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CHARACTERISATION OF PRODUCTS FORMED BY CONDENSATIONS OF PRIMARY AMINES WITH POLYGODIAL, AND THEIR SCOPE FOR ENANTIOMER ANALYSES

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SUMMARY

The sesquiterpenedial polygodial undergoes smooth reactions with primary amines, under mild conditions, to give stable derivatives (1,3,4-trisubstituted pyrroles) useful for characterisation by gas chromatography–mass spectrometry. Diastereomeric pyrroles formed from chiral amines were in suitable instances separable by gas chromatography. With hydroxy amines it may be advantageous to convert the initial products into O-trimethylsilyl or O-*tert.*-butyldimethylsilyl ethers for analysis. All the derivatives studied yielded molecular ions under electron impact, while informative fragmentations also occurred.

INTRODUCTION

(−)-Polygodial (**1a**)[1] is the simplest member of a group of natural drimenedials, many of which show interesting biological activities. A convenient method for the analysis of enantiomeric polygodials is based on their rapid reaction with chiral amines to give stable diastereomeric pyrrole derivatives (**2**), separable by gas chromatography (GC)[2]. It is possible to employ a similar process for the analysis of chiral primary amines[3]. This report is concerned with qualitative work on a wide range of amines,

1a : R,R' = H   **2**   **3**
b : R = OH, R' = OOCCH$_3$
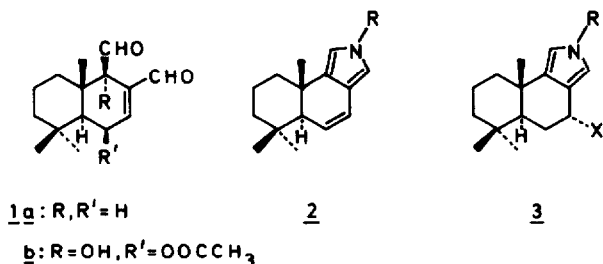
including biogenic amines and hydroxy amines. Derivatives containing hydroxyl groups were generally examined as trimethylsilyl (TMS) or *tert.*-butyldimethylsilyl (TBDMS) ethers. Of the fifteen amines included in this report, ten afforded derivatives that were either partially or fully resolved by capillary GC. Mass spectra of the pyrroles

were structurally informative, and in many cases yielded intense ions, suitable for selective detection of primary amines.

EXPERIMENTAL

Solvents and reagents

N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *tert.*-butyldimethyl-chlorosilane–imidazole (TBDMCS–imidazole) were obtained from Pierce and War-riner (Chester, U.K.). Ethyl acetate (Nanograde) was purchased from Mallinckrodt (St. Louis, MO, U.S.A.), and pyridine (AnalaR grade, BDH, Poole, U.K.) was dried over potassium hydroxide pellets and redistilled prior to use.
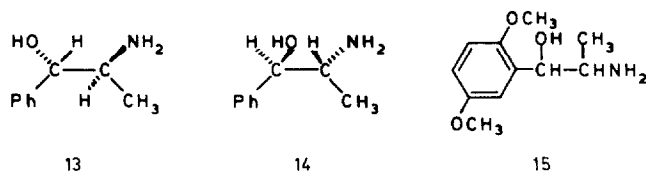
Amines and other reference compounds

The primary amines were numbered as in Fig. 1. Compound **4** was a gift from Smith Kline and French. Compound **5** was obtained from Norse Labs. (Santa

Amines
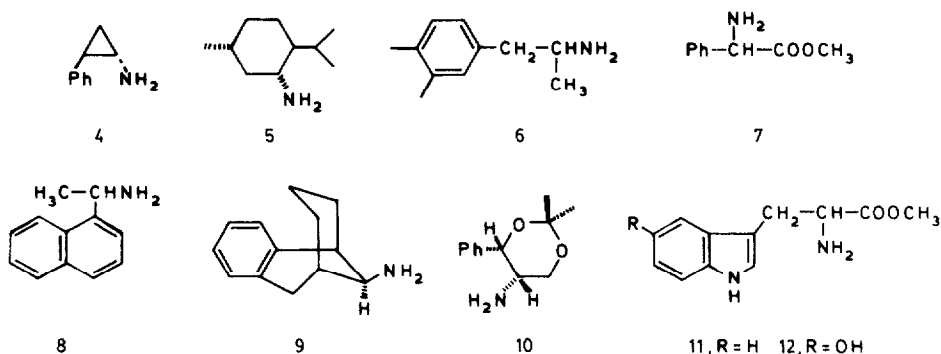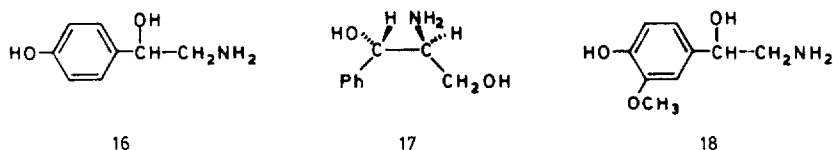

Hydroxy amines

Dihydroxy amines

Fig. 1. Structures of amines. **4** = (±)-tranylcypromine; **5** = (−)-menthylamine; **6** = xylopropamine; **7** = D-(−)-phenylglycine methyl ester; **8** = (+)- and (−)-α-1-naphthylethylamine; **9** = Organon compound 6328 [5,6,7,8,9,10-hexahydro-5,9-methanobenzocycloocten-11-amine (5α,9α,11β)]; **10** = 4S,5S-(+)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxan; **11** = (±)- and L-(−)-tryptophan methyl ester; **12** = (±)-5-hydroxytryptophan methyl ester; **13** = D-(+)-norpseudoephedrine; **14** = L-(+)-norephedrine; **15** = (±)-methoxamine; **16** = (±)-octopamine; **17** = 1S,2S-(+)-2-amino-1-phenylpropane-1,3-diol; **18** = (±)-normetanephrine. Ph = Phenyl.

Barbara, CA, U.S.A.) and **6** was a gift from Roche (Welwyn Garden City U.K.). Compound **7** was purchased from Fluka (Fluorochem, Glossop, U.K.) and **8** (+) and (−) and **9** (+) were gifts from Organon Laboratories (Newhouse, U.K.). Compounds **10** and **11** were obtained from Sigma (Poole, U.K.) and **12** was a gift from Dr. E. C. Horning. Compounds **13** and **14** were purchased from Koch Light (A. and J. Beveridge, Edinburgh, U.K.) and **15** was a gift from The Wellcome Foundation (Dartford, U.K.). Compounds **16** and **18** were obtained from Calbiochem (San Diego, CA, U.S.A.) and **17** from Aldrich (Gillingham, Dorset, U.K.). Compounds **7** and **12**, originally obtained as free acids, were methylated prior to use. Dr. J. A. Pickett (Rothamsted) provided gifts of (±)- and (−)-polygodial.

Gas chromatography

Open-tubular GC was performed with a Hewlett-Packard (Winnersh, U.K.) 5880A gas chromatograph, equipped with CP-Sil 5 CB (Chrompack, London, U.K.) and SE-54 (GC^2, Northwich, Chester, U.K.) fused-silica capillary columns (25 m × 0.32 mm I.D.). The Grob-type injectors were operated in split mode (50:1) and the helium carrier gas flow-rates were 3 ml/min. The columns were operated according to conditions in Table I, while the instrument employed flame ionisation detectors.

Gas chromatography–mass spectrometry

GC–mass spectrometry (MS) was carried out with an LKB 9000 instrument, equipped with a DB-1 fused-silica capillary column, 60 m × 0.32 mm I.D. (J. and W. Scientific, Rancho Cordova, CA, U.S.A.) and a falling-needle injection system[4]. Helium carrier- and make-up gas flow-rates were 7 ml/min (measured at atmospheric pressure) and 25 ml/min, respectively. Mass spectra (22 eV) were recorded under electron-impact conditions: filament current, 4 A; trap current, 60 μA; accelerating voltage, 3.5 kV; and source and separator temperatures, 270°C.

Preparation of amine–polygodial condensation products

Primary amines (*ca.* 200 μg) were dissolved in water (50 μl) and mixed with 50 μl of a 1-μg/μl solution of (±)- or (−)-polygodial in ethyl acetate and with pyridine (10 μl). The mixture was shaken periodically for 2 h at room temperature. The aqueous phase was removed with a syringe, and the organic phase was washed with water (50 μl) and dried over anhydrous sodium sulphate. The solution was filtered through a small plug of cotton wool and analysed by capillary GC and GC–MS. The 1,3,4-trisubstituted pyrrole obtained from methoxamine (**15**) was treated with an additional quantity of (±)-polygodial (50 μg) and extracted and analysed as above.

In a study to determine possible competitive reactions of secondary amines, additional condensation reactions were performed, using (−)-amphetamine (50 μg)/polygodial (50 μg) and (−)-amphetamine (50 μg) + N-methylamphetamine (50 μg)/(±)polygodial (100 μg). The two solutions were allowed to react, and the mixtures were extracted and analysed as above.

Trimethylsilylation. Compounds **12–18** were converted into their corresponding 1,3,4-trisubstituted pyrrole products as above. After filtration and evaporation to dryness, the products were treated with BSTFA (20 μl) and dry pyridine (10 μl) and heated at 80°C for 30 min. The reagents were removed by evaporation, and the mono- or di-TMS ethers of the substituted pyrroles were redissolved in ethyl acetate for GC and GC–MS analyses.

tert.-Butyldimethylsilylation. The 1,3,4-trisubstituted pyrrole products of compounds **13** and **17** were prepared as above. Following filtration and evaporation to dryness, the compounds were treated with TBDMCS–imidazole (20 μl) and dry pyridine (10 μl) and heated at 80°C for 30 min. After removal of solvent, the corresponding pyrrole mono- and di-TBDMS ethers were extracted into ethyl acetate and analysed by GC and GC–MS.

RESULTS AND DISCUSSION

General features of the reactions

The reaction of polygodial with methylamine in aqueous buffer has been studied in some detail, and is known to lead to compounds of type **3** [X = OH (refs. 5–7) or NHCH$_3$ (ref. 8)]: these readily undergo thermal elimination during GC to afford the unsaturated products (**2**). In the present work, the aim was to determine the extent to which diastereomers (**2**) from various types of amines (Fig. 1) would be separable by GC. Optimisation of yields was not attempted, but in general the reactions proceeded smoothly, and on the analytical scale there was little or no evidence of by-products. The products from valine methyl ester (not included in this paper) and tranylcypromine (Fig. 1, No. **4**) were shown to be stable during two years' storage in the dry state. Most of the products were stored for several weeks as solutions in ethyl acetate without undergoing any significant changes, as judged by GC–MS.

Possible interference by secondary amines has not been explored in detail, but the reaction of amphetamine with polygodial[2] yielded identical results (by GC) in the absence or the presence of N-methylamphetamine.

GC properties

Retention indices (*I*) of the condensation products, analysed on the principal column used (CP-Sil 5 CB) ranged from 2466, for the unresolved menthylamine derivatives, to 3515 and 3521 for those of 5-hydroxytryptophan methyl ester as trimethylsilyl ethers. Data are summarised in Table I.

(i) Amines lacking other functional groups. Seven amines of this type (Fig. 1, **4–10**) were studied, of which only two yielded diastereomeric pyrroles that were separable by GC under the conditions used. The racemic adrenergic compound xylopropamine (**6**) afforded well-resolved products (*ΔI* = 9: Fig. 2). An equally satisfactory separation (*ΔI* = 11: Fig. 3) was observed for the pyrroles formed from (±)-polygodial and compound **10** (the acetonide of **17**): the diastereomer from (−)-polygodial was eluted first on the CP-Sil 5 CB column.

(ii) Tryptophan and 5-hydroxytryptophan methyl esters. A partial separation (*ΔI* = 6) of diastereomeric products from (±)-tryptophan methyl ester (**11**) was observed (Fig. 4, A). As indicated in Table I, the (+)-tryptophan methyl ester (−)-polygodial product was eluted first.

Racemic 5-hydroxytryptophan methyl ester (**12**) yielded diastereomeric pyrroles, which, after trimethylsilylation, gave partially separated peaks (*ΔI* = 7; Fig. 4, B). The order of elution was not established, but probably corresponded to that of the tryptophan methyl ester derivatives.

(iii) β-Hydroxy amines. Three representative bioactive β-hydroxy amines each gave pyrroles that were sufficiently separated by GC for characterisation of the
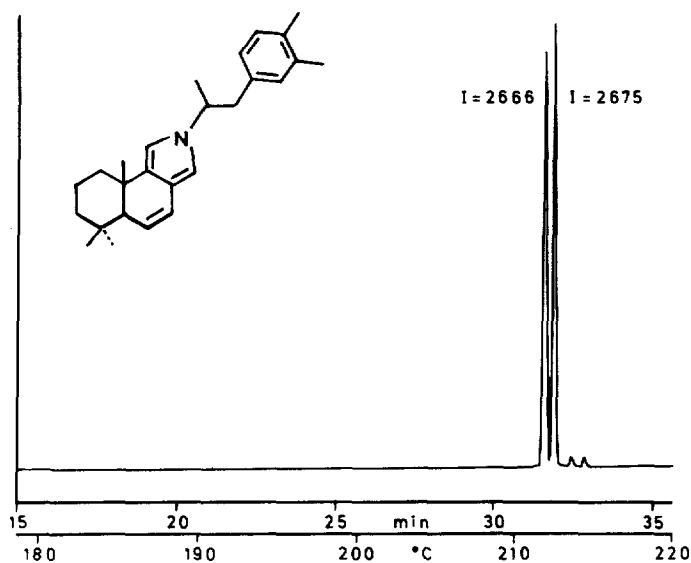
Fig. 2. GC separation of reaction products of xylopropamine with (±)-polygodial. Column, CP-Sil 5 CB fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (2 min) to 160°C (1 min) at 30°C/min, and then at 2°C/min to 270°C; helium flow-rate, 3 ml/min.

Fig. 3. GC separation of reaction products of 4S,5S-(+)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxan with (±)-polygodial. Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (2 min) to 170°C (1 min) at 30°C/min, and then at 2°C/min to 270°C; helium flow-rate, 3 ml/min. Ph = phenyl.
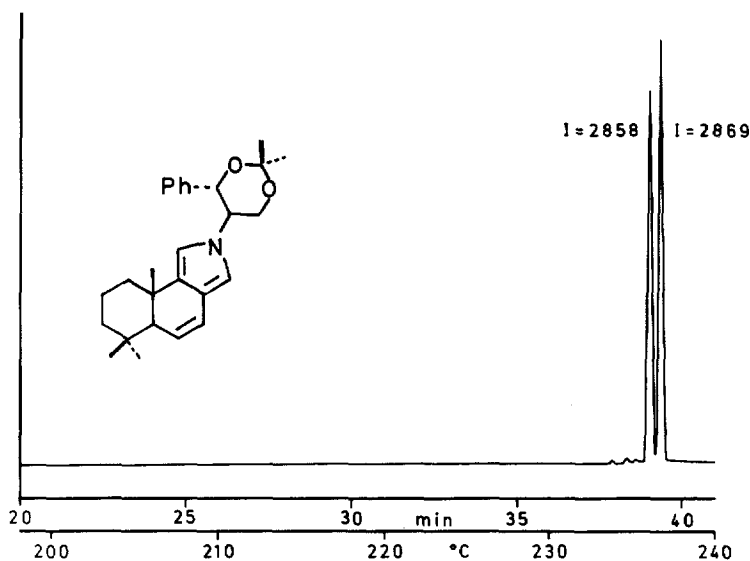
TABLE I

KOVÁTS RETENTION INDICES (I) AND MASS SPECTROMETRIC DATA (22 eV) FOR POLYGODIAL (PG) PRIMARY AMINE CONDENSATION PRODUCTS

Compound	PG type	I		M+·	Base Peak[c]	m/z for other principal ions (intensities relative to base peak in parentheses)[d]
		CP-Sil 5	SE-54			
4	(±)	2713[a]	—	331(100)	331	333(5) 332(31) 330(12) 318(3) 317(16) 316(69)(a) 262(4) 261(3) 260(4) 255(12) 254(47) 250(5) 249(21)(g) 248(24)(h) 247(4) 246(5) 195(3) 170(6)(l) 118(4) 117(33)
5	(±)	2466[a]	—	353(84)	338(a)	355(4) 354(26) 352(8) 340(5) 339(29) 284(6) 282(3) 272(15) 271(73)(g) 270(43)(h) 269(5) 268(5) 242(4)(b) 229(6) 215(3) 214(3) 201(4) 200(22)(j) 144(3) 83(5)
6	(±)	2666[a] 2675	—	361(54)	242(b)	363(3) 362(20) 360(6) 359(3) 348(3) 347(12) 346(38)(a) 345(4) 292(3) 290(3) 281(4) 280(6) 279(26)(g) 278(18)(h) 277(4) 244(7) 243(12) 200(4)(j) 160(8) 158(4)(f) 148(3) 147(13) 146(8) 144(6) 132(4)(k) 131(5) 120(4) 119(8)(d)
7	(−)	2656[a]	2705[a]	363(80)	348(a)	365(6) 364(28) 362(8) 350(7) 349(27) 306(3) 305(4) 304(10) 294(7) 293(5) 292(10) 283(3) 282(18) 281(76)(g) 280(62)(h) 279(12) 278(4) 220(3) 214(4) 150(4) 149(24)(d) 145(3) 144(5) 132(5)(k) 122(5) 121(32) 91(4)
8(−)	(−)	2977[b]	3043[b]	369(45)	155(d)	370(14) 368(3) 355(4) 354(18)(a) 288(8) 287(27)(g) 286(14)(h) 215(5) 214(5) 201(4) 156(18) 154(8) 153(4) 152(3) 145(4) 147(3) 141(3) 133(8) 132(10)(k) 131(5)
8(+)	(+)	2977[b]	3043[b]			
9	(±)	3198[b]	—	385(97)	370(a)	387(7) 386(32) 384(10) 383(4) 373(3) 372(11) 371(38) 316(5) 315(3) 314(4) 305(6) 304(29) 303(94)(g) 302(50)(h) 301(11) 300(4) 216(5) 201(4) 200(22)(j) 172(8) 171(27) 170(7)(l) 158(3)(f) 146(3) 144(6) 143(8) 142(5) 132(8)(k) 130(6) 129(20) 117(4) 115(4)
10	(±)	2798[b] 2809	2858[b] 2869	405(100)	405	407(8) 406(26) 404(8) 403(3) 392(5) 391(23) 390(63)(a) 389(5) 324(9) 323(39)(g) 322(26)(h) 321(4) 320(3) 317(5) 302(5) 298(3) 292(3) 243(3) 242(11)(b) 241(56) 240(5) 234(4) 233(4) 228(7) 227(13) 226(70)(c) 198(4) 184(3) 172(8) 171(6) 170(12)(l) 160(10) 159(67) 158(35)(f) 157(9) 156(3) 144(3) 133(4) 130(3) 106(3) 105(5) 77(3)
	(−)	2797[b]				
11(±)	(±)	3332[b] 3339	3416[b] 3423	416(52)	130(d)	418(6) 417(18) 415(8) 414(6) 403(3) 402(10) 401(29)(a) 400(6) 357(3) 355(2) 335(5) 334(20)(g) 333(12)(h) 332(3) 287(9) 286(8) 272(4) 216(3) 215(3) 205(4) 204(5) 203(3) 202(7) 201(5) 160(3) 144(3) 131(12) 129(5)
11(−)	(−)	3338[b]				
12 di-TMS	(±)	3515[b] 3521	3570[b] 3576	576(6)	290(d)	577(3) 561(3)(a) 292(9) 291(26)

Compound	Config	Kováts RI	M+	Base	Principal ions m/z (abundance)
13	(±)	2673ᵃ, 2678	349(4)	158(**f**)	347(5), 345(4), 332(15), 331(28), 330(7), 317(8), 316(22), 274(3), 265(3), 263(3), 262(3), 260(3), 250(5), 249(14), 248(14), 247(4), 246(6), 243(7), 242(68)(**b**), 241(92), 240(8), 228(4), 227(21), 226(94)(**c**), 225(6), 224(3), 215(5), 213(3), 200(8)(**j**), 198(7), 185(5), 184(9), 183(4), 173(4), 172(12), 171(9), 170(43)(**l**), 168(3), 160(8), 159(70), 157(28), 156(20), 144(10), 143(7), 133(6), 132(5)(**k**), 131(4), 130(4), 129(4), 118(5), 117(7), 107(10)(**d**), 106(64), 105(72), 78(18), 77(50)
	(−)	2672ᵃ			
13 TMS	(±)	2598ᵃ, 2839ᵃ	421(6)	242(**b**)	422(3), 244(3)
13 mono-TBDMS	(±)	2846	463(8)	242(**b**)	464(3), 406(3)
14	(±)	2641ᵃ, 2650	349(13)	242(**b**)	350(5), 348(5), 347(6), 334(4)(**a**), 333(3), 332(6), 331(10), 318(5), 316(9), 249(5), 244(7), 243(16), 241(50), 240(4), 228(6), 227(8), 226(56)(**c**), 184(3), 183(4), 172(8), 171(4), 170(11)(**l**), 160(4), 159(28), 158(26)(**f**), 157(8), 156(6), 107(3), 106(35), 105(27), 77(13)
14 TMS	(±)	2599ᵃ, 2611	421(15)	242(**b**)	422(5), 244(5), 243(22), 221(4)(**d**), 179(3)(**d**), 172(8), 171(4), 107(3)
	(−)	2598ᵃ			
	(±)	2598ᵃ			
15	(±)	2943ᵃ, 2950	409(9)	226(**c**)	407(3), 405(3), 393(5), 392(14), 391(40), 390(6), 378(4), 377(12), 376(39), 362(9), 361(22), 360(80), 359(4), 344(4), 310(4), 309(14), 308(20), 278(6), 276(7), 275(4), 244(4), 243(15), 242(64)(**b**), 241(92), 240(9), 229(3), 228(6), 227(21), 215(6), 200(5)(**j**), 198(4), 185(4), 184(10), 183(5), 173(4), 172(14), 171(11), 170(35)(**l**), 167(10), 166(76)(**m**), 165(10), 160(6), 159(50), 158(65)(**f**), 157(21), 156(15), 155(4), 151(20), 150(4), 149(8), 148(9), 144(9), 143(5), 137(7), 136(6), 135(6), 134(6), 133(6), 132(7)(**k**), 131(6), 123(15), 121(6), 120(15), 109(6), 108(6), 107(4), 106(11), 95(12), 93(4), 92(4), 80(4), 77(5), 77(4)
15 TMS	(±)	2886ᵇ, 2910	481(19)	242(**b**)	483(4), 482(8), 466(4), 244(4), 244(20), 243(20), 241(7), 240(16), 238(3), 158(3)(**f**), 77(5)
	(−)				
	(±)	2982ᵇ, 3004			
16 di-TMS	(−)	2909ᵃ, 2916	495(9)	267(**d**)	496(3), 480(3), 405(5), 390(4), 269(12), 268(23)
17 di-TMS	(±)	2746ᵇ	509(18)	330(**e**)	511(3), 510(9), 495(2), 494(4)(**a**), 397(4)(**g**), 396(5)(**h**), 374(10), 373(33), 371(4), 356(2), 332(8), 331(29), 315(3), 290(4), 288(3), 242(3)(**b**), 241(12), 179(6)(**d**), 106(3), 105(5), 77(5)
17 mono-TBDMS	(±)	3127ᵇ, 3132	479(36)	372(**e**)	481(5), 480(14), 465(7), 464(17)(**a**), 422(4)
18 di-TMS	(±)	2998ᵇ, 3006	525(9)	297(**d**)	526(4), 298(27), 239(77)(**d**)
	(±)	3029ᵇ, 3037			

ᵃ 80°C (2 min) then to 160°C (1 min) at 30°C/min and then at 2°C/min to 270°C.
ᵇ 80°C (2 min) then to 170°C (1 min) at 30°C/min and then at 2°C/min to 270°C.
ᶜ Mass spectra normalised above m/z 40.
ᵈ Only ions above m/z 75 in excess of 2% abundance have been tabulated. Letters (bold) in parentheses denote ions cited in Figs. 7 and 8.
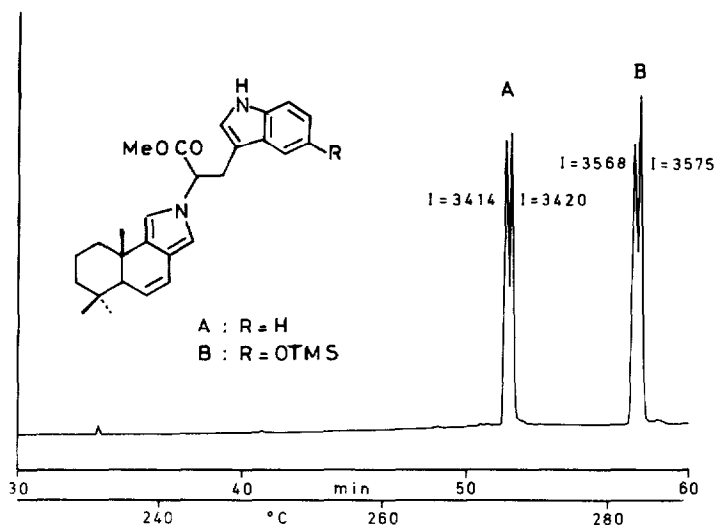
Fig. 4. GC separation of reaction products of (±)-polygodial with (A) (±)-tryptophan methyl ester and (B) (±)-5-hydroxytryptophan methyl ester TMS ether. Column and column temperature programming conditions as in Fig. 3. Me = Methyl.

enantiomers. The separation ($\Delta I = 5$) of peaks of the products from norpseudo-ephedrine (13) was lost in the TMS ethers but recovered ($\Delta I = 7$) in the TBDMS ethers (Table I). However, the *erythro* isomer norephedrine (14) afforded pyrrole diastereo-mers that were separable both in the free and trimethylsilylated forms (Table I). The (+)-amine in each instance (13 and 14) yielded, by reaction with (−)-polygodial, the earlier-eluted diastereomers.

The racemic adrenergic agent, methoxamine[9] (15), which has the *erythro* configuration, gave diastereomeric products which were exceptionally well resolved as their TMS ethers ($\Delta I = 24$; Fig. 5). This result probably reflects the steric enhancement of conformational energy differences by the *ortho*-methoxyl group. The possibility of a spurious result, due to formation of the *threo* isomer during the reaction, was not regorously excluded, but is considered unlikely. No such epimerisa-tions were observed with any of the related amines; furthermore, achiral reagents yielded single peaks from methoxamine.

(iv) Dihydroxy amines. Three examples of this class were selected: *p*-octopamine (16), *erythro*-2-amino-1-phenylpropane-1,3-diol (17) and normetanephrine (18). Sepa-rations of the diastereomeric pyrroles were achieved by means of suitable derivatives (Table I). The di-TMS ethers of the (±)-octopamine derivatives showed $\Delta I = 7$ on both columns.

The diastereomeric derivatives of 17 were partially separated as the 3-mono-TBDMS ethers, but not as the di-TBDMS ethers (Fig. 6). The marked effect of the single TBDMS group at the achiral 3-position may be ascribed to its promotion of intramolecular hydrogen bonding and consequent conformational stabilisation. In the case of normetanephrine (18) the di-TMS ethers were adequately resolved.
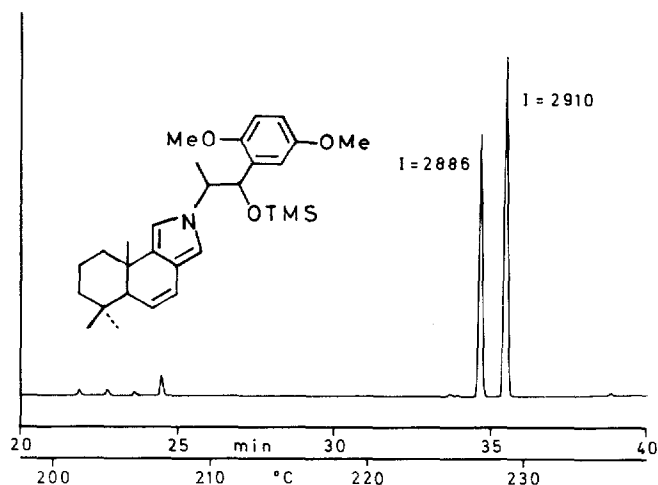
Fig. 5. GC separation of the reaction products of (±)-methoxamine with (±)-polygodial. Column, CP-Sil 5 CB fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (2 min) to 170°C (1 min) at 30°C/min, and then at 2°C/min to 270°C; helium flow-rate, 3 ml/min.

MS properties

The mass spectra showed a variety of notable features. No significant differences were seen between the spectra of the two diastereomers, produced from enantiomeric amines: accordingly, data in Table I are recorded for one of each pair. Molecular ions were observed for every derivative studied (including TBDMS ethers), ranging in relative abundance from 4 to 100%. Other base peaks mainly resulted from expected cleavages, although these were in some cases attended by unpredictable hydrogen
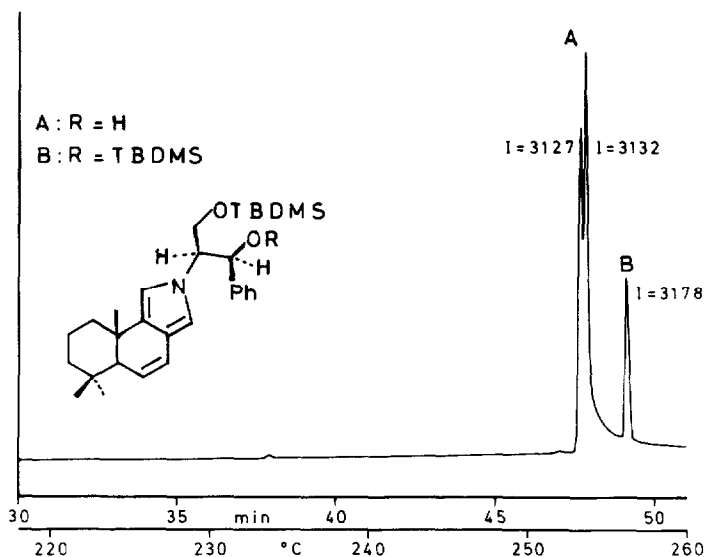
Fig. 6. GC separation of the reaction products of 1S,2S-(+)-2-amino-1-phenylpropane-1,3-diol with (±)-polygodial as TBDMS ethers. Column and column temperature programming conditions as in Fig. 3.

rearrangements. Likely ion structures for the base peaks (excluding the molecular ions from **4** and **10**) are shown in Fig. 7. The structure **a** relates particularly to parent amine **9**, which contains no methyl group. In the derivative of menthylamine (**5**), $(M-15)$ ions probably result also from loss of methyl radicals from the monoterpene ring. Of the other ion types, **b–e** all ensue from benzylic cleavages, in which the positive charge may be preponderantly on the drimane moiety or on part of the original amine substituent group, according to structural factors. Ion type **f** results from benzylic cleavage, accompanied by loss of C_6H_{12} (84 mass units) from ring A of the polygodial residue. Ions from similar scission of ring A, but mainly of types **g** ($[M-82]$) and **h** ($[M-83]$) (Fig. 8), representing losses of C_6H_{10} and $C_6H_{11}^{\cdot}$, respectively, were particularly prominent in the mass spectra of derivatives of **4–11**. Metastable ions, corresponding to the transition $[M]\rightarrow[M-82]$, were observed in this group, except for **4** and **6**. Further salient aspects of the data are outlined below, chiefly in respect of types of ion not mentioned above: some of these are also included in Fig. 8.

(i) Amines lacking other functional groups. The product from tranylcypromine (**4**) gave prominent ions at m/z 254, due to loss of a phenyl radical, and at m/z 117 ($C_6H_5C_3H_4^+$ resulting from C–N bond scission). Simple C–N cleavage also yields prominent ions —three benzylic cations and one secondary carbonium ion— from derivatives of **6, 7, 8** and **9**.

An ion at m/z 200, observed in the mass spectra of derivatives of **5, 8** and **9**, appears to be $C_{15}H_{20}^{+\cdot}$ (**j**), formed by elimination of the pyrrole nitrogen atom together

a

$\underline{m}/\underline{z}$ 199 + R

[Nos. 5, 7, 9]

b

$\underline{m}/\underline{z}$ 242

[Nos. 6; 13-TMS; 13-TBDMS; 14; 14-TMS; 15-TMS]

c

$\underline{m}/\underline{z}$ 226

[No. 15]

$$Aryl-\overset{+}{C}HR$$

d

$\underline{m}/\underline{z}$ 13 + Aryl + R

[Nos. 8, 11, 12, 16, 18]

e

$\underline{m}/\underline{z}$ = 330 (R = Me)

$\underline{m}/\underline{z}$ = 372 (R = t-Bu)

[Nos. 17-TMS, 17-TBDMS]

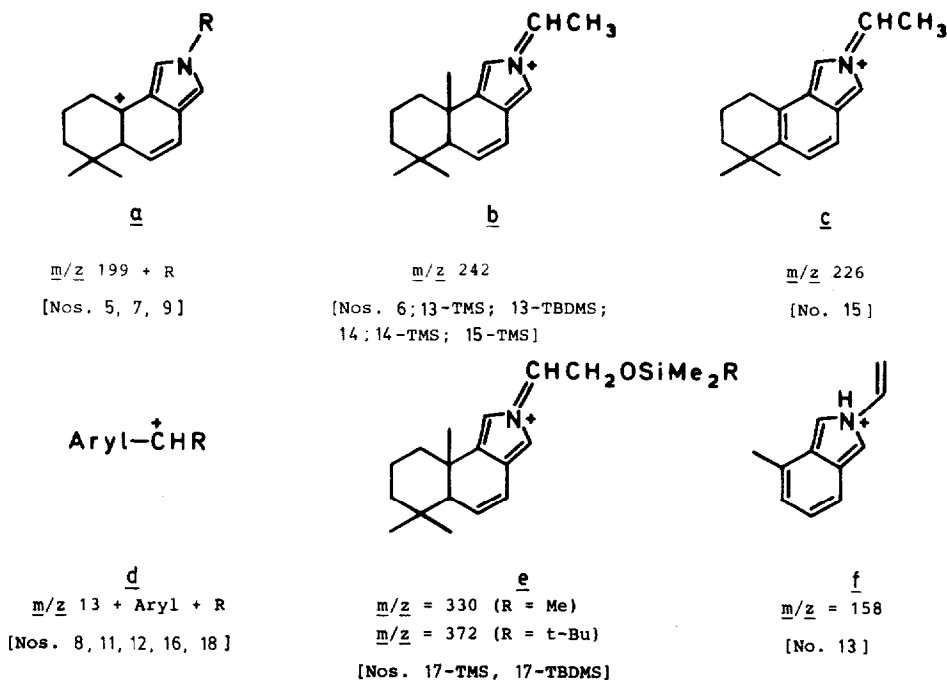**f**

$\underline{m}/\underline{z}$ = 158

[No. 13]

Fig. 7. Ion types constituting the base peaks in the mass spectra (22 eV) of derivatives of the parent amines (excluding the molecular ions of compounds **4** and **10**). Bu = Butyl.
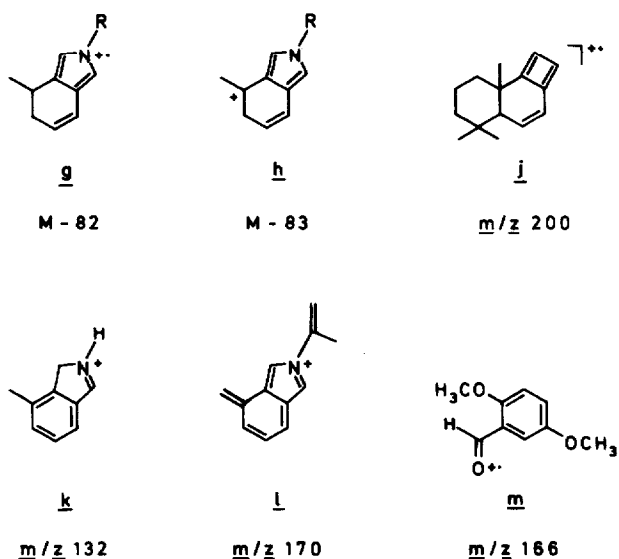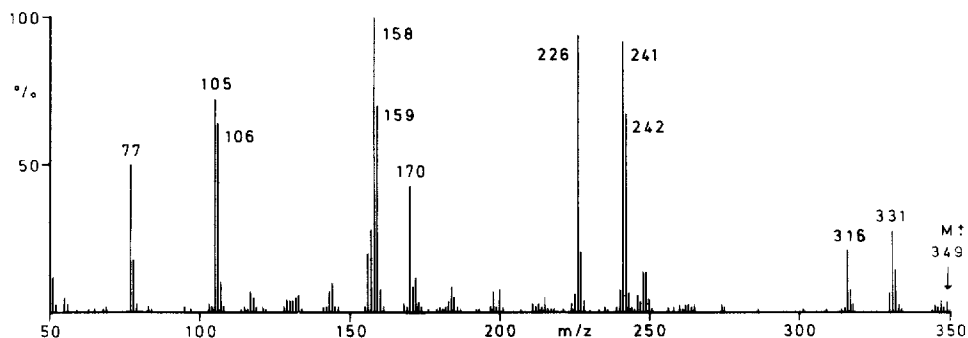
Fig. 8. Postulated structures of some fragment ions.

Fig. 9. Mass spectrum (22 eV) of the reaction products of D-(+)-norpseudoephedrine with (±)-polygodial measured on an LKB gas chromatograph–mass spectrometer. Column, DB-1 fused-silica capillary (60 m × 0.32 mm I.D.); column temperature, 240°C; helium carrier and make-up gas flow-rates, 7 ml/min and 25 ml/min, respectively; accelerating voltage, 3.5 kV; filament current, 4A; trap current, 60 μA; source and separator temperatures, 270°C.
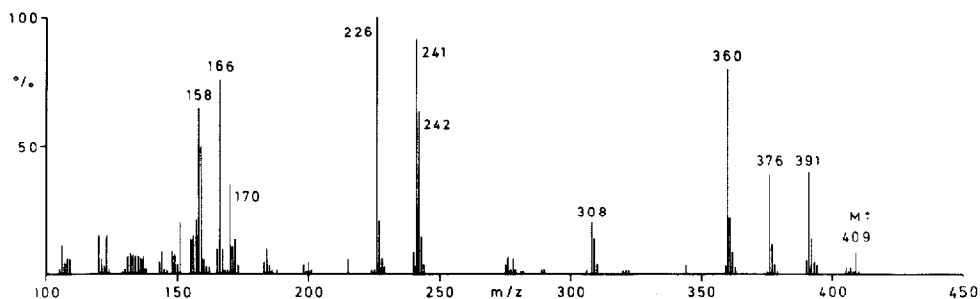
Fig. 10. Mass spectrum (22 eV) of the reaction products of (±)-methoxamine with (±)-polygodial. GC–MS conditions as in Fig. 9.

with its substituent group. An ion of isoindole type (**k**) was also produced, particularly (8–10% relative abundance) by these three derivatives.

The mass spectrum of the derivative of the acetonide (**10**) was of interest in that, besides the expected ions of type [M − 15], types **b**, **c**, **f**, **g** and **h** were all prominent.

(ii) Tryptophan and 5-hydroxytryptophan methyl esters. As expected, the indolyl methyl cations dominated the mass spectra of the derivatives of these compounds (**11** and **12**; the latter being studied as its di-TMS ether), and few other ions were present.

(iii) β-Hydroxy amines. The pyrroles formed from the *threo* and *erythro* isomers norpseudoephedrine (**13**) and norephedrine (**14**) yielded many fragment ions in common but with markedly different relative intensities: thus an ion of type **f** was the base peak in the former spectrum (Fig. 9) but only of 26% relative abundance in the latter (Table I). Ion-radicals of m/z 159 were also abundant in both spectra and are undoubtedly analogues of ions **f**. Prominent ions due to benzylic cleavages included $C_6H_5CHO^{+\cdot}$ (m/z 106) and $C_6H_5CO^+$ as well as types **b** and **c**. Ions of type [M − H_2O] also occurred. The ion of m/z 170 (see also Fig. 10) is attributable to an analogue (type **l**) of ion **f**. Conversion of the pyrrole derivative, derived from norpseudoephedrine, to the TMS or TBDMS ether suppressed most of the fragmentations except for the formation of ions of type **b**, which carried 60–80% of the total ion current.

Methoxamine (**15**) gave a pyrrole showing a very characteristic mass spectrum (Fig. 10) with a variety of prominent ions —thirteen of these having relative abundances of 20% or more. Some of these arose from the presence of the aryl methyl ethers and the benzylic hydroxyl group: thus, sequential losses of H_2O and $CH_3O^\cdot$ gave ions at m/z 391 and 360 with a strong metastable ion, indicating the latter transition (calcd. 331.5: observed 331.6). Other noteworthy ions were at m/z 158 (**f**) and m/z 159 (see above); m/z 308 ([M − 101]), ascribed to the loss of H_2O and of $C_6H_{11}^{\cdot}$ from the terpenoid moiety (type **h**); and at **m/z** 166, probably representing the dimethoxybenzaldehyde molecular ion **m** (stabilised by the *o*-methoxyl group).

(iv) Dihydroxy amines. In order to obtain satisfactory GC peaks, the condensation products from these substrates were studied as silylated derivatives. The di-TMS ether of the pyrroles formed from **16–18** gave only one major fragmentation, *viz.* the α-cleavage of the TMS ether: however, in the case of the β,β'-dihydroxyamine (**17**) the fragment affording the base peak was not the benzylic ion (m/z 179: 6%) but the complementary pyrrole moiety **e** (m/z 330: Fig. 7). A metastable ion was present for [M] → [M − 179]. The mono-TBDMS ether of the product from **17** gave a similar result: the only other notable point was the abundance of the molecular ion (36%) compared with [M − 57] (4%), having regard to the general prevalence of the latter type in the mass spectra of TBDMS ethers.

CONCLUSIONS

The qualitative studies reported here for approximately micromolar amounts of reactants show that polygodial undergoes rapid condensation with a wide variety of primary amines to yield stable substituted pyrroles. No interference in the reaction was observed from β-hydroxyl or other hydroxyl substituents: however, where these were present, it was usually advantageous to convert them into TMS or TBDMS ethers to improve their properties in GC.

Among the fifteen chiral amines examined here, five failed to show separation,

by GC, of the derived diastereomeric pyrroles: these are being further studied using more selective phases. In other instances (with silylation of OH or NH groups where appropriate) such diastereomers showed retention index differences (ΔI) ranging from 5 to 24 units. Preliminary work had indicated that chiral aliphatic amines (such as valine methyl ester) did not yield separable diastereomeric pyrroles, and it is noteworthy that all the successful resolutions reported here came from parent amines containing aromatic or heteroaromatic rings.

The mass spectra were satisfactory in yielding clear molecular ions together with a range of informative fragment ions. Several of these ensued from simple and predictable cleavages, but more complex fragmentations also showed regularities that strengthened their value for the characterisation of different amines. One drawback of the present method is that polygodial is not readily available and is somewhat unstable. The former difficulty may be solved if the compound comes into agrochemical use, as partial and total syntheses have been devised. However, other suitably constituted 1,4-dioxo compounds merit investigation, having regard to the speed and experimental simplicity of amine–carbonyl reactions under mild conditions, and particularly to the selectivity of pyrrole formation from primary amines. Preliminary work on the more stable drimenedial, cinnamodial (1b)[10,11] has shown that it undergoes similar condensations but that the products are more complex. Further studies are in progress with this reagent.

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