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ANALYSIS OF PERFLUOROACYL DERIVATIVES OF EPHEDRINE, PSEUDOEPHEDRINE AND ANALOGUES BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

I. TRIFLUOROACETYL DERIVATIVES

R. T. COUTTS*, R. DAWE, G. R. JONES and S.-F. LIU

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8 (Canada)

and

K. K. MIDHA

Drug Research Laboratories, Health Protection Branch, National Health and Welfare, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Canada)

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SUMMARY

N-Mono-trifluoroacetylated and N,O-di-trifluoroacetylated derivatives of norephedrine and norpseudoephedrine, and of some N-substituted and ring-methoxylated analogues were prepared and analyzed by gas chromatography. Diastereoisomeric mixtures were readily separated only if the components possessed an N-alkyl group were N,O-di-trifluoroacctylated. A method is described to convert rapidly β-hydroxy primary amines to β-hydroxy secondary amines prior to derivatization with trifluoroacetic anhydride. Such a procedure permits the quantitative analysis of each diastereoisomer in a mixture of β-hydroxy primary amines.

The electron-impact mass spectra of the mono- and di-trifluoroacetylated derivatives are interpreted. Most mass spectral fragmentation pathways were predictable and it was observed that ring substitution resulted in significant changes in the nature and abundances of some fragment ions. Fragmentation pathways can vary if conditions within the mass spectrometer are altered.

INTRODUCTION

The metabolism of methoxyphenamine (Fig. 1, I) has been studied in man and monkey[1,2] and various potential metabolites have been synthesized to assist in the identification of the biologically produced products. Some synthetic analogues of methoxyphenamine have already been described[1]. Two additional ephedrine-like

* To whom correspondence should be addressed.

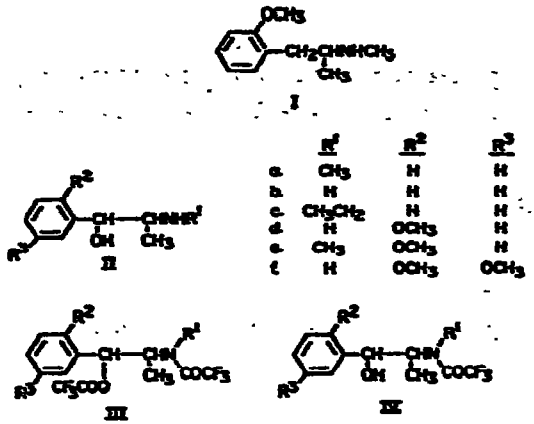

Fig. 1. Structures of methoxyphenamine (I) and related compounds.

derivatives of methoxyphenamine were also prepared and attempts were made to characterize these derivatives (IId, IIe) by gas chromatography (GC), with unexpected results. The product obtained when IId was treated with trifluoroacetic anhydride (TFAA) gave rise to a single GC peak; in contrast, when the trifluoroacetylated (TFA) derivative of the secondary amine (IIe) was gas chromatographed under identical conditions, two GC peaks were obtained (Fig. 2) and each gave rise to identical mass spectra. From this preliminary result, it appeared that the TFA-derivatized diastereoisomeric components of IIe had separated on GC whereas the derivatized diastereoisomers of IId had not. To verify this result, the GC behavior of

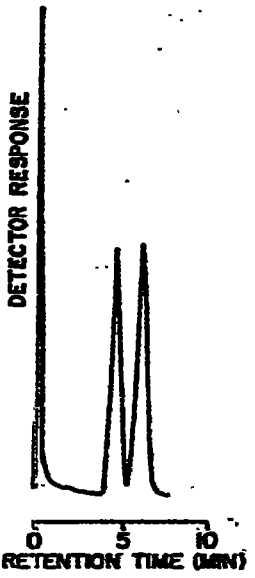

Fig. 2. Gas chromatographic separation of N,O-di-trifluoroacetylated diastereoisomeric mixture of 2-methoxy-α-[(1-methylamino)ethyl]-benzenemethanol (IIe).

the TFA derivatives (III and IV) of ephedrine (IIa), its diastereoisomer pseudoephedrine (IIa), norephedrine (IIb), norpseudoephedrine (IIb), and the sympathomimetic drug methoxamine (IIf) was investigated. The results of this GC study and the methods used to synthesize diastereoisomeric mixtures of IId and IIe (Fig. 3) are now reported.

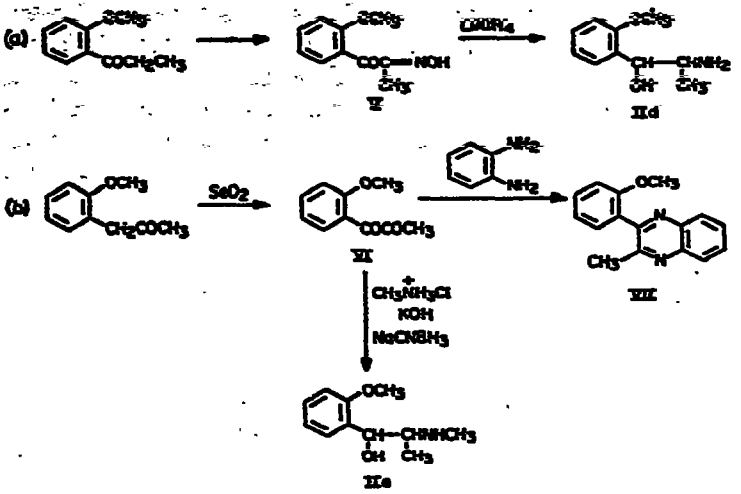

Fig. 3. Synthesis of (a) α-(1-aminoethyl)-2-methoxybenzenemethanol (IId) and (b) its N-methyl analog (IIe).

During the course of the present investigation, it was noticed that the mass spectra of the mono- and di-TFA derivatives of ephedrine were surprisingly different from that of the reported[3] mono-TFA derivative. This observation prompted us to record and interpret the mass spectra of the mono- and di-TFA derivatives of all eight ephedrine-like compounds which were available to us [ephedrine (IIa), pseudo ephedrine (IIa), norephedrine (IIb), norpseudoephedrine (IIb), α-(1-(ethylamino)-ethyl)benzenemethanol (IIc), α-(1-aminoethyl)-2-methoxybenzenemethanol (IId), 2-methoxy-α-(1-(methylamino)ethyl)benzenemethanol (IIe) and α-(1-aminoethyl)-2,5-dimethoxybenzenemethanol (methoxamine) (IIf)] and determine the effect of N- and ring-substitution on fragmentation pathways.

MATERIALS AND METHODS

Instrumentation

GC data were obtained from a Hewlett-Packard Model 5700A gas chromatograph equipped with a flame ionization detector and a 1.2 m × 6.5 mm I.D. glass column packed with 3% OV-17 on 80–100 mesh Chromosorb 750 with helium (60 ml/min)as carrier gas. The same column was used for combined GC–mass spectrometry (MS) on either a Hewlett-Packard Model 5710A gas chromatograph coupled to a Hewlett-Packard Model 5980A mass spectrometer or a Hewlett-Packard Model 5985A GC–MS system, which were operated at 70 eV. Direct-inlet high-resolution

mass spectra were recorded by Mr. A. I. Budd, Department of Chemistry, University of Alberta, on an AEI MS-50 mass spectrometer coupled to a DGC DS50S computer.

Chemistry

The hydrochlorides of ephedrine (Aldrich, Milwaukee, Wisc., U.S.A.), pseudoephedrine (Sigma, St. Louis, Mo., U.S.A.), norephedrine (Eastman-Kodak, Rochester, N.Y., U.S.A.) and norpseudoephedrine (Koch-Light, Colnbrook, Great Britain); *o*-hydroxypropiophenone (Aldrich), 1-(*o*-methoxyphenyl)-2-propanone (Aldrich) and trifluoroacetic anhydride (Sigma) were obtained from commercial sources. Methoxamine was a gift from Burroughs Wellcome (LaSalle, Canada). Other hydroxyamines were synthesized as described below. All other reagents and solvents were of superior grade.

1-(o-Methoxyphenyl)-1,2-propanedione-2-oxime (V)

Anhydrous hydrogen chloride was bubbled into a continuously stirred ethereal solution of *o*-methoxypropiophenone (1.64 g; prepared from *o*-hydroxypropiophenone using essentially the reported[4] method) while *n*-butyl nitrite (1.5 ml) was added dropwise. The stream of hydrogen chloride gas was continued for a further 15 min, then the gently refluxing solution was cooled to room temperature and left for 3 h. Evaporation of the diethyl ether gave compound V as colorless prisms (1.42 g), m.p. 133–124° (from benzene, uncorrected); lit.[4], m.p. 132°. Nuclear magnetic resonance spectrometry (NMR) (C^2HCl_3): δ 2.02 (*s*, 3H, C–CH_3), 3.70 (*s*, 3H, O–CH_3), 7.10 (*m*, 4H, ArH), 8.90 (*s*, 1H, exchanged with ^2H_2O, N–OH). Anal. calc. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.00; H, 5.66; N, 7.16.

α-(1-Aminoethyl)-2-methoxybenzenemethanol (IId)

A solution of lithium aluminium hydride in dry diethyl ether (0.5 *N*, 9 ml) was added dropwise to a stirred solution of compound V (291 mg) in the same solvent. The mixture was then heated at reflux temperature for 2 h, cooled, diluted with water, and filtered. The ether layer was separated, dried (Na_2SO_4) and evaporated to give an oil (31 mg). The precipitate of aluminum salts was digested with methanol (2 × 10 ml); evaporation of the methanol gave a further 188 mg of product. Purification of the combined products by preparative thin-layer chromatography (TLC) (silica gel G; 1.5% NH_4OH in CH_3OH; $R_F = 0.3$) gave the diastereoisomeric compound IId as a colorless oil (143 mg) which slowly crystallized, m.p. 68°. NMR (C^2HCl_3): δ 0.95 and 0.98 (*d*, 3H, $J = 6.5$ Hz, $CHCH_3$), 2.25 (*s*, 3H, exchanged with ^2H_2O, OH and NH_2), 3.20 (broad *m*, 1H, $CHNH_2$), 3.78 (*s*, 3H, OCH_3), 4.77 and 4.57 (*d*, 1H, $J = 5$ and 6 Hz respectively, CHOH), 7.10 (*m*, 4H, C_6H_4). The hydrochloride salt of the product had m.p. 239–241° (decomposition); lit.[4], m.p. 245° (decomposition). Anal. calc. for $C_{10}H_{16}ClNO_2$: C, 55.17; H, 7.41; N, 6.43. Found: C, 55.01; H, 7.78; N, 6.32.

1-(o-Methoxyphenyl)-1,2-propanedione (VI)

To a solution of 1-(*o*-methoxyphenyl)-2-propanone (8.2 g) in dioxane (40 ml) was added a solution of selenium dioxide (5.7 g) in water (10 ml). The mixture was heated at reflux temperature for 10 h then cooled and the black precipitate removed. The filtrate was evaporated under reduced pressure to 15 ml then extracted with

diethyl ether (2 × 25 ml). The combined ether solution was extracted with saturated aqueous $NaHCO_3$ (5%, 4 × 25 ml), then water (10 ml), dried (Na_2SO_4) and evaporated to give a yellow oil which was distilled and the fraction b.p. 90–116° (2.5 mm) was collected (5.9 g). This was a mixture of approximately equal amounts of starting material and desired product (GC evidence). A portion (1.5 g) was dissolved in a mixture of diethyl ether–light petroleum (b.p. 40–60°) (15:85, 5 ml) and chromatographed on a column of silica gel (60 g) using the same solvent mixture. Starting material eluted first, followed by compound VI. A quantity (438 mg) of the latter was obtained as a pale yellow oil which was characterized by the formation of the quinoxaline derivative 3-(*o*-methoxyphenyl)-2-methylquinoxaline (VII), m.p. 126–127°, using the method described for the preparation of related compounds[5]. Anal. calc. for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C, 76.56; H, 5.58; N, 11.18.

2-Methoxy-α-(1-(methylamino)ethyl)benzenemethanol (IIe)

Methylamine hydrochloride (340 mg) was dissolved in freshly distilled dry methanol (10 ml) containing potassium hydroxide (85 mg). The diketone (VI, 700 mg) in dry methanol (3 ml) was added in one portion, and to the stirred mixture, a solution of sodium cyanoborohydride (264 mg) in dry methanol (5 ml) was added dropwise over 10 min. The mixture was stirred for 2 h then diluted with a solution of potassium hydroxide (1 g) in methanol (10 ml), filtered and evaporated under reduced pressure. A saturated solution of sodium chloride (10 ml) was added and the mixture was extracted with diethyl ether (2 × 25 ml). The combined organic solution was washed with water (10 ml) dried (Na_2SO_4) and diluted with a saturated solution of oxalic acid in dry diethyl ether. A white solid (492 mg) precipitated, m.p. 175–200° (decomposition) (mixture of diastereoisomers) after recrystallization from methanol–diethyl ether. Anal. calc. for $(C_{11}H_{17}NO_2)_2(COOH)_2 \cdot H_2O$: C, 57.87; H, 7.63; N, 5.62. Found: C, 57.75; H, 7.28; N, 5.59.

Preparation of derivatives for GC and GC–MS examination

N,O-Di-trifluoroacetyl derivatives. A solution of the hydroxyamine free base (1–2 mg) in trifluoroacetic anhydride (0.1 ml) was heated to 60° for 5 min in a capped Reactivial (Pierce, 0.6 ml capacity). The cap was removed and the contents evaporated to dryness at 60° in a stream of nitrogen. The residue of N,O-di-TFA derivative was dissolved in diethyl ether (0.5 ml) for GC examination.

N-Mono-trifluoroacetyl derivatives. The residue from the above reaction was dissolved in diethyl ether (0.5 ml) and ammonium hydroxide solution (7 *N*, 2 drops) was added. The mixture was shaken for 0.5 min. The ether layer which now contained the N-mono-TFA derivative was used for GC examination.

N,O-Diacetyl derivatives of norephedrine and norpseudoephedrine (VIII). These were prepared in exactly the same manner as the di-TFA derivatives described above except that acetic anhydride was used. The products chromatographed as a single peak on the 3% OV-17 column, $t_R = 4.56$ min (180°).

α-(1-(Ethylaminoethyl))benzenemethanol (IIc). To a solution of the N,O-diacetyl mixture (VIII, 20 mg) in tetrahydrofuran (3 ml), aluminum trichloride (10 mg) and lithium aluminum hydride (10 mg) were added and the mixture was heated to reflux temperature for 30 min, then cooled and diluted with 5% aqueous sodium hydroxide (3 ml). The aqueous layer was separated and washed with tetra-

hydrofuran (2 ml). The combined organic extract was dried (Na_2SO_4) and examined by GC which showed that a greater than 85% conversion of VIII to II chad occurred. The diastereoisomeric mixture (IIc) chromatographed unresolved (Table I) and was characterized by mass spectrometry—*m/e* (% rel. abund.) (identity): 179 (absent) (M^+); 105(4) $(PhC \equiv O)^+$; 72(100) $(CH_3CH = NHCH_2CH_3)^+$; 44(11) $(CH_3CH = NH_2)^+$.

RESULTS AND DISCUSSION

Treatment of both ephedrine (E) and pseudoephedrine (PE) with TFAA gave either mono- or di-TFA derivatives (IIIa and IVa respectively) depending on reaction conditions[6]. The GC retention times of E-$(TFA)_2$ and PE-$(TFA)_2$ were significantly different (Table I) and mixtures of these derivatives were completely separated. In contrast, mixtures of the mono-TFA derivatives, E-TFA and PE-TFA, could not be separated on GC. When the mono- and di-TFA derivatives of norephedrine (IIb, NE) and norpseudoephedrine (IIb, NPE) were similarly examined by GC, no separation of diastereoisometic components was observed. The mono- and di-TFA derivatives of methoxamine (IIf), another primary amine, also had similar GC properties. Both derivatives of this diastereoisomeric mixture of compounds chromatographed as single peaks (Table I). The results indicate that the diastereoisomeric components of compounds of general structure II, in which R′ is an alkyl group, can be separated only after di-derivatization with TFAA. In contrast, neither the mono- nor the di-TFA derivatives of primary amines of general structure II (R′ = H) are separated into their diastereoisomeric components on GC.

Related studies by Lin *et al.*[7] and by Cummins and Fourier[8] have demonstrated that whereas the heptafluorobutyryl (HFB) derivatives of ephedrine and pseudoephedrine can be separated by GC, the HFB derivatives of NE and NPE cannot. In the former study[7], it was not determined whether mono- or di-HFB derivatives had been formed; in the latter study it was claimed[8] that only mono-HFB derivatives could be prepared. Studies in progress[9] are not in agreement with this conclusion by Cummins and Fourier.

Gilbert and Brooks[10] have also shown that mixtures of E and PE can be separated on GC, after chemical derivatization of the hydroxyamines. The N-acetyl derivatives of the hydroxyamines were prepared by reaction of the bases with one equivalent of acetic anhydride; subsequent silylation of the product gave the N-acetyl-O-trimethylsilyl derivatives of E and PE which had different GC t_R values. A disadvantage of this method is that a knowledge of the exact amounts of the hydroxyamines under investigation is necessary in order that complete monoacetylation can be achieved. The alternative method of derivatization described in the present study might be preferable since complete derivatization of E and PE is achieved by reaction with an excess of one reagent (TFAA). It is interesting, and complementary to the present study, that mixtures of N-acetyl-O-trimethylsilyl derivatives of the primary amines, NE and NPE, did not separate when gas chromatographed[10].

In view of the fact that only diastereoisomeric secondary amines separate on GC after derivatization, the possibility of rapidly converting a primary amine to a secondary amine, prior to derivatization with TFAA, was explored. The reaction sequences illustrated in Fig. 4 were performed on a mixture of NE and NPE. All products were characterized by means of combined GC–MS. Each compound in

TABLE I

GC RETENTION TIMES

GC column and conditions are given in Materials and methods section; column temperature was 140° unless stated otherwise, and helium flow-rate was 60 ml/min. Abbreviations: E = ephedrine, PE = pseudoephedrine, NE = norephedrine, NPE = norpseudophedrine, ENE = N-ethylnorephedrine, ENPE = N-ethylnorpseudoephedrine, TFA = N-trifluoroacetyl derivative; $(TFA)_2$ = *N,O*-ditrifluoroacetyl derivative.

Compound	t_R *(min)*	*Compound*	t_R *(min)*
E (IIa)	2.84	Mixture of NE-TFA and NPE-TFA	5.40
PE (IIa)	2.98	NE-$(TFA)_2$ (IIIb)	1.42
Mixture of E/PE	2.95	NPE-$(TFA)_2$ (IIIb)	1.49
NE (IIb)	2.62	Mixture of NE-$(TFA)_2$ and NPE-$(TFA)_2$	1.47
NPE (IIb)	2.72	ENE-TFA (IVc)	7.76
Mixture of NE/NPE	2.68	ENPE-TFA (IVc)	8.50
ENE (IIc)	3.54	Mixture of ENE-TFA and ENPE-TFA	8.28
ENPE (IIc)	3.81	ENE-$(TFA)_2$ (IIIc)	2.24
Mixture of ENE/ENPE	3.70	ENPE-$(TFA)_2$ (IIIc)	3.17
IId*	8.17	Mixture[§] of ENE-$(TFA)_2$ and ENPE-$(TFA)_2$	2.24 and 3.17
IIe*	8.41	IId-TFA (IVd)*	5.19**
IIf*	10.55**	IId-$(TFA)_2$ (IIId)*	2.99
E-TFA (IVa)	7.77	IIe-TFA (IVe)*,***	7.09** and 7.69**
PE-TFA (IVa)	8.39	IIe-$(TFA)_2$ (IIIe)[§]	4.60 and 6.11
Mixture*** of E-TFA and PE-TFA	7.77 and 8.39	IIf-TFA (IVf)*	14.33**
E-$(TFA)_2$ (IIIa)	1.97	IIf-$(TFA)_2$ (IIIf)	7.65
PE-$(TFA)_2$ (IIIa)	2.81		
Mixture[§] of E-$(TFA)_2$ and PE-$(TFA)_2$	1.97 and 2.81		
NE-TFA (IVb)	5.35		
NPE-TFA (IVb)	5.42		

* Diastereoisomeric mixture.
** 160°.
*** Incomplete resolution.
§ Complete resolution.

Fig. 4 was a diastereoisomeric mixture; with one exception (IIIc), no separation of diastereoisomers was achieved by GC analysis (Table I). From this, and related studies[7,8,10] it would appear that only N- and O-disubstituted N-alkylated hydroxyamines of general structure II can be rapidly and completely separated into individual diastereoisomers by GC.

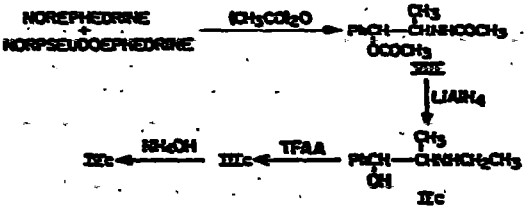

Fig. 4. N-Ethylation and subsequent derivatization of a mixture of norephedrine and norpseudoephedrine.

The appreciable differences in the GC t_R values of di-TFA derivatives (III; R′ = alkyl) can be used to determine rapidly the quantitative diastereoisomeric composition of a mixture of secondary amines of general structure II (R′ = alkyl). Mixtures of E and PE in the ratios 1:3, 3:1 and 1:1 were prepared and treated with TFAA under conditions which produced di-TFA derivatives. The results of subsequent GC analysis of these derivatized mixtures are illustrated in Fig. 5.

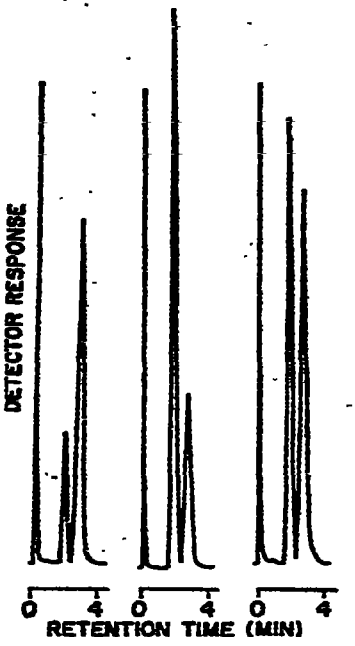

Fig. 5. Gas chromatographic separations of N,O-di-trifluoroacetylated mixtures of ephedrine and pseudoephedrine in ratios 1:3, 3:1 and 1:1 respectively. Peak areas were 23:77, 73:27 and 48:52 respectively.

The structures of the mono- and di-TFA derivatives listed in Table I required confirmation, and therefore, each compound was subjected to mass spectral analysis. Numerous spectra were recorded of compounds III (a–f) and IV (a–f) in two different laboratories over a six-month period during which time the mass spectrometers were routinely retuned. With one exception (IIIf), reproducible spectra were obtained for all compounds, and relative abundances of ions were within ± 10% of those reported in Figs. 6 and 7. A reproducible spectrum of the di-TFA derivative (IIIf) could not be obtained; whereas the masses of the fragment ions were reproducible, their relative abundances varied. Two typical spectra are illustrated in Fig. 8. On no occasion was a mass spectrum obtained for the mono- or di-TFA derivative of ephedrine which was comparable to the published[3] spectrum of what was claimed to be the mono-TFA derivative (mol. wt. 261) of ephedrine.

Diagnostic ions in the relatively complex mass spectra of the mono- and di-trifluoroacetylated compounds investigated are listed in Figs. 6 and 7, together with suggested fragmentation pathways which explain the mechanisms of formation of

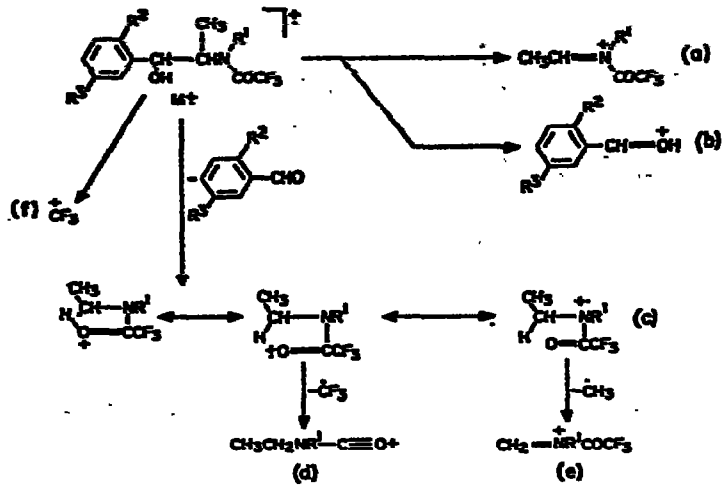

Fig. 6. Mass spectral fragmentation pathways and fragment ion abundances of N-trifluoroacetylated compounds:

Compound	*Fragment ions m/e (% relative abundance)*							
	M^+	*a*	*b*	*c*	*d*	*e*	*f*	*Other fragments***
IVa (E)	261(—)	154(56)	107(14)	155(100)	86(38)	140(27)	69(7)	110(32), 105(9), 79(18), 77(18)
IVa (PE)	261(—)	154(58)	107(13)	155(100)	86(44)	140(26)	69(9)	110(32), 105(9), 79(21), 77(19)
IVb (NE)	247(1)	140(10)	107(98)	141(100)	72(32)	126(9)	69(12)	134(12), 105(17), 79(80), 77(43)
IVb (NPE)	247(2)	140(16)	107(90)	141(100)	72(23)	126(9)	69(11)	134(9), 105(15), 79(45), 77(26)
IVc*	275(—)	168(91)	107(17)	169(92)	100(31)	154(6)	69(14)	140(100), 105(16), 79(20), 77(31), 72(25)
IVd*	277(1)	140(5)	137(100)	—	—	—	69(12)	121(15), 109(6), 107(61), 77(11)
IVe*	291(1)	154(10)	137(100)	155(8)	86(11)	140(7)	69(5)	121(8), 110(9), 107(44), 77(9)
IVf*	307(6)	140(14)	167(100)	—	—	—	69(16)	152(15), 139(56), 137(32), 124(24), 109(10), 107(5)

* Mixtures of diastereoisomers.

** Ions *m/e* 105, 79 and 77 are *m/e* 107-H_2, 107-CO and 105-CO respectively[11]; *m/e* 110 and 140—see Fig. 9; *m/e* 139, 124—see Fig. 10; *m/e* 152 (167-CH_3); *m/e* 137 (167-CH_2O); *m/e* 134 ($PhC(OH)=CHCH_3^+$); *m/e* 109 (137-CO); *m/e* 107 (137-CH_2O); *m/e* 72 (100-$CH_2=CH_2$); *m/e* 121 (origin unknown).

most of the fragment ions. The proposed fragmentations are consistent with those described in the literature for similar compounds[3,11–15]. Additional fragment ions were present in all spectra; appropriate structures for these are now suggested.

The fate of ion (a) in the spectra of the mono- and di-TFA derivatives is of interest since it varies according to the nature of the N-alkyl (R′) substituent (Fig. 9). If R′ is a hydrogen atom, the ion does not fragment further to any great extent. If R′ is an ethyl group, that group is expelled as an ethylene molecule. If, however, R′ is a methyl group, the fragment rearranges as illustrated in Fig. 9, and expels an acetaldehyde molecule to give the ion $(CH_3N \equiv CCF_3)^+$. This observation has been made by us[1] and others[14] who examined the mass spectra of TFA derivatives of amphetamines and related drugs. Kamei *et al.*[3] identified the fragment arising from the decomposition of ion (a), R′ = CH_3, as being $(CH_3C \equiv NCF_3)^+$ but it is difficult to imagine

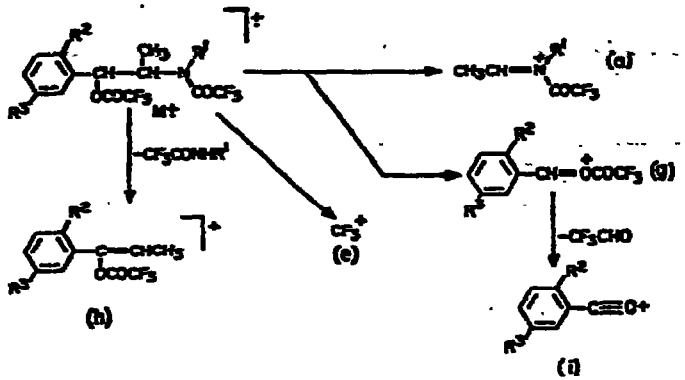

Fig. 7. Mass spectral fragmentation pathways and fragment ion abundances of N,O-di-trifluoroacetylated compounds:

Compound	*Fragment ions m/e (% relative abundance)*						
	M⁺	*a*	*e*	*g*	*h*	*i*	*Other fragments*
IIIa (E)	357(—)	154(100)	69(11)	203(—)	244(1)	105(5)	110(28)***
IIIa (PE)	357(—)	154(100)	69(7)	203(—)	244(2)	105(5)	110(31)***
IIIb (NE)	343(—)	140(100)	69(10)	203(5)	230(15)	105(8)	
IIIb (PNE)	343(—)	140(100)	69(8)	203(5)	230(12)	105(7)	
IIIc*	371(—)	168(100)	69(7)	203(2)	258(1)	105(4)	140(32)***
IIId*	373(7)	140(34)	69(8)	233(100)	260(25)	135(32)	203(15)§
IIIe*	387(2)	154(100)	69(4)	233(1)	260(4)	135(4)	110(31)***
IIIf*,**	403	140	69	263	290	165	289‡‡, 192‡‡‡, 166‡‡ 138‡‡, 123‡‡

* Mixtures of diastereoisomers.
** Ion abundances variable; see Fig. 8 and text.
*** See Fig. 9.
§ *m/e* 233-CH_2O.
‡‡ See Fig. 11.
‡‡‡ Origin unknown.

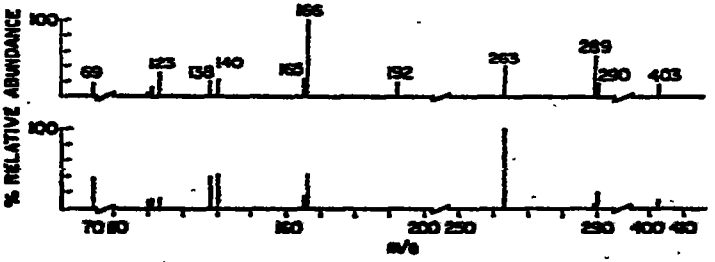

Fig. 8. Mass spectra of the di-trifluoroacetyl derivative (IIIf) of methoxamine depicting the result of minor changes in operating conditions within the mass spectrometer.

a mechanism which would readily permit the formation of this latter ion. A mechanism similar to the one depicted in Fig. 9 for the formation of the ion, *m/e* 110, has been proposed by other investigators[24].

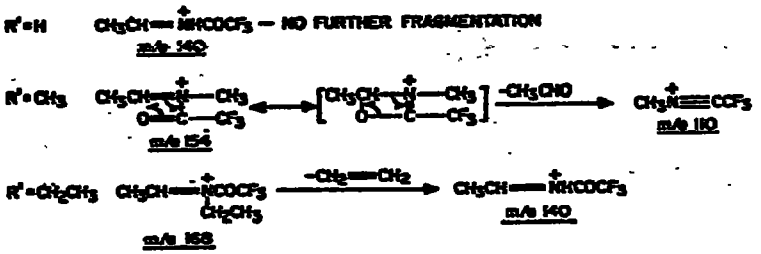

Fig. 9. Further fragmentation of ion (a) $CH_3CH{=}N^+R^1COCF_3$ (see Figs. 6 and 7).

The mass spectra of N-monotrifluoroacetylated and N,O-di-trifluoroacetylated methoxamine (IIIf) contained fragment ions which were unique to these two spectra. The ions, *m/e* 139 and 124, in the spectrum of the mono-TFA derivative (IVf) were shown by accurate mass measurements to be $C_8H_{11}O_2^+$ and $C_7H_8O_2^+$, respectively and were presumably formed as outlined in Fig. 10. These fragmentations must be influenced by the presence of the two methoxyl groups on the aromatic ring since comparable fragmentations do not occur to any great extent with other trifluoroacetylated ring-methoxylated compounds (IVd, IVe). The formation of ion *m/e* 124 from ion *m/e* 139 requires the expulsion of a radical from an even-electron ion. This is not a common fragmentation sequence, but a similar transition was observed in our previous studies[12,16].

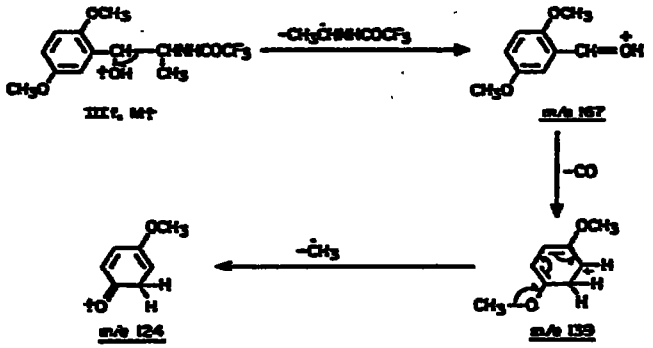

Fig. 10. Suggested fragmentations to explain the presence of ions *m/e* 139 and 124 in the mass spectrum of N-trifluoroacetylated methoxamine.

Five additional ions were present in the mass spectrum of the di-TFA derivatives (IIIf) and were not identified in the fragmentation pathways depicted in Fig. 7. One of these ions, *m/e* 192 ($C_{11}H_{14}NO_2$ by accurate mass measurement) was formed by the expulsion of a CF_3COOH molecule and a CF_3CO radical from the molecular ion. The remaining four ions, *m/e* 289, 166, 138 and 123, can also be explained as a result of an initial expulsion of a CF_3COOH molecule from the molecular ion of IIIf (Fig. 11). Accurate mass measurements of all four fragment ions confirmed their elemental compositions; the ion *m/e* 123 was a mixture of two fragments, $C_7H_7O_2^+$ and $C_4H_4F_3N^+$, but mainly the former. If this loss of a CF_3COOH molecule occured at the GC–MS interface, it is presumably influenced by the presence of the two

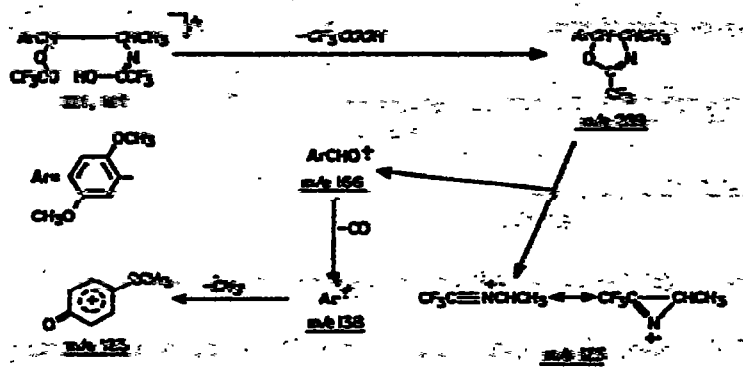

Fig. II. Suggested fragmentation pathways to explain the presence of ions *m/e* 289, 166, 138 and 123 in the mass spectrum of N,O-di-trifluoroacetylated methoxamine.

electron-donating methoxyl groups in the aromatic ring and it may not occur to the same extent each time a spectrum is recorded. This would, at least in part, explain the difficulty in obtaining reproducible mass spectra of IIIf.

The spectrum claimed by Kamei *et al.*[3] to be that of the mono-TFA derivative of ephedrine had abundant fragment ions of *m/e* (% relative abundance) 243 (68), 154 (43), 110 (100) and 56 (70). If the derivative prepared by them was, in fact, the di-TFA derivative (and their method of preparing TFA derivatives would permit it to be so), then mechanisms can be deduced which would enable these fragments to be formed (Fig. 12), though some of these ions (Fig. 7) were not observed by us. It is conceivable, however, that the conditions existing within the mass spectrometer used by Kamei *et al.* differed appreciably from those existing in both instruments employed in the present study.

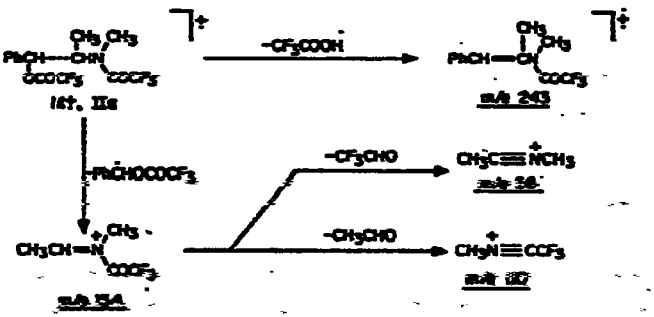

Fig. 12. Possible mass spectral fragmentations which explain a reported[3] spectrum of trifluoroacetylated ephedrine.

Finally, although it is possible that ephedrine analogs fragment by different mechanisms, depending on the conditions which exist within the mass spectrometer, it may be concluded from the present study that the mono- and di-trifluoroacetylated derivates of ephedrine, pseudoephedrine and their analogs fragment mainly in predictable fashion in the mass spectrometer. It may be further concluded that the

presence of methoxyl groups on the aromatic ring, and the nature of the substituent on the nitrogen atom, have a profound influence on some of the fragmentation pathways.

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