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CYCLIC DI-*tert.*-BUTYLSILYLENE DERIVATIVES OF SUBSTITUTED SALICYLIC ACIDS AND RELATED COMPOUNDS

A STUDY BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

In this extension of earlier work, the main compounds studied have been di-*tert.*-butylsilylene (DTBS) derivatives of hydroxylic and phenolic acids. Preparative procedures were based on those originally applied by Trost and Caldwell [*Tetrahedron Lett.*, 22 (1981) 4999] to diols. Most substrates gave apparently quantitative yields in analytical-scale preparations. Derivatives containing five-, six-, and seven-membered rings were prepared. With a variety of substituted salicylic acids, good gas chromatographic separations were obtained for pairs or sets of isomeric substrates: examples included 3-allyl- and 3-propenylsalicylic acid; carvacrotic and thymotic acid; 1- and 3-hydroxy-2-naphthoic acid and 2-hydroxy-1-naphthoic acid. 2,5-Dihydroxyterephthalic acid yielded a bis-DTBS derivative. Among other substrates studied were 3-(2,3-dihydroxypropyl)salicylic acid and its 2-methyl homologue, some catechol derivatives, and the drugs mephenesin and chlorphenesin. The mass spectra showed general features established earlier. Most salicylate DTBS derivatives gave abundant molecular ions and base peaks resulting from loss of C_4H_8. Isomers afforded very similar mass spectra because of the preponderant fragmentations of the DTBS groupings. (However, DTBS derivatives of the isomeric catechols, methyl 2,3- and 3,4-dihydroxybenzoate, showed distinctive differences in their mass spectra.) The bis-DTBS derivatives of the two (3-dihydroxyalkyl)salicylic acids examined were exceptional in that fragmentations of the side-chain moieties preponderated in the mass spectra.

The results confirm the value of DTBS derivatives for the characterisation of hydroxy-acids. They also show that bis-DTBS derivatives, even with masses above 450, are quite satisfactory for gas–liquid chromatography, with typical retention indices in the region of 3000. Most fragment ions from bis-DTBS derivatives, in the group studied, retain one intact cyclic silylene moiety.

INTRODUCTION

Our earlier studies[1,2] have shown that cyclic di-*tert.*-butylsilylene (DTBS) derivatives are usefully complementary to cyclic boronates for the gas-phase analyses of diols and related bifunctional substrates. It was noted[2] that DTBS derivatives of

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the four isomeric methylsalicylic (cresotic) acids gave sharp and well separated gas chromatographic peaks, and appeared to be more stable in solution than analogous alkaneboronates. Accordingly, a wider range of substituted salicylic acids has been examined by gas chromatography–mass spectrometry. The work has been extended also to some compounds containing two bifunctional groupings and affording bis-DTBS derivatives. Among these were two typical metabolites of biogenic amines, *viz.* (3,4-dihydroxyphenyl)ethane-1,2-diol and 3,4-dihydroxymandelic acid. The main aim of this investigation was to verify that DTBS derivatives could be effectively used for the separation and characterisation of relatively complex substrates.

EXPERIMENTAL

Solvents and reagents

Acetonitrile (AnalaR) was obtained from BDH (Poole, U.K.), N-methylmorpholine from Ventron Alfa Products (Coventry, U.K.) and ethyl acetate (Nanograde) from Mallinckrodt (St. Louis, MO, U.S.A.). Di-*tert.*-butyldichlorosilane was purchased from Petrarch Systems (Bristol, PA, U.S.A.), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) from Pierce and Warriner (Chester, U.K.) and 1-hydroxybenzotriazole from Fluka (Fluorochem, Glossop, U.K.).

Substituted salicylic acids and other reference compounds

The parent compounds were numbered as in Fig. 1. Compounds 19–26, 29 and 30 were mixtures of enantiomers. The following samples were available from previous work: compounds 1, 7–10, 12, 14–16[3]; 24–26[4]; 3–5[5]. Compound 6 was a gift from Dr. G. T. Newbold. The remaining samples were obtained from commercial sources.

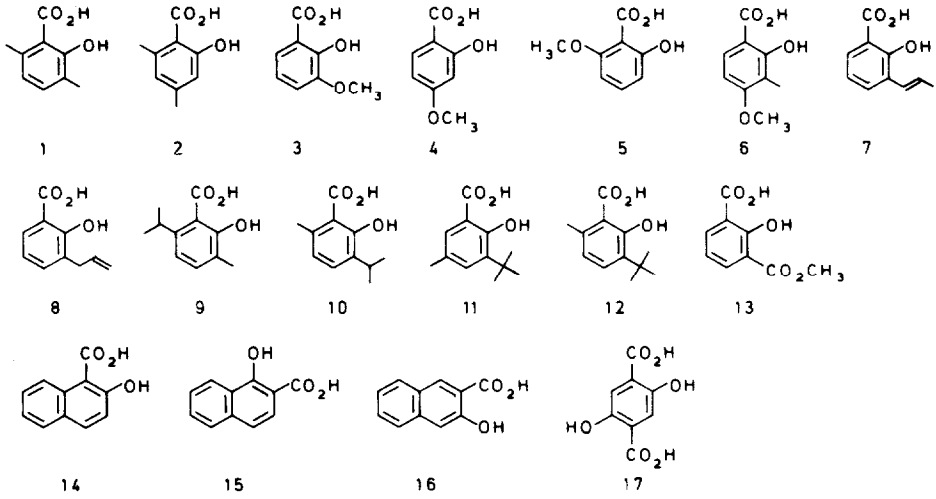
Gas–liquid chromatography

Packed-column gas–liquid chromatography (GLC) was carried out with a Perkin-Elmer (Beaconsfield, U.K.) F11 chromatograph equipped with a silanised glass column (1.8 m × 4 mm I.D.) packed with 1% OV-1 coated on Gas-Chrom Q, 100–120 mesh (Phase Separations, Queensferry, U.K.). A variety of column temperatures were used (see Table I for details) and the nitrogen carrier gas flow-rate was 40 ml/min. Open-tubular GLC was performed with a Hewlett-Packard (Winnersh, U.K.) 5880A gas chromatograph fitted 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 injector was 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 Figs. 3, 5–7, 10, 11 and 13. Both instruments employed flame-ionisation detectors.

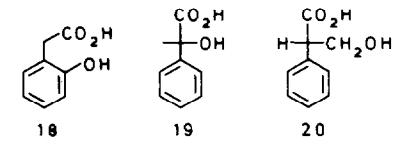
Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–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. Helium carrier and make-up gas flow-rates were 7 ml/min and 25 ml/min, respectively. Mass spectra (22 eV) were recorded under electron im-

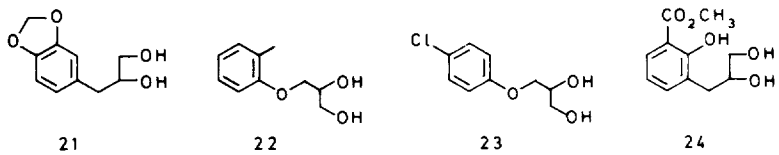
Substituted salicylic acids



Side-chain hydroxy-acids



Side-chain diols



Dihydroxyalkyl salicylic acids Catechols

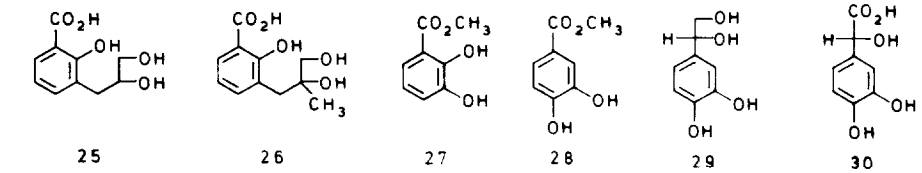


Fig. 1. Structures of parent hydroxy acids and diols. 1 = 3,6-dimethylsalicylic acid; 2 = 4,6-dimethylsalicylic acid; 3 = 3-methoxysalicylic acid; 4 = 4-methoxysalicylic acid; 5 = 6-methoxysalicylic acid; 6 = 4-methoxy-3-methylsalicylic acid; 7 = 3-(prop-1-enyl)salicylic acid; 8 = 3-(prop-2-enyl)salicylic acid; 9 = 6-isopropyl-3-methylsalicylic acid; 10 = 3-isopropyl-6-methylsalicylic acid; 11 = 5-methyl-3-*tert.*-butylsalicylic acid; 12 = 6-methyl-3-*tert.*-butylsalicylic acid; 13 = 3-methoxycarbonylsalicylic acid; 14 = 2-hydroxy-1-naphthoic acid; 15 = 1-hydroxy-2-naphthoic acid; 16 = 3-hydroxy-2-naphthoic acid; 17 = 2,5-dihydroxyterephthalic acid; 18 = *o*-hydroxyphenylacetic acid; 19 = atrolactic acid; 20 = tropic acid; 21 = 3-(3,4-methylenedioxyphenyl)propane-1,2-diol; 22 = 3-(2-methylphenoxy)propane-1,2-diol (mephenesin); 23 = 3-(4-chlorophenoxy)propane-1,2-diol (chlorphenesin); 24 = methyl 3-(2,3-dihydroxypropyl)salicylate; 25 = 3-(2,3-dihydroxypropyl)salicylic acid; 26 = 3-(2-methyl-2,3-dihydroxypropyl) salicylic acid; 27 = methyl 2,3-dihydroxybenzoate; 28 = methyl 3,4-dihydroxybenzoate; 29 = 3,4-dihydroxyphenylethane-1,2-diol; 30 = 3,4-dihydroxymandelic acid.

TABLE I

KOVÁTS RETENTION INDICES (*I*) AND MASS SPECTROMETRIC DATA (22 eV) FOR DI-*tert.*-BUTYLSILYLENE DERIVATIVES OF DIOLS, TRIOLS, A TETROL AND HYDROXY-ACIDS

Superscripts a–h correspond to the fragment ions in Fig. 2. Values in parentheses are intensities relative to base peak.

Compound	*I (OV-1)*	*Temperature (°C)*	$M^{+\cdot}$	*Base peak**	*m/z for other principal ions***								
1	1865	170	306(57)	250[c]	291(1) 149(3)[h]	249(8)[a]	247(3)	207(16)[d]	205(72)[b]	194(24)[f]	193(12)[g]	192(4)	163(36)[e]
2	1890	170	306(60)	250[c]	291(1) 163(40)[e]	249(11)[a] 149(4)[h]	247(3)	207(18)[d]	205(93)[b]	194(25)[f]	193(13)[g]	192(5)	177(3)
3	1870	150	308(52)	252[c]	251(6)[a] 151(3)[h]	209(27)[d]	207(28)[b]	196(14)[f]	195(12)[g]	194(3)	192(7)	167(5)	165(16)[e]
4	1965	150	308(45)	207[b]	252(89)[c] 151(4)[h]	251(8)[a]	249(4)	209(14)[d]	196(28)[f]	195(22)[g]	194(6)	193(3)	165(32)[e]
5	1940	150	308(68)	207[b]	293(1) 189(3)	279(2) 181(3)	252(73)[c] 177(3)	251(16)[a] 165(51)[e]	249(3) 151(5)[h]	234(3)	223(21)	195(24)[g]	194(3)
6	2045	170	322(42)	221[b]	266(92)[c]	265(7)[a]	263(3)	220(3)	210(22)[f]	209(16)[g]	179(32)[c]	165(5)[h]	
7	1995	170	318(60)	262[c]	261(4)[a]	219(24)[d]	217(45)[b]	206(12)[f]	205(15)[g]	204(3)	175(23)[c]	161(3)[h]	160(3)
8	1950	170	318(49)	262[c]	261(10)[a]	219(29)[d]	217(42)[b]	206(13)[f]	205(14)[g]	189(3)	175(19)[c]	161(3)[h]	160(3)
9	1945	170	334(93)	278[c]	319(5) 205(5)	277(19)[a] 191(63)[e]	275(4) 177(3)[h]	263(23)	233(81)[b]	221(33)[g]	220(5)	219(3)	207(3)
10	1965	170	334(72)	278[c]	319(10) 177(3)[h]	277(9)[a]	275(3)	263(56)	233(91)[b]	221(11)[g]	220(2)	205(3)	191(45)[e]
11	2110	170	348(95)	247[b]	333(32) 191(4)[h]	292(96)[c] 175(3)	291(6)[a]	289(4)	277(84)	235(16)[g]	234(2)	219(3)	205(40)[e]
12	2060	170	348(56)	247[b]	333(53) 191(7)[h]	292(48)[c] 175(6)	291(7)[a]	289(3)	277(75)	235(15)[g]	231(3)	219(4)	205(53)[e]
13	2000	170	336(33)	280[c]	305(11) 179(5)[h]	289(3) 166(6)	279(20)[a] 165(6)	247(73)	237(18)[d]	235(4)[b]	224(52)[f]	223(27)[g]	180(11)
14	2250	190	328(70)	272[c]	271(4)[a] 170(3)	269(3)	227(77)[b]	216(18)[f]	215(20)[g]	214(6)	201(3)	185(23)[e]	171(6)[h]
15	2235	190	328(46)	272[c]	271(2)[a] 170(6)	269(2)	227(94)[b]	216(27)[f]	215(27)[g]	214(9)	201(3)	185(50)[e]	171(11)[h]

16	2280	190	328(66)	272[c]	271(6)[a]	269(2)	229(41)[d]	227(45)[b]	216(17)[f]	215(14)[g]	214(7)	185(33)[e]	171(3)[h]
					170(2)								
17	2730	235	478(100)	478	422(29)[c]	421(3)[a]	419(3)	379(23)[d]	377(28)[b]	366(42)[f]	365(11)[g]	335(8)[e]	323(7)
					321(9)[h]	310(3)	309(4)						
18	1845	155	292(37)	165	277(2)	248(6)	236(50)	235(49)	207(9)	192(66)	191(10)	189(9)	180(3)
					179(4)	151(19)	149(8)	147(3)	136(19)	135(3)	134(6)		
19	1825	170	306(1)	262	291(2)	261(36)	260(5)	249(4)	221(6)	207(10)	206(11)	205(30)	193(3)
					179(5)	165(3)	164(3)	163(13)	149(3)	127(9)	105(3)	103(3)	101(3)
20	2000	180	306(1)	249	304(1)	291(2)	276(4)	262(74)	231(3)	207(80)	206(9)	205(19)	179(3)
					177(3)	163(70)	161(7)	149(4)	145(5)	137(8)	131(9)	117(5)	105(3)
					104(24)	103(13)	102(4)	101(8)	95(3)				
21	2105	180	336(48)	201	279(79)	249(4)	237(19)	223(5)	222(4)	219(15)	207(18)	200(5)	193(3)
					189(5)	179(5)	161(15)	145(8)	136(8)	135(41)	131(5)	103(46)	101(3)
22	1990	170	322(3)	265	247(2)	237(8)	235(3)	209(8)	205(8)	193(10)	191(8)	179(30)	175(5)
					167(20)	165(4)	163(3)	157(5)	149(3)	117(4)	115(3)	103(4)	
23	2110	170	342(3)	285	287(38)	259(4)	257(8)	250(3)	243(4)	231(5)	229(15)	227(3)	225(5)
			344(1)		215(7)	213(17)	211(6)	201(12)	199(29)	197(3)	195(4)	189(12)	187(34)
					157(11)	117(5)	115(8)	103(5)	101(4)				
24	2295	190	366(0.2)	277	335(1)	319(1)	309(17)	201(14)	145(2)	103(7)			
24a***	2360	190	438(0.1)	277	423(20)	407(2)	393(2)	391(4)	381(12)	351(3)	349(5)	238(2)	222(2)
					206(2)	201(48)	200(2)	191(3)	149(2)	145(4)	103(15)	89(5)	
25	2745	235	492(4)	435[a]	477(2)	393(4)[d]	379(2)[g]	351(3)	321(2)	292(68)	277(16)	265(2)	235(4)
					201(68)	159(2)	145(4)	103(11)					
26	2835	235	506(2)	215	491(2)	449(8)[a]	291(4)	173(2)	117(3)				
27	1910	150	308(55)	252	277(9)	219(42)	209(17)	196(19)	195(51)	194(11)	179(5)	163(27)	153(5)
					152(4)	136(3)	133(3)	119(3)					
28	1795	150	308(80)	252	277(9)	251(4)	221(9)	209(58)	196(12)	195(23)	194(6)	163(3)	151(3)
29	2465	235	450(58)	393	449(4)	448(2)	351(25)	337(9)	333(20)	332(3)	317(6)	309(7)	291(5)
					277(3)	276(4)	267(9)	264(3)	263(4)	225(3)	143(19)	115(5)	101(5)
					100(3)								
30	2615	235	464(100)	464	420(80)	419(21)	418(6)	408(3)	407(3)	379(11)	363(55)	321(17)	307(4)
					304(16)	289(24)	263(8)	248(4)					

* Mass spectra normalised above m/z 40.

** Only ions in excess of 2% abundance have been tabulated (above m/z 80) unless of specific importance.

*** TMS ether.

pact conditions: accelerating voltage, 3.5 kV; filament current, 4 A; trap current, 60 μA; and source and separator temperatures, 270°C.

Preparation of derivatives

DTBS derivation. Aliphatic diol substrates (100 μg) were dissolved in acetonitrile (30 μl). N-Methylmorpholine (20 μl), 1-hydroxybenzotriazole (3 μg) (stock solution: 3 mg, dried *in vacuo* at 40°C, was dissolved in 1 ml of acetonitrile) and di-*tert.*-butyldichlorosilane (3.5 μl) were added sequentially and the mixture was heated in a Reactivial at 80°C overnight (15 h). For the aromatic diols and hydroxy-acids, addition of the 1-hydroxybenzotriazole catalyst was omitted, and the reactants were heated at 80°C for only 1–2 h. The solutions derived above were diluted to 100 μl with ethyl acetate and used without delay (usually within 1–2 h) for GLC and GC–MS analyses.

Trimethylsilylation. Compound 24 was converted to its DTBS derivative as above. After filtration and evaporation to dryness, the product was treated with BSTFA (15 μl) and heated at 80°C for 15 min. The solution was immediately evaporated to dryness, and redissolved in ethyl acetate (100 μl) for GLC and GC–MS analyses.

RESULTS AND DISCUSSION

Preparation and stability of DTBS derivatives

For the majority of the substrates examined, the derivatives were prepared (on an analytical scale with ca. 100 μg of sample) essentially by the original procedure of Trost and Caldwell[6]. Compounds containing tertiary hydroxylic groups (19 and 26) were successfully derivatised under the same conditions without recourse to the more powerful reagent (di-*tert.*-butylsilyl ditriflate) of Corey and Hopkins[7]. Earlier related work on dimethylsilylene and other 1,3-dioxa-2-sila groupings was cited in our first report[1].

The derivatives (in solutions retaining some excess reagent) were submitted to GLC and GC–MS analyses without undue delay after their preparation. In most instances, no significant decomposition was observed within 1–2 h. Notable exceptions were the DTBS derivatives of 2-hydroxy-1-naphthoic acid (partially decomposed after 30 min, totally after 6 h) and of atrolactic acid (partially decomposed after 30 min, totally after 24 h).

GLC retention data for a packed column, together with salient features of DTBS derivatives of the thirty compounds studied, are given in Table I. Open-tubular GLC was used for illustrative figures.

DTBS derivatives of simple substituted salicylic acids

The acids in this group (compounds 1–17) were selected primarily to check the suitability of DTBS derivatives for isomer separations. Regularities of the mass spectra, and any significant differences in fragmentations of isomers, were also of interest.

The mass spectra of the derivatives showed several general features: these are noted below and illustrated in Fig. 2, in which the postulated ion structures are of course only formal possibilities.

(i) Molecular ions were invariably prominent, constituting the base peak for compound 17.

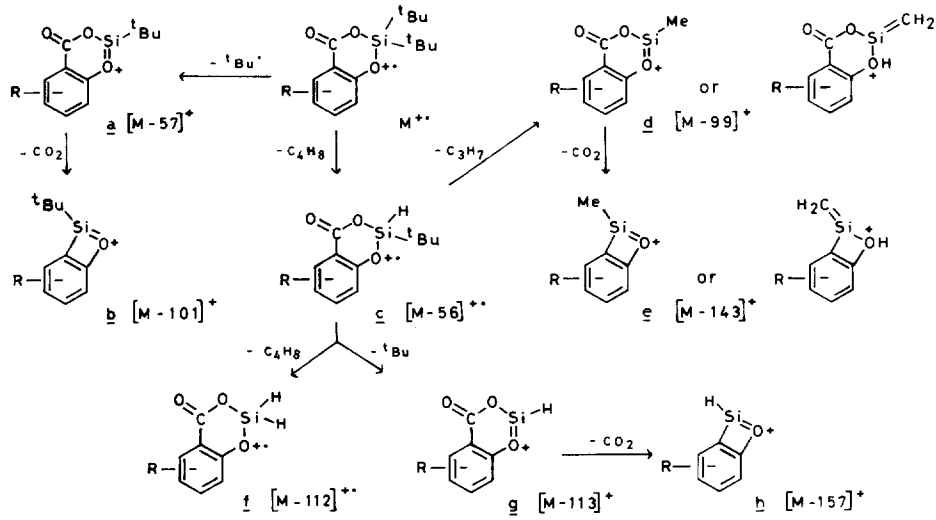


Fig. 2. Formal representations of fragment ions.

(ii) In eleven other instances, the base peak was [M − 56], as was the case for the four methylsalicylic acid DTBS derivatives studied earlier[2]: this ion represents loss of isobutene, from one of the *tert.*-butyl groups.

(iii) Ions of the type [M − 57] formed by loss of a *tert.*-butyl radical occurred at modest intensities (2–20%) in all the mass spectra, and evidently underwent ready decarboxylation to afford the generally strong ions [M − 101], which were base peaks in the remaining five spectra.

(iv) Other major ions, common to all the spectra from compounds 1–17, were derived from ions of type [M − 56] as outlined in Fig. 2. Three alternative pathways were apparent: successive losses of $C_3H_7^{\bullet}$ to give [M − 99] and carbon dioxide yielding the abundant [M − 143] ions; elimination of $C_4H_9^{\bullet}$ to give [M − 113], followed by minor loss of carbon dioxide to afford [M − 157] ions; and elimination of isobutene yielding [M − 112] ions.

The routes of fragmentation proposed above were in accordance with evidence from metastable ions, which were observed for at least one example of each process. Other ions, reflecting individual structural features, are referred to later.

Notable features of the properties of the derivatives in respect of GC–MS are outlined below (*cf.* Table I).

Two isomeric dimethylsalicylic acids (1 and 2) gave DTBS derivatives well separated by GLC but with similar mass spectra. Three methoxy acids (3–5) gave, as DTBS derivatives, excellent gas chromatographic separation (Fig. 3), and somewhat distinctive mass spectra. Two of the latter are shown in Fig. 4: the ions [M − 99] and [M − 101] were of markedly different abundance. The third isomer (6-methoxy: compound 5) was uniquely distinguished by the presence of a strong ion (*m/z* 223) formed, on the evidence of a metastable peak, by the loss of a CHO radical from [M − 56]. The eliminated moiety is presumed to contain the carbonyl group, perhaps extruded as a result of a steric effect from the adjacent methoxy substituent.

DTBS derivatives of a pair of acids representing sidechain isomerism, 3-allyl-

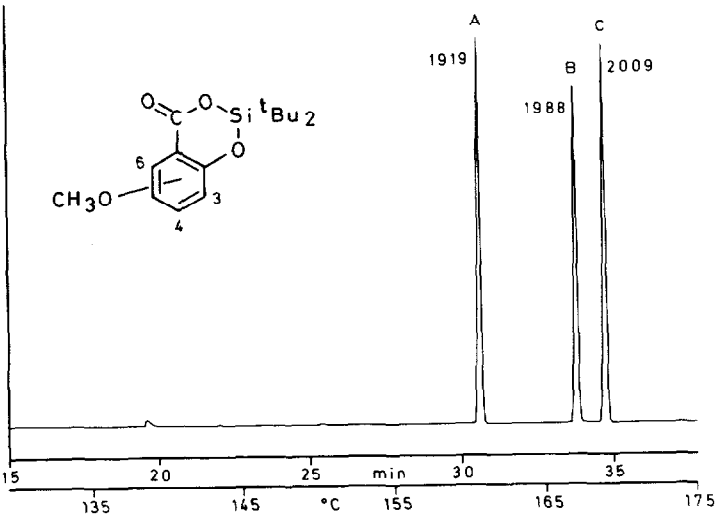


Fig. 3. Gas chromatographic separation of the DTBS derivatives of 3-methoxysalicylic acid (A), 6-methoxysalicylic acid (B) and 4-methoxysalicylic acid (C). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 105°C (1 min) at 30°C/min, and then at 2°C/min to 190°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

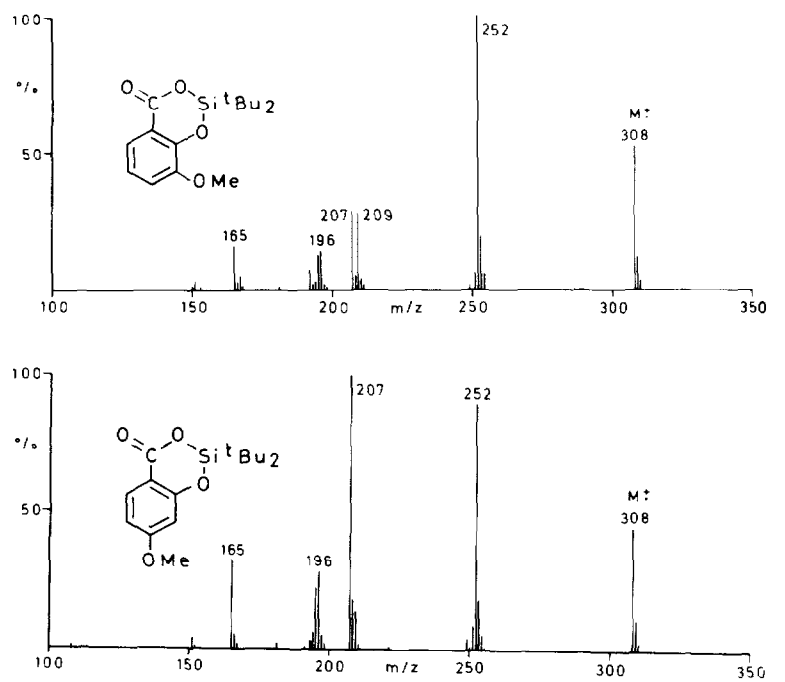


Fig. 4. Mass spectra (22 eV) of the DTBS derivatives of 3-methoxysalicylic acid (top) and 4-methoxysalicylic acid (bottom) measured on an LKB9000 gas chromatograph–mass spectrometer. Column, DB-1 *fused-silica capillary* (60 m × 0.32 mm I.D.); column temperatures as in Table I; helium carrier gas and make-up gas flow-rates, 7 ml/min (measured at room temperature) and 25 ml/min, respectively; accelerating voltage, 3.5 kV; source and separator temperatures, 270°C; filament current, 4 A; trap current, 60 μA. ^{t}Bu = *tert.*-butyl, Me = methyl.

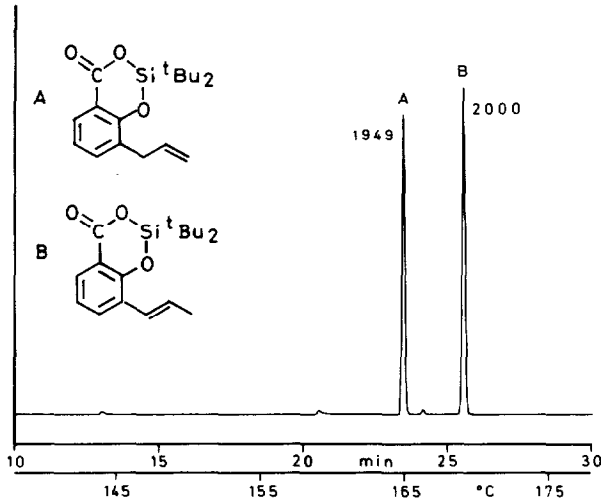


Fig. 5. Gas chromatographic separation of the DTBS derivatives of 3-(prop-2-enyl)salicylic acid (A) and 3-(prop-1-enyl)salicylic acid (B). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 125°C (1 min) at 30°C/min, and then at 2°C/min to 200°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

and 3-(prop-1-enyl)salicylic acid, were widely separated by GLC (Fig. 5), the non-conjugated compound being eluted earlier, as expected. The mass spectra were practically identical and showed only the common fragments discussed above. Further types of isomerism were exemplified by carvacrotic and thymotic acid (9 and 10) and the 3-*tert.*-butyl-5-(or 6-)methylsalicylic acids (11 and 12). Within each pair, the isomers were easily distinguished by GLC of their DTBS derivatives (see Fig. 6 for 9

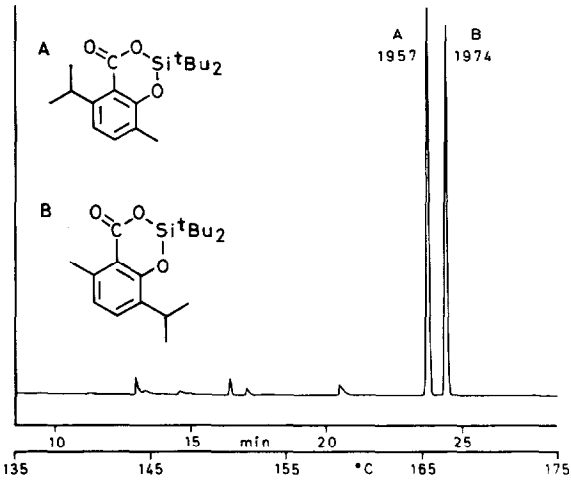


Fig. 6. Gas chromatographic separation of the DTBS derivatives of 6-isopropyl-3-methylsalicylic acid (A) and 3-isopropyl-6-methylsalicylic acid (B). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 125°C (1 min) at 30°C/min, and then at 2°C/min to 180°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

and 10), but not by the corresponding mass spectra which showed only minor differences (Table I). Ions of type [M − 15] and [M − 56 − 15] —the latter sequence shown by appropriate metastable ions— were features of all four spectra.

Within the group of simple alkylated salicylic acids, the chromatographic data were in accordance with the consideration that the buttressing effects of substituents at the 3 and 6 positions would tend to reduce retention times: thus, orders of elution for the following compounds were 1 < 2; 9 < 10; 12 < 11.

The DTBS derivative of 3-methoxycarbonylsalicylic acid (2-hydroxyisophthalic acid monomethyl ester, 13) showed, together with an ion at *m/z* 305 due to loss of a methoxy radical, and ions shown in Fig. 2, a prominent ion at *m/z* 247. Observation of a metastable ion indicated that this arose by loss of methanol from [M − 57], but the origin of the extra hydrogen atom remains obscure.

The three hydroxynaphthoic acids of salicylate type (14–16) gave DTBS derivatives that were well separated by GLC (Fig. 7): the earlier elution of the 3,4-benzosalicylate (15) than of the 5,6-benzosalicylate (14) was unexpected. As in other instances, the importance of the gas chromatographic characterisation was apparent, in view of the largely similar mass spectra. Some differences in relative intensities, among the ions resulting from regular fragmentations, were noted: Fig. 8 indicates the markedly higher abundance of *m/z* 229 [$M - C_4H_8 - C_3H_7^{\bullet}$] in the spectrum of the 3-hydroxy-2-naphthoic acid derivative than of its 2-hydroxy-1-naphthoic isomer: the spectrum of the latter was virtually identical with that of the 1-hydroxy-2-naphthoic isomer. The effect of inclusion of two *o*-hydroxy acid groups in one ring was examined for the example of 2,5-dihydroxyterephthalic acid (17). This readily formed a bis-DTBS derivative that showed good gas chromatographic behaviour. The mass spectrum (Fig. 9) showed the molecular ion to be the base peak, but in other respects

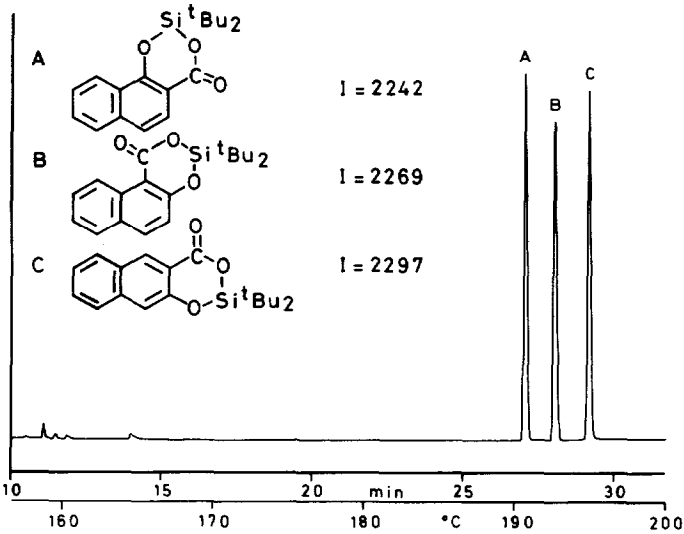


Fig. 7. Gas chromatographic separation of the DTBS derivatives of 1-hydroxy-2-naphthoic acid (A), 2-hydroxy-1-naphthoic acid (B) and 3-hydroxy-2-naphthoic acid (C). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 145°C (1 min) at 30°C/min, and then at 2°C/min to 225°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

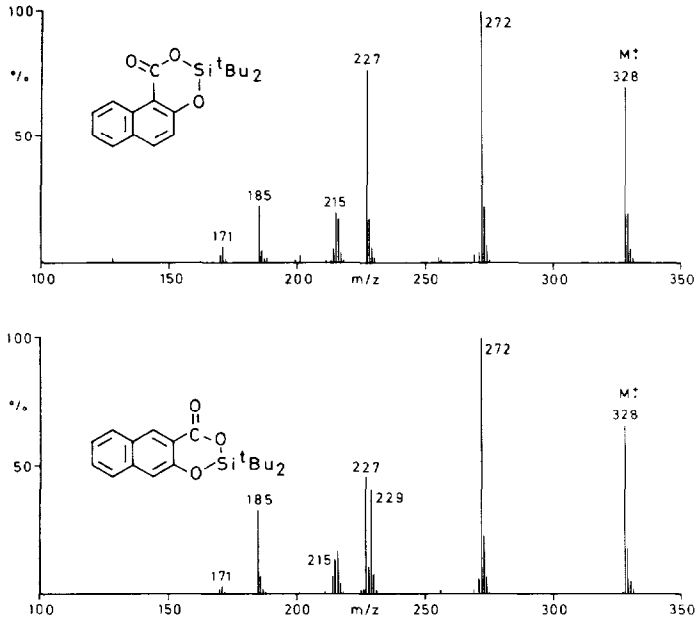


Fig. 8. Mass spectra (22 eV) of the DTBS derivatives of 2-hydroxy-1-naphthoic acid (top) and 3-hydroxy-2-naphthoic acid (bottom). GC–MS conditions as in Fig. 4. ^{t}Bu = *tert.*-butyl.

there were few fragmentations beyond those already discussed (Fig. 2); the second DTBS ring apparently remained largely intact, except for its probable contribution, via loss of C_4H_8, to the ion at *m/z* 366.

Side-chain hydroxy-acids

As a preliminary to the examination of some salicylic acids with hydroxyalkyl side chains, three compounds (18–20) were chosen to check the formation of DTBS derivatives with different ring sizes. Each hydroxy-acid gave a satisfactory derivative for GLC: the separation of the isomeric pair derived from atrolactic acid (19) and tropic acid (20) was very large (ΔI = 184 on the open-tubular column: Fig. 10).

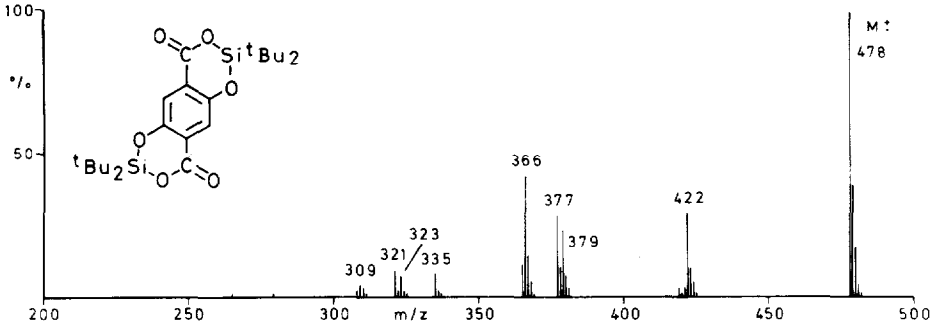


Fig. 9. Mass spectrum of the bis-DTBS derivative of 2,5-dihydroxyterephthalic acid. GC–MS conditions as in Fig. 4. ^{t}Bu = *tert.*-butyl.

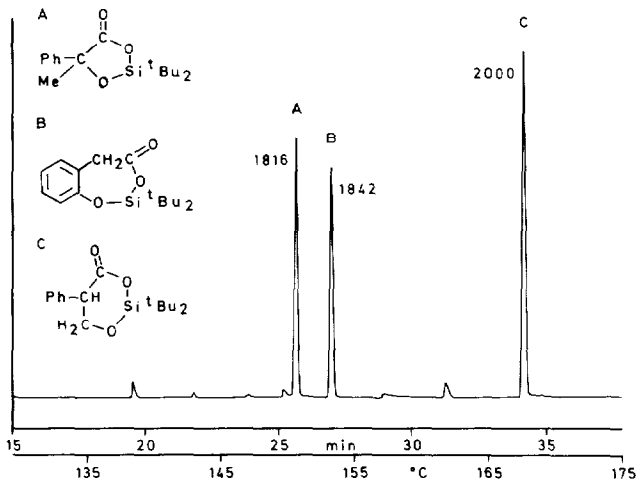


Fig. 10. Gas chromatographic separation of the DTBS derivatives of atrolactic acid (A), *o*-hydroxyphenylacetic acid (B) and tropic acid (C). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 105°C (1 min) at 30°C/min, and then at 2°C/min to 185°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl, Ph = phenyl, Me = methyl.

The mass spectra of these compounds reflected their individual structures. The phenolic acid (18) gave, as its DTBS derivative, some fragmentations analogous to those of Fig. 2. In addition, loss of carbon dioxide from the [M − 56] ion, as confirmed by a metastable peak, gave the prominent ion at *m/z* 192.097; while the base peak at *m/z* 165.037 ($C_8H_9O_2Si$) is ascribed to an alternative sequence (*m/z* 292 → 235 → 207 → 165) involving loss of carbon monoxide.

The derivatives of the non-phenolic acids (19 and 20) gave major fragments resulting from losses of carbon dioxide from the molecular ions. In the former example, a further loss of a $C_4H_9^{\cdot}$ radical, from metastable ion evidence, gave rise to the ion at *m/z* 205. The origin of the prominent ion at *m/z* 261 [M − 45] remains unclear: there were no metastable ions indicative of loss of hydrogen atoms either from [M] or from [M − 44]. The DTBS derivative of tropic acid (20) yielded [M − 57] as the base peak, and this lost carbon dioxide to give a prominent ion at *m/z* 207. A strong ion at *m/z* 163 [M − 143] probably corresponded to that noted in Fig. 2. Loss of CH_2O from the molecular ion was a minor process affording the [M − 30] ion at *m/z* 276.

In summary, the five-, six- and seven-membered heterocyclic rings in the derivatives of 19, 20 and 18 respectively were readily formed, and gave mass spectra combining general types of fragmentation with some more specifically characteristic modes.

Side-chain diols

In this group, compounds 21–23 were diols lacking reactive groups in the aromatic moiety. All gave very good gas chromatographic peaks. Compound 27 was studied as a simple model for the side chain in compound 25 (see next section), while compound 24 contained a phenolic group that might have contributed to form a

large-ring cyclic derivative: in fact the mass spectral evidence, noted below, gave no indication of such a structure.

The DTBS derivative of compound 21 underwent major benzylic cleavages, yielding the dialkylsilylene ion (*m/z* 201.131) as the base peak, and the complementary piperonyl ion (*m/z* 135) with relative abundance 41%. The other major ion was the [M − 57] ion typical of diol DTBS derivatives.

The DTBS derivatives of mephenesin (22) and chlorphenesin (23) gave complex mass spectral fragmentations which remain largely uninterpreted. The base peaks were [M − 57] ions, and only chlorphenesin showed a significant silylene ion at *m/z* 201 from benzylic cleavage.

Methyl 3-(2,3-dihydroxypropyl)salicylate (24) apparently formed only the five-membered DTBS derivative. The base peak of the mass spectrum resulted from the combined loss of methanol (typical of the salicylate moiety) and C_4H_9 (typical of the side-chain silylene group); and benzylic cleavage giving the ion at *m/z* 201 was enhanced in the trimethylsilylated derivative (24a).

Dihydroxyalkyl salicylic acids

Two compounds of this class, 25 and 26, were examined in order to assess the competitive fragmentation modes of the DTBS groupings derived from the diol and hydroxy-acid moieties. The bis-DTBS derivatives were readily obtained, even from the compound containing a tertiary hydroxylic group in the side chain (26), and both gave good peaks in GLC (Fig. 11). The mass spectra (Fig. 12) were dominated by side-chain fragmentations. Benzylic cleavage afforded intense ions comprising the DTBS moieties. In the tertiary/primary diol derivative of compound 26 the complementary ion was produced in low abundance at *m/z* 291, but in the secondary/primary diol (25) a hydrogen atom rearrangement accompanied the cleavage to yield

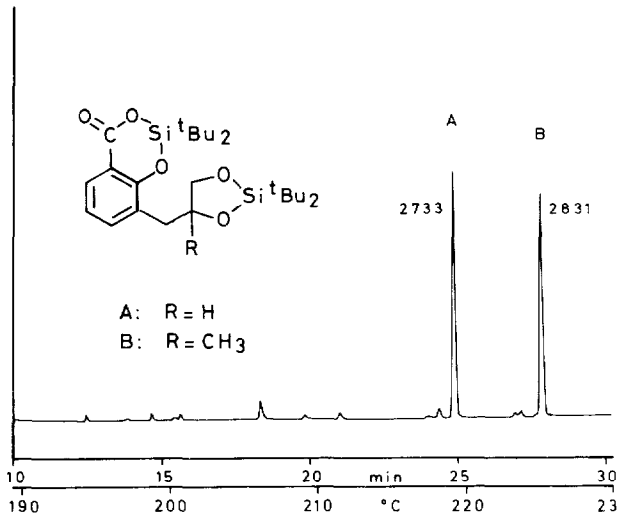


Fig. 11. Gas chromatographic separation of the bis-DTBS derivatives of 3-(2,3-dihydroxypropyl)salicylic acid (A) and 3-(2,3-dihydroxy-2-methylpropyl)salicylic acid (B). Column, CP Sil5 CB fused-silica capillary (25 m × 0.32 mm I.D.); column temperature programmed from 80°C (1 min) to 180°C (1 min) at 30°C/min and then at 2°C/min to 240°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

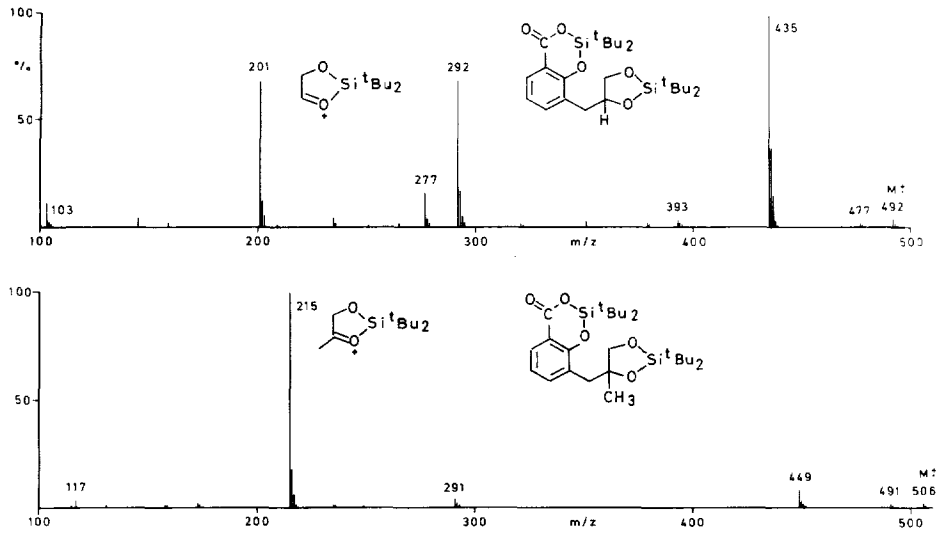


Fig. 12. Mass spectra (22 eV) of the bis-DTBS derivatives of 3-(2,3-dihydroxypropyl)salicylic acid (top) and 3-(2,3-dihydroxy-2-methylpropyl)salicylic acid (bottom). GC–MS conditions as in Fig. 4. ^{t}Bu = *tert.*-butyl.

m/z 292 as an abundant ion. The facility of these fragmentations led to the unusually low abundances of molecular ions, and to the virtual absence of typical ions from the salicylate DTBS groupings.

Catechols

In this last group of compounds, our main interest focused on the two tetrafunctional metabolites of catecholamines (29 and 30), but the two simpler catechols (27 and 28) were included for comparison. The DTBS derivatives of methyl 2,3- and 3,4-dihydroxybenzoates were remarkably well separated by GLC: the retention index difference of 115 was the largest found in our laboratory for any derivatives of these isomers. The mass spectra gave many common ions as expected from previous work[1] and by analogy with Fig. 2, but showed an important difference: only the derivative of the 2,3-isomer gave an intense ion at *m/z* 219, evidently representing [M − 57 − 32] as supported by a weak metastable ion at 191.1. This combined loss of a *tert.*-butyl radical and methanol was also noted for the derivative of 13, in which the methoxycarbonyl group was *ortho* to a cyclic silylene moiety, and appears to be usefully diagnostic of this structure.

The natural metabolites 3,4-dihydroxyphenylethane-1,2-diol (29) and the corresponding hydroxy-acid (30) were found to yield bis-DTBS derivatives that had excellent gas chromatographic properties (Fig. 13). In their mass spectra (Fig. 14), side-chain fragmentations took precedence, as in the earlier related examples. For the tetrol derivative, the base peak, [M − 57], and its further fragmentation products, [M − 57 − 42] at *m/z* 351 and [M − 57 − 42 − 18] at *m/z* 333 were typical of DTBS derivatives of saturated diols, as previously described[1]. The bis-DTBS derivative of 3,4-dihydroxymandelic acid (30) likewise gave rise to fragmentations largely of the side-chain moiety. The molecular ion was the base peak, and the ion from initial loss

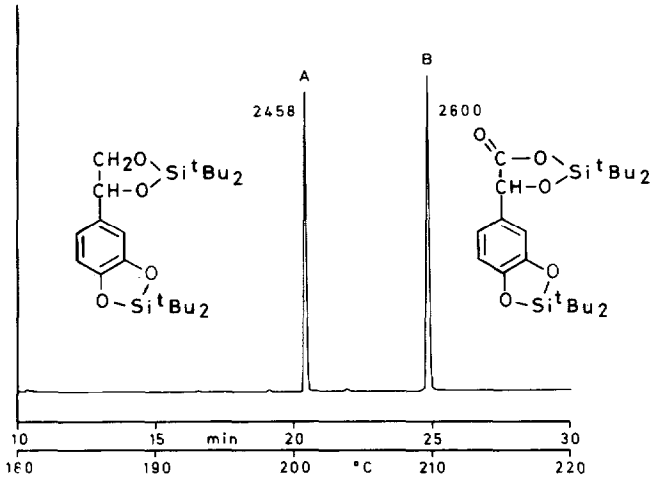


Fig. 13. Gas chromatographic separation of the bis-DTBS derivatives of 3,4-dihydroxyphenylethane-1,2-diol (A) and 3,4-dihydroxymandelic acid (B). Column, SE-54 fused-silica capillary (25 m × 0.32 mm I.D.); column temperature, programmed from 80°C (1 min) to 170°C (1 min) at 30°C/min, and then at 2°C/min to 230°C; helium flow-rate, 3 ml/min. ^{t}Bu = *tert.*-butyl.

of carbon dioxide, at *m/z* 420, was unusually prominent. The formation of [M − 101] by loss of C_4H_9 from [M − 44] was verified by a metastable peak at the expected position (313.7). Initial loss of a *tert.*-butyl radical gave only a minor ion.

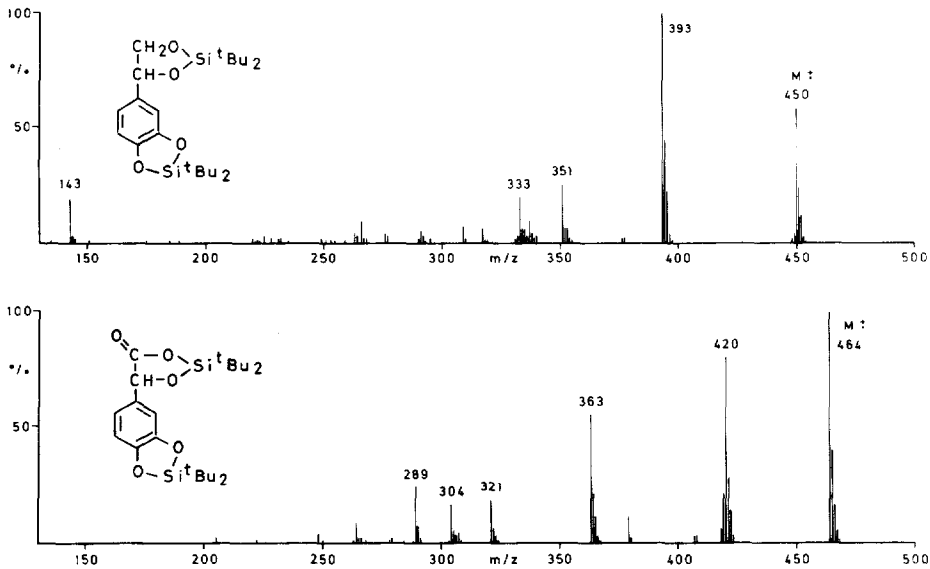


Fig. 14. Mass spectra (22 eV) of the bis-DTBS derivatives of 3,4-dihydroxyphenylethane-1,2-diol (top) and 3,4-dihydroxymandelic acid (bottom). GC–MS conditions as in Fig. 4. ^{t}Bu = *tert.*-butyl.

CONCLUSIONS

This survey has confirmed the value of DTBS derivatives in the following respects.

(i) The derivatives (with ring sizes of five, six or seven) were readily preparable from a wide variety of phenolic acids, hydroxy-acids and diols; compounds containing two such bifunctional groupings formed bis-DTBS derivatives.

(ii) Very good gas chromatographic peaks were observed from all the products.

(iii) Among the substituted salicylic acids, which formed the largest group of parent compounds, separations of isomers by GLC of DTBS derivatives were complete.

(iv) Practically all the mass spectra showed abundant molecular ions; these had intensities of at least 50% of the base peak in more than half of the derivatives studied. Only compound 24 and its trimethylsilyl ether yielded molecular ions of less than 1% abundance from their DTBS derivatives, by reason of an extremely facile side-chain cleavage.

(v) In general, DTBS derivatives of isomers of the types studied here gave very similar mass spectra, differing only in some ion abundances.

Gas chromatographic separations, and correlations of retention data, were normally essential for the differentiation of isomeric derivatives. In some instances, however —notably the DTBS derivatives of methyl 2,3- and 3,4-dihydroxybenzoates— distinctive ions occurred in the mass spectra.

(vi) This survey of model compounds has indicated that DTBS derivatives have potential applications in analytical work on natural metabolites or xenobiotics that contain salicylic acid or other *o*-phenolic acid moieties. Several examples of the ease of formation of bis-DTBS derivatives have also been given. Our characterisation of a group of sesquiterpenoid diols, elicited in tobacco tissue cultures, was greatly aided by their conversion into DTBS derivatives[8,9], and there is clearly a basis for similar applications of DTBS derivatives to hydroxy-acids.

The analogous diethylsilylene (DES) derivatives, introduced earlier by Miyazaki *et al.*[10] have been shown to have valuable properties for the analysis, by GC–MS, of 1,2- and 1,3-diols. These authors have prepared DES derivatives by using the reagent bis-(diethylhydrogen silyl)trifluoroacetamide, which simultaneously converts free hydroxylic groups into diethylhydrogensilyl (DEHS) derivatives. Their method has been successfully applied to steroidal diols and triols[10,11], to corticosteroids of the 17α,21-dihydroxy-20-oxopregnane type[12], and to thromboxane B2[13]. These investigations, together with the studies on DTBS derivatives, and with earlier work cited in our first papers[1,2] on the theme, show that there is scope for a variety of substituted silanes as reagents for GC–MS of bi- and multi-functional substrates.

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