrum consistent with that of structure (I), but was not the amine (Ib). The coupling constant (J_{ab} = 3·0 Hz) indicated an *erythro* configuration (Ic) (Schmidt, 1968); the spectrum was not strong enough to resolve any *threo* isomer (IIc). Mass spectra (solid probe) showed a *m/e* 60 base peak, which is characteristic of many aralkyl hydroxylamines (Beckett & others, 1973) and a metastable ion at 29·4 from the breakdown of fragment *m/e* 60 to *m/e* 42:



The present results therefore establish the structure of the metabolic *N*-oxidation product of norephedrine as the hydroxylamine (Ic) in a species known to have a well developed *N*-oxidation pathway. This metabolite is readily converted chemically to the oxime (IV) which in acidic solution breaks down to the ketone (III). Thus the possibility arises that the ketone observed by Sinsheimer & others (1973) could have arisen metabolically from attack upon the nitrogen as well as from α-carbon oxidation.

The presence of the hydroxylamine metabolite Ic indicates that the introduction of a β-hydroxyl group onto an aralkylamine does not block the *N*-oxidation metabolic route which has been demonstrated to be of such importance in these compounds (Beckett, 1973), irrespective of whether they possess one (Beckett & Al-Sarraj, 1972a, 1973) or two (Beckett & Bélanger, 1974a, b) methyl groups on the carbon atom α to the nitrogen group.

(±)-Amphetamine is stated to be metabolized in animal and man to norephedrine and *p*-hydroxynorephedrine (Dring, Smith & Williams, 1970; Caldwell, Dring & Williams, 1972) but the stereochemical configuration of the metabolites does not seem to have been established unequivocally. The role of a false transmitter at

noradrenergic nerve endings in the peripheral sympathetic nervous system and brain (Gill, Mason & Bartter, 1967; Cavanaugh, Griffith & Oates, 1970) has been proposed. Hitherto used techniques would not have indicated the presence of norephedrine hydroxylamine (Ic) and thus the possibility that amphetamine hydroxylamine and norephedrine hydroxylamine are implicated in some of the actions of amphetamine requires investigation.

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