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Metabolic oxidation of aralkyl oximes to nitro compounds by fortified 9000*g* liver supernatants from various species

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Incubation of 'amphetamine oxime' (IIa, *anti*-benzyl methyl ketoxime) with fortified rabbit liver 9000 *g* supernatants gave the nitro compound (Ie) and the β-hydroxylated oxime (IIc) in addition to the previously reported ketone (IIb) and alcohol (Ic) metabolites. Formation of the products was cofactor dependent. The nitro compound was also formed using mouse, hamster and guinea-pig 9000 *g* liver supernatants and to a minor extent by rat liver. The oximes of 2-phenethylamine (IIe) and norfenfluramine (IIg) were also metabolized to the corresponding nitro compounds, ketones and alcohols with rabbit 9000 *g* liver supernatants; however, no nitro compound (IIIb) was detected after the incubation of 'mexiletine oxime' (IVa). The metabolic products were identified and characterized by g.l.c., t.l.c. and g.l.c. linked mass spectrometry by comparison with synthetic materials.

The metabolism of amphetamine has been extensively studied in recent years as a model for elucidating routes and mechanisms of *N*- and α-*C*-oxidations (Beckett, Van Dyk & others, 1971; Hucker, Michniewicz & Rhodes, 1971; Beckett & Al-Sarraj, 1972; Hucker, 1973; Lindeke, Cho & others, 1973; Parli & McMahon, 1973); the hydroxylamine (Ib), oxime (IIa), ketone (IIb) and alcohol (Ic) have been isolated after incubation of the amine (Ia) with fortified liver 9000 *g* supernatants from rabbits and other species. Whilst some of the oxime (IIa) reported may have arisen partly from breakdown of the labile hydroxylamine (Ib) metabolite during the analytical procedure, oxime formation has been demonstrated to be at least partly independent of the hydroxylamine (Ib), since the ratio of oxime (IIa) to hydroxylamine (Ib) detected varied with the species used even when employing the same analytical methods (Parli, in the discussion following Parli & McMahon, 1973). Also different ratios of oxime (IIa) to hydroxylamine (Ib) were obtained following incubations of α-carbon deuterated amphetamine (Parli & McMahon, 1973) with microsomal suspensions from different species. Beckett & Bélanger (1975) proposed a model of the metabolic formation of the oxime and hydroxylamine metabolic products from aliphatic primary amines possessing an α-C-H, by separate routes, involving a common flavoprotein ion/radical complex.

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Parli & McMahon (1973) reported extensive metabolism of synthetic 'amphetamine oxime' (IIa) by fortified rabbit liver microsomal suspensions; however, the ketone (IIb) was reported as a product which accounted for only 3·7% of the oxime (IIa) incubated, while only 53% of oxime was recovered. Hucker & others (1971) also found that 'amphetamine oxime' (IIa) was extensively metabolized to the ketone (IIb) and alcohol (Ic) by fortified rabbit liver homogenates.

Our preliminary results on the metabolism of 'amphetamine oxime' (IIa) by fortified rabbit liver supernatants have been reported (Beckett & Jones, 1975). An independent study of the *in vitro* metabolism of IIa was published almost simultaneously by Coutts, Dawe & others (1976) but these workers employed fortified liver supernatants from rat homogenized liver only. In the present work, the metabolism of various oximes was studied using fortified liver supernatants from five different species.

MATERIALS AND METHODS

Compounds and reagents

'Amphetamine oxime' (1-phenyl-2-propanone oxime, IIa) and 'norfenfluramine oxime' (1-[3′-trifluoromethylphenyl]propanone oxime, IIg) were synthesized by standard methods by a colleague Dr K. Haya. Norfenfluramine and the corresponding alcohol (1-[3′-trifluoromethylphenyl]-2-propanol, Ij) and ketone (1-[3′-trifluoromethylphenyl]-2-propa-

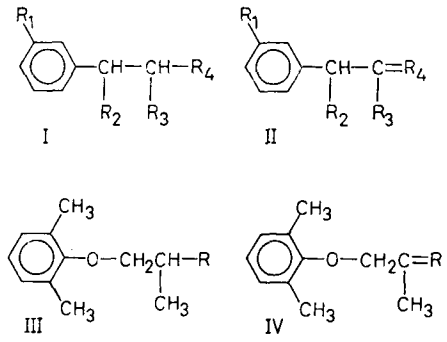

FIG. 1. Structures of some aralkylamines and their possible metabolites.

	R_1	R_2	R_3	R_4
Ia	H	H	CH_3	NH_2
Ib	H	H	CH_3	NHOH
Ic	H	H	CH_3	OH
Id	H	H	CH_3	NO
Ie	H	H	CH_3	NO_2
If	H	OH	CH_3	NHOH
Ig	H	OH	CH_3	OH
Ih	H	H	H	OH
Ii	H	H	H	NO_2
Ij	CF_3	H	CH_3	OH
Ik	CF_3	H	CH_3	NO_2

	R_1	R_2	R_3	R_4
IIa	H	H	CH_3	NOH
IIb	H	H	CH_3	O
IIc	H	OH	CH_3	NOH
IId	H	OH	CH_3	O
IIe	H	H	H	NOH
IIf	H	H	H	O
IIg	CF_3	H	CH_3	NOH
IIh	CF_3	H	CH_3	O

	R
IIIa	OH
IIIb	NO_2
IIIc	NH_2

	R
IVa	NOH
IVb	O

none, IIh) analogues of norfenfluramine were gifts from Servier (England); mexiletine, 'mexiletine oxime' (1-[2′,6′-dimethylphenoxy]-2-propanone oxime, IVa) and the alcohol (IIIa) and ketone (IVb) analogues were gifts from Boehringer (Ingelheim). The following compounds were purchased: phenylacetaldehyde (BDH), 1-phenyl-2-propanone (Koch Light), 1-phenyl-2-propanol (Fluka), 3-chloroperbenzoic acid (Koch Light). The corresponding hydroxylamines were synthesized as described previously (Beckett, Haya & others, 1975). Microanalyses were by Strauss (Oxford) and Dr A. J. Leyton, University College, London.

Preparation of liver homogenates

Incubations were carried out using 9000 *g* supernatant liver homogenates from rabbits (male New Zealand White, 2–3 kg), guinea-pigs (Duncan Hartly, 400–500 g), hamsters (Syrian, 100 g), rats (Wistar, 350 g) and mice (Laca, 30 g), prepared according to Beckett & Bélanger (1974a).

Incubations with liver homogenates

Incubations were carried out using the system described by Beckett & Bélanger (1974a) containing 9000 *g* supernatant, NADP, glucose-6-phosphate, nicotinamide and magnesium chloride in phosphate buffer pH 7·4. The oxime substrates were each added in 0·1 ml dioxan and incubated for 60 min with fortified 9000 *g* supernatants equivalent to 0·5 g of original liver. Where the sample was required for mass spectrometry, the incubation time was doubled. Control incubations were carried out in which (a) substrate (b) cofactor and (c) liver homogenate were separately omitted.

Extraction and analysis

Flasks were placed in ice after incubation and the whole incubates were normally extracted at pH 7·4 in 2·24 × 7·5 cm stoppered centrifuge tubes with *ca* 2 × 10 ml freshly distilled diethyl ether; (when required, solutions were rendered acidic, < pH1), with 0·5 ml conc. hydrochloric acid, alkaline (pH 9–10) with 50–100 mg anhyd. sodium carbonate and 'strongly alkaline' (pH 13–14) with 0·5 ml 5N sodium hydroxide before extraction). The ethereal extracts were concentrated at 45° to about 20 μl and placed in an ice bath.

Some extracts of 'amphetamine oxime' metabolism were further evaporated to dryness under nitrogen, 10 μl of dry acetonitrile (over $CaCl_2$) plus 25 μl *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) added and mixed well with the metabolic residue.

Gas liquid chromatography (g.l.c.)

G.l.c. was carried out on a Perkin Elmer F11 instrument which incorporated a flame ionization detector. Columns: glass i.d. 0·40 cm; the carrier gas N_2; air and hydrogen pressures 145 kNm^{-2}. *System A1*: 1 m column, packed with 5% Carbowax 20M on Chromosorb W 100–120 mesh, acid washed and DMCS treated; nitrogen 50 ml min^{-1} (pressure, 105 kNm^{-2}); oven temperature, 130°. *System A2*: As for A1 but nitrogen 120 ml min^{-1} and oven 160°. *System B*: 2 m column, packed with 2% XE60 on Chromosorb W 80–100 mesh, acid washed and DMCS treated; nitrogen 20 ml min^{-1} (pressure, 75 kNm^{-2}); oven temperature, 90°.

Thin layer chromatography (t.l.c.)

Plates were spread to a thickness of 250 μm with silica gel GF_{254}, dried for 1 h at 110° and run in benzene–ethyl acetate (3 : 1). The plates were viewed under ultraviolet light (λ 254 nm) and either sprayed with 1% copper(II) chloride, 1% iron(III) chloride or placed in an iodine tank.

G.l.c. linked mass spectrometry (g.l.c.-ms)

G.l.c.-ms spectra were obtained using a Perkin

Elmer model 270 g.l.c.—mass spectrometer. *System A*: packed as for g.l.c., system A1; helium (carrier gas) 100–140 kNm^{-2}; oven temperature 120–160°. *System B*; packed as for g.l.c. system B; helium, 75–105 kN^{-2}; oven temperature, 90–120°.

Spectroscopy

Infrared spectra were recorded as films between rock salt plates.

Nuclear magnetic resonance (nmr)

Nmr spectra were recorded as 10% solutions in $CDCl_3$ in a Perkin Elmer R32 spectrometer, incorporating a field lock on the TMS (tetramethylsilane) internal standard signal. All δ values are quoted in ppm and are the measured centres of groups and not corrected for non-first-order conditions. The methylene protons of the 2-nitroisopropyl compounds are reported as CH_A and CH_B.

Syntheses

(±)-*2-Nitro-1-phenylpropane* (*Ie*). (±)-2-Amino-1-phenylpropane (Ia, extracted from the sulphate, 10·0 g 54 mmol) was slowly added in chloroform (100 ml) to 3-chloroperbenzoic acid (CPBA, 60 g, 347 mmol) under reflux, in chloroform (150 ml) and conditions maintained for 4 h. The solution was washed with aqueous sodium metabisulphite (10%, 150 ml), followed by potassium carbonate solution (10%, 5 × 250 ml). The aqueous layers were discarded and the chloroform layer evaporated *in vacuo* to leave a yellow oil (8·9 g) which contained about 90% of the title compound (nmr evidence). The oil was then purified by steam distillation to give a pale yellow oil (6·4 g, 72% yield). Further purification by continuous column chromatography (silica gel; eluted with benzene–ethyl acetate 3:1), gave a product estimated to be >98% of the title compound; b.p. 74°, 0·25 mm Hg (cf. Gilsdorf & Nord, 1952; 103–104°, 4·0 mm Hg); nmr δ1·50 (d, 3, CH_3) 2·95 (q, 1, CH_A) 3·31 (q, 1, CH_B) 4·77 (m, 1, $CHCH_3$) 7·1–7·4 (m, 5, *Ar*); infrared (film ν_{max} (main bands) 705 (m), 755 (m), 1360 (m, sym. NO_2 stretch), 1390 (m), 1455 (m), 1545 (s, asym. NO_2 stretch) cm^{-1}; g.l.c.-ms, *m/e* (% rel. abund): 165 ($M^{+\cdot}$, 0·5),1 19 (15), 118 (82), 117 (17), 91 (100), 65 (10), 41 (18), m* (metastables): 69·6, 46·4 (in good agreement with that reported by Nibberling & de Boer, 1969). Found: C, 65·3; H, 6·7; N, 8·0. Calc. for $C_9H_{11}NO_2$: C, 65·5; H, 6·7; N, 8·5%.

(±)-*2-Nitro-1-(3′-trifluoromethylphenyl)propane* (*Ik*). This was prepared from (±)-2-amino-1-(3′-trifluoromethylphenyl)propane (Id, extracted from the hydrochloride salt, 3·0 g, 12·5 mmol) and CPBA (20 g, 116 mmol) as described for Ie above, to give a pale yellow oil. The oil was steam distilled to give the title product (estimated to be 95% by g.l.c. and nmr; 2·33 g, 80% yield); nmr δ 1·54 (d, 3, CH_3) 3·04 (q, 1, CH_A) 3·38 (q, 1, CH_B) 4·79 (m, 1, $CHCH_3$) 7·2–7·6 (m, 4, *Ar*); infrared (film) ν_{max} (main bands) 710 (m), 1080 (m), 1130 (s) and 1170 (s, CF_3), 1335 (s), 1550 (s, asym. NO_2 stretch cm^{-1}; g.l.c.-ms; *m/e* (% rel. abund.): $M^{+\cdot}$ absent, 214 (M^+–19, 4%), 187 (15), 186 (54), 159 (100) 117 (20), 109 (10), 41 (22), m* (metastable) 135·2.

2-Nitro-1-phenylethane (*Ii*). This was prepared from 2-phenylethylamine base (5·0 g, 41 mmol) and CPBA (40 g, 232 mmol) as described for Ie, as a yellow oil which was distilled *in vacuo* to give a pale yellow oil (estimated to be 95% of the title compound by nmr, 4·0 g, 65% yield), b.p. 78–80° (0·3 mm Hg); nmr δ 3·28 (t, 2, CH_2-Ar) 4·58 (t, 2, CH_2-NO_2) 7·1–7·3 (m, 5, *Ar*); infrared (film) ν_{max} (main bands) 700 (m), 755 (m), 1380 (m, sym. NO_2 stretch), 1545 (s, asym. NO_2) (stretch) cm^{-1} (cf. Gottlieb & Magalhaes, 1959; nitro absorption 1380 and 1550 nm); g.l.c.-ms, *m/e* (% rel. abund.): 151 ($M^{+\cdot}$, 1), 105 (38), 104 (100), 103 (16), 79 (23), 77 (27), 51 (14).

(±)-*2-Nitro-1-(2′-6′-dimethylphenoxy)propane* (*IIIb*). This was prepared, but not isolated, from the oxidation of (±)-2-amino-1-(2′,6′-dimethylphenoxy)propane (IIIc) with CPBA as described for Ie. A moderate quantity of at least one other oxidation product was formed (unidentified, g.l.c. Rt 1·7 min—system A2) but not the corresponding ketone, (Rt 2·5 min) in addition to the nitro compound (IIIb, Rt 9·0 min). Purification was not attempted and the nitro compound (IIIb) was identified by its g.l.c. retention time (system A2) relative to the corresponding ketone and oxime (Rt 21·2 min, system A2) (cf. those of the corresponding amphetamine and norfenfluramine analogues) and by interpretation of its g.l.c.-ms; *m/e* (% rel. abund.): 209 ($M^{+\cdot}$ 5), 122 (100), 121 (55), 107 (95), 105 (18), 91 (32), 79 (29), 77 (45), 41 (50), 39 (47). No reference to the title compound could be found in the literature.

2-Phenylacetaldehyde oxime (*IIe*). This was prepared by condensation of the corresponding

aldehyde (IIf) with hydroxylamine by a standard method (Vogel, 1966). The resulting white solid was characterized: m.p. 92–94° (cf. Gilsdorf & Nord, 1952, 97–99°); nmr δ 3·73 (d, 2, CH_2) 6·88 (t, 1, *CH*) 7·26 (s, 5, *Ar*) *ca* 9·2 (s, broad, 0·5?, *OH*); infrared (Nujol) ν_{max} 695 (s), 740 (m), 930 (s), 1060 (m), 1460 (s, partly Nujol), 1675 (C=N str., m), 2860–3080 (s, Nujol and aliph. CH), 3200 (s, b, OH); mass spectrum (direct linet), *m/e* (% rel. abund.): 135 ($M^{+\cdot}$, 20), 118 (35), 117 (100), 116 (22), 91 (99), 90 (70), 89 (30), 65 (29), 51 (20), 39 (22).

RESULTS AND DISCUSSION

Examination of the incubates

G.l.c. analysis (system A1 or B) of underivatized or derivatized (with BSTFA) extracts made at acidic (pH <1) and at alkaline (pH 9–10 and 13–14) pH values, of preliminary 'amphetamine oxime' (IIa) incubates, showed no peaks that were not observed on analysis of the extract made at neutral pH (7·4). Comparison of g.l.c. traces (of underivatized and derivatized samples, systems A1 and B respectively) of extracts of 'amphetamine oxime' (IIa) incubates with those of reference compounds excluded the following potential metabolites: amphetamine (Ia), 'amphetamine hydroxylamine' (Ib), benzoic acid (the extract at pH <1 was examined), benzaldehyde, and the β-hydroxylated ketone (IId) and alcohol (Ig). Ring hydroxylated metabolites were concluded to be absent following 1% iron (III) chloride treatment of t.l.c. plates spotted with extracts made at neutral and acidic pH values.

Further examination of the incubate of 'amphetamine oxime' (IIa) was made using neutral (pH 7·4) ethereal extracts, except where derivatization was required, when the addition of *ca* 50–100 mg of sodium carbonate removed an interfering ether

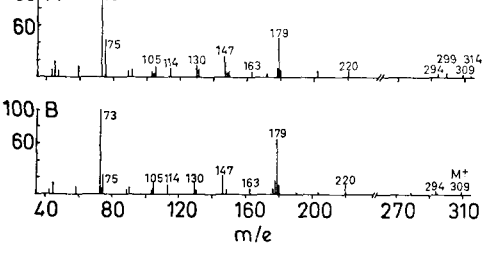

FIG. 2. G.l.c.-ms spectra of the TMS derivatives of A—'norephedrine oxime' (IIc), formed from the metabolism of amphetamine oxime (IIa), and B—synthetic 'norephedrine oxime' (IIc).

impurity of similar g.l.c. (system B) retention time to that of the di-silylated 'norephedrine oxime' (IIc).

Identification of the metabolic products of 'amphetamine oxime' (IIa)

Incubation of 'amphetamine oxime' (IIa) with fortified rabbit liver 9000 *g* supernatants gave the ketone (IIb), alcohol (Ic), nitro compound (Ie) (g.l.c. Rt 3·6 min, system A1; cf. oxime, IIa, 8·8 min) and 'β-hydroxylated oxime' ('norephedrine oxime', IIc) (Beckett & Jones, 1975). None of the products were detected in the absence of cofactor or liver homogenate, except for a very small quantity of the ketone (IIb). G.l.c.-ms (system A1) of the metabolic ketone (IIb), alcohol (Ic) and nitro compound (Ie) were identical with those of authentic materials. The nitro compound (Ie) mass spectrum showed a very weak molecular ion (*m/e* 165, 0·3%), the base peak occurring at *m/e* 118 (M^+–47) due to the loss of nitrous acid from the molecular ion (in agreement with the spectrum in Nibberling & De Boer, 1969, and in contrast to the mass spectrum of the corresponding synthetic nitroso compound, where the base peak was *m/e* 119 due to the loss of an NO radical from the monomer molecular ion (Beckett, Jones & Coutts, 1976). The metabolically formed nitro compound (Ie) was located on t.l.c. with the aid of ultraviolet light λ 254 nm (silica gel GF_{254}), development with iodine vapour and comparison of its R_F value (R_F 0·62) with that of synthetic material. The untreated 'nitro spot' was scraped off and extracted with freshly distilled diethyl ether; the extract gave one peak on g.l.c. (system A1) with a retention time identical to that of the synthetic nitro compound (Ie).

T.l.c. of the 'amphetamine oxime' (IIa) metabolic extract, after spraying with 1% copper(II) chloride, gave two green spots of R_F values 0·50 and 0·18, corresponding to unchanged 'amphetamine oxime' (IIa) and the β-oxidized oxime, 'norephedrine oxime' (IIc) respectively. Authentic 'norephedrine oxime' (IIc) gave a t.l.c. spot of identical R_F value (0·18) to that of the metabolic material. On g.l.c. system A2, 'norephedrine oxime' (IIc) had a much longer retention time than 'amphetamine oxime' (IIa), almost identical to that of nicotinamide (a cofactor constituent). However on g.l.c. system B, after silylation with BSTFA, the β-hydroxy-oxime ('norephedrine oxime', IIc) metabolite gave rise to a peak of retention time 22·3 min (cf. oxime, IIa-TMS derivative, Rt 11·3 min) corresponding to that of the di-TMS-ether derivative of the oxime (g.l.c.-ms evidence) (see Fig. 2). Meta-

bolic and synthetic 'norephedrine oxime'-TMS-derivative g.l.c. retention times and g.l.c.-ms were comparable.

Preliminary work indicates that metabolic formation of the nitro compound (3·7%) is at least as important as the formation of the dealkylated products (ketone 1·7, and alcohol 1·0%) after a 30 min incubation of 5 μmol 'amphetamine oxime' (IIa) with fortified 9000 *g* supernatant equivalent to 0·5 g of rabbit liver; about 90% of oxime was recovered unchanged. The amount of β-hydroxy oxime (IIc) was difficult to determine due to the very different analytical conditions used from that for the nitro compound (Ie) and interference from extraneous silylated material, but was comparable to the amount of nitro compound (Ie) formed.

In vitro metabolism of 'amphetamine oxime' by liver homogenates from five different species

'Amphetamine oxime' (IIa) was incubated with fortified liver 9000 *g* supernatants from rabbits, mice, hamsters, guinea-pigs and rats. The nitro compound (Ie) was formed in comparable quantities to that in rabbit liver incubates, in guinea-pig and hamster incubates, less in mouse and very little in the rat incubate. (Contrast the considerable metabolism of the oxime (IIa) to the nitro compound (Ie), by fortified rat liver homogenate, 12 000 *g* supernatant, found by Coutts & others, 1976). The ketone (IIb) was formed by all species, but little alcohol (Ic) was formed except when rabbit liver homogenate was used. The poor ability of rat liver homogenate to *N*-oxidize many substrates is recognized (Beckett & Al-Sarraj, 1972; Cho, Lindeke & Sum, 1974; Beckett & Gibson, 1975).

Metabolism of other oximes by rabbit liver homogenates

'Amphetamine oxime' (IIa), phenylacetaldehyde oxime (IIe), 'norfenfluramine oxime' (IIg; 1-[3′-trifluorophenyl]-2-propanone oxime) and 'mexiletine oxime' (IVa; 1-[2′,6′-dimethylphenoxy]-2-propanone oxime), were incubated (5 μmol of each) with rabbit liver fortified 9000 *g* supernatants (equivalent to 0·5 g liver) for 60 min. The corresponding nitro compound and alcohol were formed from phenylacetaldehyde oxime (IIe) and 'norfenfluramine oxime' (IIg) in comparable quantities to those formed from 'amphetamine oxime' (IIa) under the same conditions; however, little, if any, of the nitro compound (IIIb) was formed from 'mexiletine oxime' (IVa). The corresponding carbonyl compounds were present only in small quantities after incubation of phenylacetaldehyde oxime (IIe) and 'mexiletine oxime' (IVa) but were present from 'norfenfluramine oxime' (IIg) in a quantity similar to that formed from 'amphetamine oxime' (IIa).

DISCUSSION

The *in vitro* metabolism of acetophenone oxime and 'amphetamine oxime' has been reported. Apparently acetophenone oxime was not metabolized under aerobic conditions on incubation with fortified 9000 *g* liver supernatant from rabbit and other species (Hes & Sternson, 1974); however, reduction to the corresponding hydroxylamine and amine did occur under anaerobic conditions; the anaerobic biotransformation was strongly inhibited by the presence of oxygen (Hes & Sternson, 1974; Sternson & Hes, 1975). In contrast, extensive aerobic metabolism of 'amphetamine oxime' (IIa) by rabbit microsomes gave the ketone (IIb) (Parli & McMahon, 1973) and with 9000 *g* homogenate also the alcohol (Ic) (Hucker & others, 1971). However, Parli & McMahon (1973) recovered only 53% of the oxime (IIa) substrate and 4% of the ketone (IIb). Since microsomes were used, the amount of alcohol (Ic) formed would be negligible; thus at least some of the remainder could have been the nitro compound (Ie).

Recently Coutts, Dawe & others (1975) reported the nitro compound (Ie) as a metabolic product of *N*-methylamphetamine using rat liver homogenate. Coutts & others (1976) later reported the metabolism of 'amphetamine oxime' (IIa) to the corresponding ketone (IIb), nitro compound (Ie) and a small amount of benzyl alcohol by fortified 12 000 *g* supernatants from rat liver.

Many nitro compounds are known to occur in nature, though most have been isolated from plants (e.g. β-nitropropionic acid: Carter & McChesney, 1949; Morris, Pagan and Warmke, 1954) or incubates of micro-organisms (e.g. chloramphenicol: Rebstock, Crooks & others, 1949). There are few reports of nitro compounds arising from mammalian hepatic oxidation.

Some nitro compounds can easily arise from oxidation of the corresponding nitroso compound, particularly if the latter is in the monomeric form (e.g. aromatic nitroso compounds or those with a tertiary α-carbon atom, in solution) which, in turn, could arise by auto-oxidation of the corresponding hydroxylamine, or by metabolic *N*-oxidation of the amine. Thus, the nitro compound identified after metabolism of phentermine (Beckett & Bélanger, 1974a) and chlorophentermine (Beckett & Bélanger,

1974a, b) are recognized by the authors as possible auto-oxidation products of the nitroso metabolites. However, the nitro compounds identified in the present study must arise largely, if not wholly, as a direct result of metabolism. If formation of the nitro compound, from the oxime (IIa), occurs via the nitroso compound (Id) the isomerization step would be metabolic since the oxime (IIa) is not oxidized in more than small amounts to the nitro compound (Ie) by 3-chloroperbenzoic acid (Jones, 1976); the nitroso compound (Id) can be oxidized chemically, or possibly by enzymic oxidation, to the nitro compound (Ie). However, metabolic oxidation of the oxime (IIa) to the nitro compound (Ie) may occur directly by an obscure mechanism.

Part of this work was presented at the 1975 British Pharmaceutical Conference in Norwich (Beckett & Jones, 1975).

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