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The identification and analysis of the metabolic products of mephentermine

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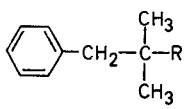
Phentermine (Ib), *N*-hydroxymephentermine (Ic) and *N*-hydroxyphentermine (Id) were identified as metabolic products after *in vitro* incubation of mephentermine (Ia) with rabbit liver microsomal fractions. Compounds Ia, Ib and Ic were also identified as excretion products in the urine of a human subject given a single dose of mephentermine (Ia) sulphate. Derivatization with acetic anhydride, trifluoroacetic anhydride and the trimethylsilyl donor reagent *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) or hexamethyldisilazane (HMDS) were used for qualitative identification of the metabolic products Ib–Id by g.l.c.-mass spectrometry and for quantitative determination of Ia–Id after extraction from rabbit hepatic homogenates. The synthesis of *N*-hydroxymephentermine (Ic) and the properties of the metabolic products are reported.

Two possible metabolic routes for arylalkylamines are through *N*-oxidation and α-carbon oxidation (Beckett, 1971). The primary amine phentermine (Ib), having no hydrogen on the α-carbon, is metabolized *in vitro* and *in vivo* in animals and man to *N*-hydroxyphentermine (Id), α,α-dimethyl-α-nitroso-β-phenylethane (Ie) (Beckett & Bélanger, 1974a,b) and *p*-hyroxyphentermine (Cho, 1974). Compound Id has also been confirmed as a metabolite after *in vitro* studies with phentermine (Ib) using liver preparations (Cho, Lindeke & Hodshon, 1972). The secondary amine mephentermine (Ia) was therefore chosen to study the characteristics of the above two metabolic routes; its *in vitro* metabolism has not been reported hitherto. Phentermine (Ib) i.e. the *N*-demethylated product of Ia has been identified using paper and gas-liquid chromatography as an excretion product of mephentermine (Ia) in the extracts of urine of dog, rat, rabbit (Walkenstein, Chumakow & Seifter, 1955) and man (Beckett & Brookes, 1971). The former authors also identified *p*-hydroxymephentermine and *p*-hydroxyphentermine in urine extracts.



(I)

N-Oxygenated metabolic products of primary and secondary amines have not been reported until recently because of their labile nature under the usual experimental conditions (Beckett, 1974). Various methods including alkaline oxidation, reduction to the parent amine, t.l.c., g.l.c. and g.c.-mass spectrometry have been used recently to identify and analyse these compounds (Cho & others, 1972; Beckett & Al-Sarraj, 1973; Beckett, Coutts & Ogunbona, 1973a; Lindeke, Cho & others, 1973; Beckett &

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Bélanger, 1974a,b; Becket & Midha 1974a). We now report the structures, properties and quantitative analysis of three new *in vitro* metabolic products of mephentermine (Ia): phentermine (Ib), *N*-hydroxymephentermine (Ic) and *N*-hydroxyphentermine (Id). The synthesis of α,α-dimethyl-α-nitro-β-(4-chlorophenyl)ethane, an excretion product of chlorphentermine in man (Beckett & Bélanger, 1974b) and animals (Köster, Caldwell & Smith, 1974), is described since this compound was used as reference g.l.c. standard in the present study.

MATERIALS AND METHODS

Compounds and reagents

Phentermine hydrochloride, mephentermine sulphate and chlorphentermine hydrochloride were kindly supplied by Riker Laboratories (Loughborough), John Wyeth and Brother (Berkshire) and William R. Warner and Co. Limited (Hampshire), respectively. *N*-Hydroxyphentermine (Id), α,α-dimethyl-α-nitroso-β-phenylethane (Ie) and α,α-dimethyl-α-nitro-β-phenylethane (If) were prepared as before (Beckett & Bélanger, 1974a) with the exception that Id was prepared as the oxalate and recrystallized from absolute ethanol, m.p. 169–171° (Found: C, 62·8; H, 7·7; N, 6·6. Calculated for $C_{22}H_{32}N_2O_6$: C, 62·8; H, 7·6; N, 6·7%). Also used was 4-chlorophenylcyclopropylketone (Ralph N. Emanuel Limited), *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, Pierce Chemical Co., U.S.A.) and hexamethyldisilazane (HMDS, Koch–Light Laboratories Limited), freshly distilled, acetonitrile (Fisons), redistilled over P_2O_5 and kept over molecular sieve at room temperature. All the drugs and their metabolic products were checked for purity by g.l.c.

N-*Hydroxymephentermine* (*Ic*). Mephentermine base (Ia, 0·015 mol) in sodium dry ether (100 ml) was refluxed overnight with benzoyl peroxide (0·0041 mol). The solution was washed with $NaHCO_3$ (10% in water, 4 times) and with water (twice) then phosphate buffer pH 6·2 (3 times). After drying ($MgSO_4$ anhydrous) the ether was removed to yield a yellowish oil (3·6 g): infrared 1740 cm^{-1} due to -NOCO- (Denney & Denny, 1960). This oil was reduced with lithium aluminium hydride (1 g) in sodium dry ether (50 ml; 1 h; room temp.; Beckett, Coutts & Ogunbona, 1973b). The excess $LiAlH_4$ was destroyed with cautious addition of water and the mixture extracted with ether. T.l.c. of the ethereal extract (system 1) yielded one main spot (iodine vapour or ammoniacal silver nitrate). Evaporation of the dried extract gave a residue (absence of 1740 cm^{-1} but with 3400 cm^{-1} due to hydroxyl). Addition of ethereal oxalic acid to this residue also in ether slowly precipitated the acid oxalate (1·54 g, yield 38%); recrystallized from EtOH/ether: m.p. 62–63°; nmr (DMSO) τ2·7 (s, 5, Ar), 6·94 (s, 3, CH_3), 7·0 (s, 2, CH_2), 8·8 (s, 6, C-$(CH_3)_2$); mass spectrum *m/e* 88 (100), 56 (22), 91 (20), 45 (13), 46 (9), 89 (6). Found: C, 55·6; H, 7·5; N, 5·0. Calculated for $C_{13}H_{19}NO_5$: C, 58·0, H, 7·1, N, 5·2%. Compound Ic was stable when extracted from phosphate buffer at pH 7–9·5, however at pH 14 a 40% decrease in recovery of Ic was obtained with the appearance of a peak on g.l.c. analysis (column D) corresponding to *N*-hydroxyphentermine (Id).

α,α-*Dimethyl-α-nitro-β-(4-chlorophenyl)ethane* (*reference standard*). The method of Kornblum, Clutter & Jones (1956) for the synthesis of 2-methyl-2-nitropropane was used; chlorphentermine base (α,α-dimethyl-*p*-chlorophenethylamine, 5 mmol) yielded (α,α-dimethyl-α-nitro-β-(4-chlorophenyl)ethane (200 mg, yield 19%); m.p. 29–32°; nmr ($CDCl_3$)τ 2·63–3·05 (quartet, 4, Ar), 6·72 (s, 2, CH_2), 8·5 (s, 6, C-$(CH_3)_2$); infrared

1550 and 1357 cm^{-1} (medium, NO_2); mass spectrum (g.l.c.-m.s.) *m/e* 125 (100), 32 (90), 55 (65), 41 (47), 58 (44), 127 (38), 166 (35), 96 (30), 167 (28). Found: C, 56·1; H, 5·8; N, 6·5. Calculated for $C_{10}H_{12}NO_2Cl$: C, 56·2; H, 5·6; N, 6·5.

Physical measurements. M.p.s (capillary tubes) are uncorrected. All mass spectra were recorded using a Perkin-Elmer Model 270 gas chromatograph-mass spectrometer system at an ionization potential of 70 eV. Two one-meter $\frac{1}{4}$ in o.d. glass columns, and Apiezon L (10% on Chromosorb G 80–100 mesh, T 160–180°) and an OV-17 (2% on Chromosorb G 80–100 mesh, T 120–160°) were used; helium (10 lb in^{-2}) was the carrier gas. The direct inlet technique was also used for reference compounds. Elemental analysis were carried out at the Department of Chemistry, University College, University of London, U.K.

Gas-liquid chromatography. A W.G. Pye "Series 104" chromatograph model 84 (Pye Unicam Limited, Cambridge) equipped with a flame ionization detector was used; the detector temperature was 250°. All columns were glass tubing $\frac{1}{4}$ in o.d. and the solid supports were acid-washed and treated with dimethyldichlorosilane before handling. Column A, 5 feet, was packed with Chromosorb G (80–100 mesh) coated with OV-17 (3% w/w). Column B, 5 feet, was packed with Chromosorb W (100–120 mesh) coated with a mixture of Silicone Elastomer E-301 (3% from Griffin & George, London) and Triton X-305 (0·01% from Lenning Chemicals Limited, Croydon). Both columns were operated under the following conditions: H_2, 10 lb in^{-2}; air, 15 lb in^{-2}; N_2, 15 lb in^{-2} (37·5 ml min^{-1}). Column C and D, 5 and 3 feet long respectively, were packed with Chromosorb Q (100–120 mesh); the former was coated with Carbowax 20M (7·5%) and the latter with Apiezon L (5%). The operating conditions were: H_2, 15 lb in^{-2}; air, 15 lb in^{-2}; N_2, 15 lb in^{-2} (60 ml min^{-1}) for column C and 10 lb in^{-2} (75 ml min^{-1}) for column D. These columns were conditioned at 250, 240, 175 and 220° respectively for 24 h before use. Each of these columns was silanized with 2 × 5 μl of hexamethyldisilazane (HMDS) before use. Samples were injected directly on the columns.

Thin-layer chromatography. Glass plates (20 × 20 cm) were spread to a thickness of 0·25 mm with a mixture of silica gel G (Merck) and water (1:2). The plates were first allowed to dry at room temperature and heated for 1 h at 110° before use. The solvent systems used were: chloroform–acetone (80:20), and ethanol 96%–pyridine–dioxane–water (20:10:60:5). The various spots were visualized after contact with iodine vapour or sprayed with $AgNO_3$(ammoniacal) solution.

Liver homogenates incubation experiments. Aqueous solutions of mephentermine sulphate (Ia: 5 μmol base ml^{-1}, 1 ml) were incubated in open 25 ml conical flasks at 37° for 60 min with rabbit (New Zealand male, white) liver microsomal fractions (9 000 *g* supernatant and microsomes) in phosphate buffer (pH 7·4) containing the appropriate cofactor requirements as recently described (Beckett & Bélanger, 1974a). Each incubate contained 1 ml of substrate, 1 ml of cofactor solution, 1 ml of microsomal fraction in tris/KCl buffer (pH 7·4) equivalent to 0·5 g of orginal liver, and 3 ml of phosphate buffer (pH 7·4). The incubate reactions were stopped by putting the flasks in ice and the mixtures were extracted at alkaline and neutral pH values,

oxidized with $KMnO_4$ (1%, 1 ml) then extracted and analysed on g.l.c. as described below.

In vivo *oral administration of mephentermine* (*Ia*). A man (age 27) with normal urine pH (6–7·3) was given a dose of 25 mg of mephentermine sulphate and the urine collected over 24 h was bulked and analysed for the excretion products. Samples of urine (10 ml) were extracted at neutral and alkaline (addition of NaOH 4 M, 1 ml) pH values with ether; hydrolysed at pH 4·5 and 37° overnight in the presence of sulphatase/β-glucuronidase (*Suc d'Elix Pomatia*, 0·1 ml) followed by extraction at neutral pH (7–7·5) with ether; oxidized with $KMnO_4$ (1%, 1 ml) at room temperature for 20 min and extracted with ether. The organic extracts were further treated and analysed on g.l.c. as below.

Determination of mephentermine (*Ia*) *and phentermine* (*Ib*) *as their trifluoroacetyl derivatives* (*TFA*). Water (1 ml) or phosphate buffer (pH 7·4) containing rabbit liver homogenates at the same dilution used in incubation experiments (1 ml), both containing phentermine HCl (Ib, 0·0216–0·26 μmol) and mephentermine sulphate (Ia, 0·05–1 μmol base) were placed in glass-stoppered centrifuge tubes. Chlorphentermine HCl (0·5 μmol ml^{-1} in water, 1 ml) was added as reference standard followed by NaOH (4 M, 1 ml) to adjust the pH to 12·5 and the mixture extracted with freshly distilled ether (3 times with 20 min shaking each time). The collective ethereal extract of each tube was concentrated to a low bulk (20–40 μl) in a water bath (42–45°), trifluoroacetic anhydride (5 μl) added and the mixture stood at room temperature for 1–5 min; the resulting trifluoroacetyl derivatives (TFA) of Ia, Ib and of chlorphentermine were analysed on g.l.c. column A.

Determination of N-*hydroxyphentermine* (*Id*) *as the acetyl derivative* (*AC*). Mixtures of *N*-hydroxyphentermine oxalate in water (Id, 0·1–2 μmol base ml^{-1}, 1 ml) in phosphate buffer pH 7·4 (5 ml) or in phosphate buffer (pH 7·4) containing rabbit liver microsomal fraction at the same dilution used in incubation experiments (5 ml) and benzophenone (1·1 μmol ml^{-1} in water, 1 ml) as reference standard were placed in 20 ml centrifuge tubes and extracted at pH 7–7·4 with ether (3 times with 20 min shaking each). The organic extracts were concentrated, acetic anhydride (5 μl) added to the ether bulk and the mixture allowed to stand at room temperature for 1–5 min; then the acetyl derivative (AC) of Id was analysed on g.l.c. column B.

Determination of N-*hydroxymephentermine* (*Ic*) *and* N-*hydroxyphentermine* (*Id*) *as their trimethylsilyl derivatives* (*TMS*). Aqueous solution (1 ml) containing *N*-hydroxymephentermine acid oxalate (Ic, 0·01–0·2 μmol base ml^{-1}) and *N*-hydroxyphentermine oxalate (Id, 0·02–0·2 μmol ml^{-1}) were added to phosphate buffer (5 ml) or to phosphate buffer (pH 7·4) containing rabbit liver microsomal fraction at the same dilution used in incubation experiments (5 ml) along with α,α-dimethyl-α-nitro-β-(4-chlorophenyl)ethane (0·14 μmol ml^{-1} in water, 1 ml) as reference standard and extracted at neutral pH as described above. The ethereal extracts were concentrated and allowed to dry at room temperature; dry acetonitrile (10 μl) or pyridine (10 μl) and the silylating reagent BSTFA or HMDS (5 μl) were added and the mixture allowed to stand at room temperature for 5–15 min; then the trimethylsilyl derivatives (TMS) of compounds Ic and Id were analysed on g.l.c. column D.

Quantitative oxidation of N-*hydroxymephentermine* (*Ic*), N-*hydroxyphentermine* (*Id*) *and* α,α-*dimethyl*-α-*nitroso*-β-*phenylethane* (*Ie*) *and determination of* α,α-*dimethyl*-α-*nitro*-β-*phenylethane* (*If*). To phosphate buffer pH 7·4 (5 ml) or phosphate buffer containing rabbit liver microsomal fraction at the same dilution used in incubation experiment (5 ml) were added known quantities of α,α-dimethyl-α-nitro-β-phenylethane. (If, 0·026–0·26 μmol ml^{-1} in water, 1 ml) and potassium permanganate ($KMnO_4$ 1% w/v in water, 1 ml); the mixture was shaken at room temperature for 20 min, then extracted with ether (3 times with 20 min shaking each). 4-Chlorophenylcyclopropylketone (0·27 μmol ml^{-1} in ether, 1 ml) as reference standard was added to the bulked ethereal extracts, the solutions concentrated and analysed on g.l.c. column C. Similar experiments were carried out with compounds Ia, Ib (5 μmol ml^{-1}, 1 ml), Ic (0·0892, 0·12 and 0·189 μmol base ml^{-1}, 1 ml), Id (0·048, 0·0725, 0·095, 0097 and 0·145 μmol base ml^{-1}, 1 ml) and Ie (4·11 μmol ml^{-1} methanol, 0·1 ml).

Quantitative analysis

Standard solutions were freshly prepared in the range of concentration described above. Calibration curves based on the peak height ratios of compounds Ia, Ib, Ic, Id and If to their g.l.c. reference standard using the methods described above were obtained from 15–21 points representing 5–7 different concentrations in triplicate analysis. The data were subjected to linear regression analysis to give the appropriate calibration factors.

RESULTS AND DISCUSSION

Structure of the metabolic products of mephentermine (*Ia*). Incubation of mephentermine (Ia) with rabbit hepatic microsomal fractions with the co-factor solutions (Beckett & Bélanger, 1974a) and subsequent extraction at pH 7–7·4 with ether, gave two spots with R_F values of 0·28 (A) and 0·40 (B) in addition to a base-line spot on t.l.c. using solvent 1 (Table 1); spraying with ammoniacal $AgNO_3$ converted A and B to black spots. The R_F values of spots A and B were identical to those of *N*-hydroxymephentermine (Ic) and *N*-hydroxyphentermine (Id), respectively, by comparison with reference compounds. Furthermore, trimethylsilyl derivatization of the ethereal extract of the incubation mixture of mephentermine (Ia) led to two peaks upon g.l.c. analysis using column D (Table 1); these peaks were not present upon similar treatment following incubation in the absence of mephentermine (Ia). G.l.c.-mass spectrum of one peak gave a mass fragment ion at *m/e* 146 corresponding to structure i (Fig. 1) while the second peak yielded a mass fragment ion at *m/e* 160 (ii); the two peaks were those of the trimethylsilyl derivatives of *N*-hydroxyphentermine (Id) and *N*-hydroxymephentermine (Ic), respectively. The mass spectrum of the hydroxylamines Id and Ic gave base peaks at *m/e* 74 (iii, Fig. 1) and 88 (iv), respectively; these two characteristic ions are shifted to mass fragments i and ii upon trimethylsilylation of the hydroxylamino group of Id and Ic. Moreover, oxidation with aqueous permanganate of Ic and Id in phosphate buffer solution and of mixtures of the incubation of mephentermine (Ia) led to complete disappearance of the g.l.c. peaks of Ic and Id in favour of that of the nitro compound If.

When the extraction after incubation of mephentermine (Ia) was carried out at pH 12·5 with ether, one spot corresponding to phentermine (Ib) on t.l.c. using solvent 2 and one peak corresponding to phentermine (Ib) on g.l.c. column A was observed in addition to that of the parent amine (Ia). The structure Ib was further confirmed by

Table 1. *G.l.c. and t.l.c. characteristics of mephentermine (Ia), phentermine (Ib), their metabolic products and related derivatives.*

Compound	Derivative	G.l.c. Rt values (min) Column A 140°	Column B 120°	Column C 170°	Column D 130°	T.l.c. R_F values Solvent 1	Solvent 2
Ia		3·9	—	—	3	0·1	0·41
Ib		2·7	2·2	1·7	1·8	0·1	0·56
Ic		—	—	—	—	0·28	—
Id		—	—	—	—	0·40	—
Ie		2·0	—	—	1·5		
If		11·2	3·6	6·4	4·5		
Ia	TFA	8·0	5·8	—	5·2		
Ib	TFA	4·0	3·1	—	3·4		
Ia	AC	—	15·3	—	15·5		
Ib	AC	16·0	8·6	—	9·4		
Ic	AC	—	13·6	—	—		
Id	AC	—	6·8	—	8·0		
Ic	TMS	—	10·2	—	8·8		
Id	TMS	—	5·3	—	4·3		

TFA: trifluoroacetyl derivative, AC: acetyl derivative, TMS: trimethylsilyl derivative. The following reference standards were used: chlorphentermine (as TFA, column A, R_F 11·2 min), benzophenone (column B, R_F 13·8 min), 4-chlorophenylcyclopropylketone (column C, R_F 10·4 min) and α,α-dimethyl-α-nitro-β-(4-chlorophenyl) ethane (column D, R_F 13·4 min).

its g.l.c.-mass spectrum which gave a base peak at *m/e* 58 (v, Fig. 1). G.l.c.-mass spectra of the acetyl and trifluoroacetyl derivatives of phentermine (Ib) gave characteristic mass fragment ions at *m/e* 100 and 154 subsequently identified as vi and vii (Lindeke & Cho, 1973), respectively. Likewise, Ia gave a base peak at *m/e* 72 (viii) and its acetyl and trifluoroacetyl derivatives gave characteristic mass fragment ions at *m/e* 114 (ix) 168 (x) respectively.

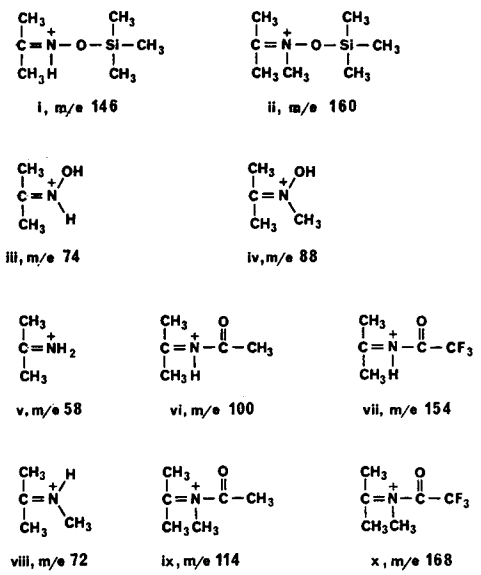

FIG. 1. Characteristic mass fragment ions of mephentermine (Ia), its metabolic products (Ib, Ic, Id) and their related derivatives.

In all the above cases, synthetic and metabolically produced materials behaved similarly. Hyroxylamines of medicinal amines are known to be relatively unstable during g.l.c. analysis (Beckett, 1974). Derivatization of the hydroxylamino group stabilized these metabolites; furthermore, the derivative gave characteristic mass fragment ions on g.l.c.-mass spectrometry.

Quantitative analysis of the metabolic products of mephentermine (Ia). The amines Ia and Ib gave tailing peaks when injected on neutral columns A-D (Table 1). Formation of the acetyl (AC) and trifluoroacetyl (TFA) derivatives of these amines improved their g.l.c. separation but only the latter derivatives (TFA) gave symmetrical peaks suitable for quantitative analysis (O'Brien, Zazulak, & others, 1972; Cho, Hodshon & others, 1973).

N-Hydroxyphentermine (Id) has been analysed by g.l.c. using various stationary phases (Beckett & Bélanger, 1974a). However, upon use for a few months, these columns became unsatisfactory although still functional normally for the analysis of Ia, Ib, Ie and If. Suitable derivates for Id were therefore sought to overcome this problem and permit continual use of the g.l.c. columns for the analysis of Id. The acetyl derivative (AC) of the hydroxylamino Id gave a single symmetrical peak on g.l.c. column B; the acetyl derivative of the parent amine (Ib) gave a peak which interfered and therefore precluded the use of acetylation for the determination of Id in metabolic studies. However, reaction of the hydroxylamines Ic and Id with the silylating reagents BSTFA or HMDS in dry acetonitrile or pyridine at room temperature gave peaks on column D (Table 1) identified as their trimethylsilyl derivatives (TMS) by g.l.c.-mass spectrometry. Under these conditions, maximum peak height of the trimethylsilyl derivative of Ic and Id was immediate and these derivatives were stable for at least 60 min. Conversion of Id to its trimethylsilyl derivative (TMS) in dichloromethane according to a published method (Cho & others, 1972) failed to be complete in our hands and two other major peaks were observed on g.l.c.

The hydroxylamines Ic and Id and the *C*-nitroso compound Ie were oxidized quantitatively to the nitro product, If (Table 2, method 3) by potassium permanganate in phosphate buffer in the absence or presence of rabbit liver homogenates at pH 7·4. Under these conditions, compounds Ia, Ib and If were not oxidized; thus If is the final oxidation product of Ic, Id and Ie and this oxidation constitutes the basis of quantitative determination of *N*-oxidized metabolites of Ia (and of Ib, see Beckett & Bélanger, 1974a). Compound If is stable, extractable into ether at pH 1 to 14 and is conveniently analysed quantitatively on g.l.c. column C (Beckett & Bélanger, 1974a).

In all the above methods, straight line calibration graphs were obtained for compounds Ia—If (excluding Ie) using concentration ranges (see experimental) similar to those found in solutions from biological experiments. Furthermore, reproducible quantitative recoveries of compounds Ib—If were obtained from their freshly prepared solutions in phosphate buffer and in phosphate buffer containing rabbit liver microsomal fractions at the same dilution used in incubation experiments (Table 2).

The analytical scheme outlined in Fig. 2 thus permitted the quantitative analysis in metabolic studies of Ia—If in the presence of each other without mutually interfering peaks or interfering peaks from the liver homogenates studied.

The scheme shown in Fig. 2 was applied to analyse the excretion products in the urine of a subject taking mephentermine (Ia). Addition of trifluoroacetic anhydride to an

Table 2. *The analysis and recovery by various methods of phentermine (Ib), N-hydroxymephentermine (Ic), N-hydroxyphentermine (Id), α,α-dimethyl-α-nitroso-β-phenylethane (Ie) and α,α-dimethyl-α-nitro-β-phenylethane (If) separately added to phosphate buffer pH 7·4 (A) and phosphate buffer containing rabbit hepatic microsomal fraction at the same dilution used in incubation experiments (B).* Results are expressed as percentage of quantitative recovery ± standard error of the mean value; the number of measurements is in parentheses.

Methods	Ib		Ic		Id		Ie		If	
	A	B	A	B	A	B	A	B	A	B
1	102 ±7·4(5)	109·2 ±4·9(4)	—	—	—	—	nil	nil	nil	nil
2	nil	nil	105·3 ±16·3(2)	101·7 ±5·3(8)	106·4 ±5·1(6)	93·7 ±0·8(3)	nil	nil	nil	nil
3	nil	nil	100·3 ±4·2(4)	102·8 ±4·3(4)	105·3 ±7·7(3)	103·5 ±4·6(8)	90·4 ±5·7(2)	101·4 ±0·4(2)	104·3 ±2·3(5)	106 (1)
4	—	—	—	—	99·8 ±2·3(4)	98·1 ±0·9(6)	nil	nil	nil	nil

Method 1: analysis of Ib as trifluoroacetyl derivative (TFA) using g.l.c. column A. Method 2: analysis of Ic and Id as their trimethylsilyl derivatives (TMS) on g.l.c. column D. Method 3: oxidation of Ic, Id, Ie and If with $KMnO_4$ (1%) for 20 min at room temperature followed by extraction with ether and subsequent analysis of If on g.l.c. column C. Method 4: analysis of Id as acetyl derivative (AC) on g.l.c. column B.

ethereal extract of alkalinized urine sample, followed by g.l.c. analysis on column A, gave peaks identical in retention times with those of the trifluoroacetyl derivatives (TFA) of mephentermine (Ia) and phentermine (Ib). The g.l.c.-mass spectra of these two peaks confirmed their identification. No free hydroxylamines Ic or Id

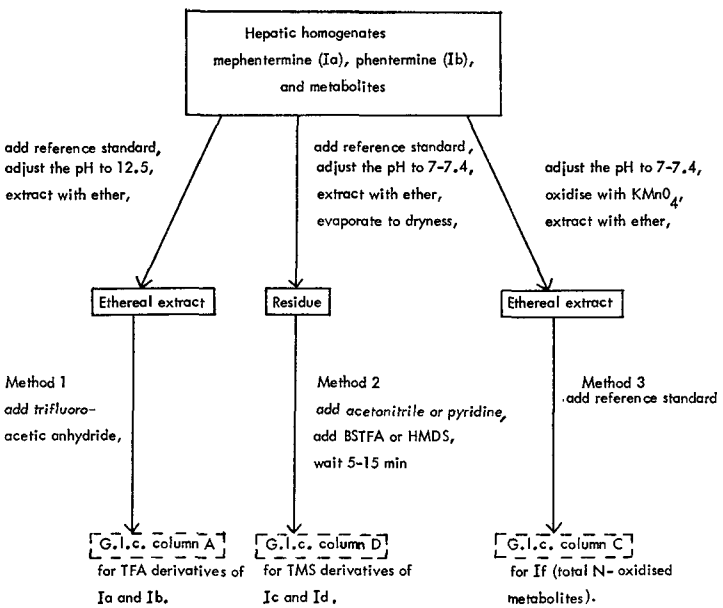

FIG. 2. Scheme for the analysis of mephentermine (Ia) and phentermine (Ib) and their metabolic products in metabolic studies.

could be detected in the neutral extract of the urine after silylation. However, hydrolysis of the urine with a mixture of sulphatase and β-glucuronidase at pH 4·5 followed by extraction at pH 7·4 with ether, derivatization and g.l.c. analysis on column D gave a peak identified as *N*-hydroxymephentermine (Ic, 1 % of the dose). Oxidation of a same volume of urine with potassium permanganate led to the disappearance of the peak corresponding to Ic in favour of that of the nitro compound (If), thus further indicating the presence of Ic in the urine.

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