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CHARACTERIZATION OF DIHYDROARENEDIOLS AND RELATED COMPOUNDS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY: COMPARISON OF DERIVATIVES

C. J. W. BROOKS*, W. J. COLE, J. H. BORTHWICK and G. M. BROWN
Chemistry Department, University of Glasgow, Glasgow G12 8QQ (Great Britain)

SUMMARY

Gas chromatographic and mass spectrometric properties are reported for some dihydrodiols and tetrahydrodiols, and for their diacetates, di(trimethylsilyl) ethers and cyclic alkaneboronates. The diols studied include typical metabolites formed via epoxidation of arylalkenes (indene, acenaphthylene) and arenes (anthracene, phenanthrene). The results confirm that trimethylsilylation is the most satisfactory general procedure for the protection of "metabolic" diols and for their analysis by combined gas chromatography–mass spectrometry. The formation of cyclic alkaneboronate esters is practicable from *cis*-diols and (somewhat less readily) from certain *trans*-diols of sufficiently flexible conformations. Where they can be formed, cyclic methaneboronates afford the advantages of short retention times, low mass increments and, generally, informative mass spectra with abundant molecular ions.

INTRODUCTION

One of the most important modes of metabolism of alkenes and arenes is their oxidation (by formal addition of the elements of H_2O_2) to vicinal dihydrodiols. The first such metabolite, 1,2-dihydroanthracene-1,2-diol, was isolated in 1935 from the urine of rats treated with anthracene[1]. The analogous dihydrodiol from naphthalene[2], and three positionally isomeric dihydrophenanthrenediols from phenanthrene (the preponderant metabolite being the 9,10-isomer) were also characterised in early work[2,3]. Typically, the dihydroarenediols of mammalian metabolism have *trans*-configurations[4,5], and Boyland's postulate[6] of the intermediacy of arene epoxides in dihydrodiol formation has been amply confirmed by the extensive studies of the N.I.H. group, following the isolation of 1,2-naphthalene epoxide as a microsomal metabolite of naphthalene[7]. Dihydroarenediols of *cis*-configuration are encountered particularly in the microbial metabolism of aromatic hydrocarbons via the action of dioxygenases[8,9]. Metabolism of the olefinic bond in conjugated benzocycloalkenes affords both *cis*- and *trans*-dihydrodiols, as was first observed for indene[10].

The characterisation of dihydrodiols in extracts of metabolites has been aided by the application of combined gas chromatography–mass spectrometry (GC–MS). Since the free dihydrodiols are susceptible to elimination of the elements of water by

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thermal or catalytic action (especially in the case of dihydroarenediols which thereby afford highly stable phenols), derivatives are generally employed for GC–MS. Trimethylsilyl (TMS) ethers have been used, for example, in the analysis of mixtures of naphthalene metabolites, including the preponderant dihydrodiol, extracted from rat urine after hydrolysis[11]. Dihydrodiols formed by microsomal metabolism of the carcinogen 7,12-dimethylbenz(*a*)anthracene, and separated by high-performance liquid chromatography (HPLC), have also been trimethylsilylated for analysis by GC–MS[12]. Protection of suitably constituted vicinal diols in the form of cyclic derivatives is also possible: thus indane-*cis*-1,2-diol was included in the first study of the value of cyclic boronate derivatives in GC–MS[13], and cyclic methaneboronates have since been applied to the analysis of metabolic dihydrodiols, *e.g.*, from naphthalene[11] and iminostilbene[14].

The aim of this paper is to compare the properties, in respect of GC–MS, of various types of dihydro- and tetrahydrodiols in their free forms and as their acetates, trimethylsilyl ethers and (where practicable) cyclic alkaneboronates.

MATERIALS AND METHODS

Solvents and reagents

Ethyl acetate (Nanograde) was obtained from Mallinckrodt (St. Louis, MO, U.S.A.). Pyridine (AnalaR: BDH, Poole, Great Britain) was dried over KOH pellets and redistilled prior to use. Acetic anhydride (AnalaR) and *p*-toluenesulphonic acid were supplied by BDH. 2,2-Dimethoxypropane (Fluka, Buchs, Switzerland) was obtained from Fluorochem (Glossop, Great Britain). Hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), N,O-bis(trimethylsilyl)acetamide (BSA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and methaneboronic acid were obtained from Pierce and Warriner (Chester, Great Britain). 1-Butaneboronic acid was supplied by Ventron (Karlsruhe, G.F.R.).

Diols and other reference compounds

The compounds are denoted below by the numbers shown in Figs. 1 and 18 (chiral compounds were racemic except for No. 10). Compounds 1, 2, 6 and 7 were available from earlier work[10,15]; compounds 3, 4 and 8–18 were donated by the late Prof. J. D. Loudon and were synthetic samples, with the exception of compound 10, which had been isolated by Prof. E. Boyland from the urine of rabbits dosed with anthracene. Compound 5 was purchased from Alfred Bader Chemicals (Aldrich, Gillingham, Dorset, Great Britain), and compound 19 (Nadolol: SQ 11725) was donated by E. R. Squibb and Sons (Princeton, NJ, U.S.A.).

Gas–liquid chromatography

Gas–liquid chromatography (GLC) was carried out with a Perkin-Elmer (Beaconsfield, Great Britain) F-11 gas chromatograph (used for packed column studies) and a Pye Model 104 gas chromatograph (Pye Unicam, Cambridge, Great Britain) equipped with a falling needle injection system (used for open-tubular GLC). Silanized glass columns (1.8 m × 4 mm I.D.) were packed with 1% OV-1 or 1% OV-17 on Gas-Chrom Q, 100–120 mesh (Pierce and Warriner, Chester, Great Britain); the nitrogen carrier gas flow-rate was 40 ml/min. Open-tubular GLC was performed on a

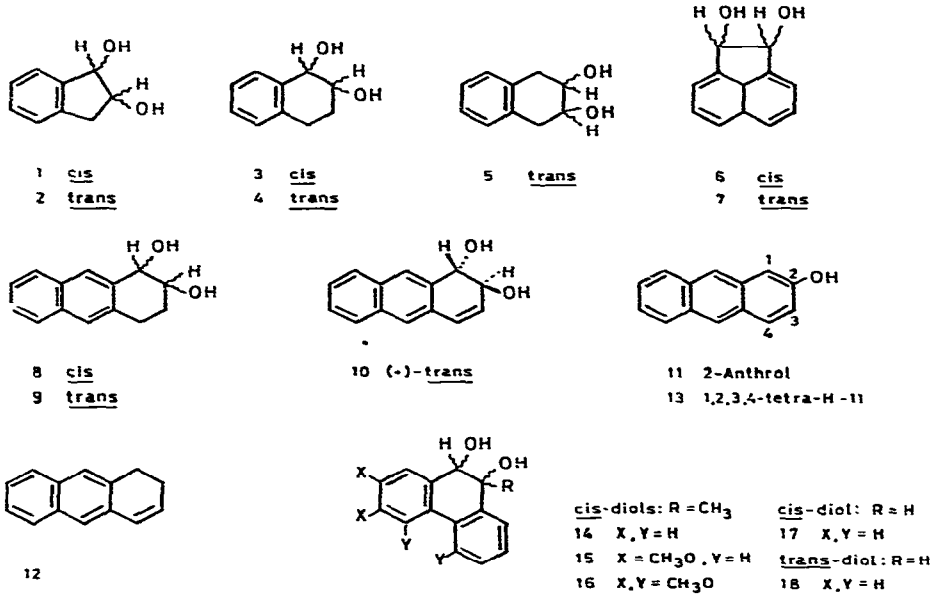

Fig. 1. Structures of dihydrodiols, tetrahydrodiols, and related compounds. 1 = Indane-*cis*-1,2-diol; 2 = indane-*trans*-1,2-diol; 3 = 1,2,3,4-tetrahydronaphthalene-*cis*-1,2-diol; 4 = 1,2,3,4-tetrahydronaphthalene-*trans*-1,2-diol; 5 = 1,2,3,4-tetrahydronaphthalene-*trans*-2,3-diol; 6 = acenaphthene-*cis*-1,2-diol; 7 = acenaphthene-*trans*-1,2-diol; 8 = 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol; 9 = 1,2,3,4-tetrahydroanthracene-*trans*-1,2-diol; 10 = 1,2-dihydroanthracene-*trans*-1,2-diol; 11 = 2-anthrol; 12 = 1,2-dihydroanthracene; 13 = 1,2,3,4-tetrahydroanthracen-2-ol; 14 = 9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol; 15 = 2,3-dimethoxy-9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol; 16 = 2,3,4,5-tetramethoxy-9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol; 17 = 9,10-dihydrophenanthrene-*cis*-9,10-diol; 18 = 9,10-dihydrophenanthrene-*trans*-9,10-diol.

flexible fused silica column (12 m × 0.25 mm I.D.) coated with OV-1 (Hewlett-Packard, Altrincham, Great Britain); the helium carrier gas flow-rate was 2 ml/min. Both instruments employed hydrogen flame-ionization detectors.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was carried out on an LKB 9000 instrument fitted with a silanized glass column (1.8 m × 4 mm I.D.) packed with 1 % OV-1 on Gas-Chrom Q, 100–120 mesh; the helium carrier gas flow-rate was 30 ml/min. Mass spectra (20 eV) were recorded under electron-impact conditions: accelerating voltage, 3.5 kV; trap current, 60 μA; source and separator temperatures, 260°C.

Preparation of derivatives

Methaneboronate esters. Methaneboronic acid (1.1 mol proportion) in dry pyridine was added to the diol (100 μg) (compounds 1–3, 5–8 and 10–17) and the mixture heated at 60°C for 30 min. After removal of solvent under nitrogen the residue was redissolved in ethyl acetate (100 μl) for GLC; compounds 4 and 9 required 2.2 mol proportions of methaneboronic acid to effect complete derivative formation. Compounds 18 and 19 (100 μg), however, were dissolved in 2,2-dimethoxypropane and

TABLE I

KOVÁTS RETENTION INDICES (*I*) AND INCREMENTS FOR DERIVATIVE FORMATION (*ΔI*) FOR INDANE-, TETRAHYDRONAPHTHALENE- AND ACENAPHTHENEDIOLS

Stationary phase	*Derivative*	*Compound no.*													
		1		*2*		*3*		*4*		*5*		*6*		*7*	
		*I***	*ΔI*	*I***	*ΔI*	*I***	*ΔI*	*I***	*ΔI*	*I****	*ΔI*	*I*§*	*ΔI*	*I*§§*	*ΔI*
OV-1	Diol	1400		1440		1515		1535		1550		1790		1830	
	Diacetate (a)*	1610	+210	1610	+170	1710	+195	1705	+170	1730	+180	1990	+200	1975	+145
	Di-TMS (b)	1580	+180	1610	+170	1660	+145	1650	+115	1675	+125	1925	+135	1965	+135
	Me boronate (c)	1295	−105	–	–	1400	−115	1445	−90	1415	−135	1670	−120	–	–
	Bu boronate (d)	1565	+165	–	–	1680***	+165	1730***	+195	1730	+180	1935§§	+145	–	–
OV-17	Diol	1670		1710		1790		1820		1850		2145		2245	
	Diacetate (a)	1890	+220	1880	+170	2005	+215	2010	+190	2030	+180	2345	+200	2335	+90
	Di-TMS (b)	1685	+15	1695	−15	1770	−20	1760	−60	1770	−80	2100	−45	2120	−125
	Me boronate (c)	1500	−170	–	–	1620	−170	1640	−180	1640	−210	1965	−180	–	–
	Bu boronate (d)	1780	+110	–	–	1920***	+130	1950***	+130	1950	+100	2260§§	+115	–	–

* Letters denote derivative types.
** 120°C.
*** 140°C.
§ 150°C.
§§ 170°C.

heated at 60°C for 30 min in the presence of methaneboronic acid (2.2 mol proportion). The resultant solution was diluted with 2,2-dimethoxypropane (40 μl) and used directly for GLC analysis (1 μl).

n-Butaneboronate esters. Compounds 1–3, 5–8 and 10–17 (100 μg) were treated with *n*-butaneboronic acid (1.1 mol proportion) and heated at 60°C for 30 min. After removal of solvent under nitrogen, the residue was redissolved in ethyl acetate (100 μl) and used for GLC (1 μl). Compounds 4, 9 and 18, however, required 2.2 mol proportions of the boronic acid for complete derivative formation.

Trimethylsilyl ethers. For non-hindered hydroxyl groups, the compound (100 μg) was dissolved in BSA (BSTFA for compound 19) and heated at 60°C for 30 min. The reagent was removed in a stream of nitrogen and the product redissolved in EtOAc (100 μl), an aliquot (1 μl) being used for GLC. For hindered hydroxyl groups, the compound (100 μg) was heated with BSA–HMDS–TMCS (10:10:5 μl) in a Reactivial at 90°C for 24 h. After solvent removal the residue was taken up in ethyl acetate (100 μl) and analysed by GLC (1 μl).

Acetylation. Compounds 1–15, 17 and 18 (100 μg) were treated with acetic anhydride (20 μl) and pyridine (10 μl) and heated at 60°C for 30 min. After reagent removal under nitrogen the product was taken up in ethyl acetate (100 μl) and used for GLC (1 μl). Compound 16 required heating for 24 h at 90°C together with the reagents in a Reactivial to effect full derivative formation. Nadolol (100 μg) required heating with the reagents at 60°C for 48 h in the presence of *p*-toluenesulphonic acid as catalyst to effect complete acetylation.

RESULTS

The compounds studied are grouped (*cf.* Fig. 1) broadly in order of molecular size. Unless otherwise stated, diacetates and di-TMS ethers were readily formed from the diols. The comments on derivative formation therefore refer chiefly to the more selective process of cyclic boronate preparation.

Indanediols, tetrahydronaphthalenediols and acenaphthenediols (compounds 1–7)

The conformational rigidity of substituents in the five-membered rings of indane and acenaphthene renders the *trans*-diols incapable of forming simple cyclic esters, but leads to the ready formation from the *cis*-diols of exceptionally stable cyclic boronates[13]. In the case of the six-membered ring diols (compounds 3–5) derived from tetrahydronaphthalenes, cyclic boronates were afforded by the 1,2-*cis*-diol, by the 2,3-*trans*-diol and (incompletely) by the 1,2-*trans*-diol. It is well known that both *cis*- and *trans*-cyclohexane-1,2-diol yield cyclic boronates[16], but the virtually planar ring-fused portions of the cyclohexenoids 3–5 make it difficult for the *trans*-diols (especially at the 1,2-position) to accommodate the conformations required in cyclic ester formation.

Gas chromatographic data for derivatives of diols 1–7 are cited in Table I. The free diols usually gave unsatisfactory "tailing" peaks, but their retention index values are included to facilitate consideration of the increments attending derivative formation. The most noteworthy feature is the marked decrement in retention time ($\Delta I \approx -120$), on the OV-1 phase, for the conversion of a diol to its cyclic methaneboronate. The satisfactory nature of the latter derivative for acenaphthene-*cis*-1,2-diol is il-

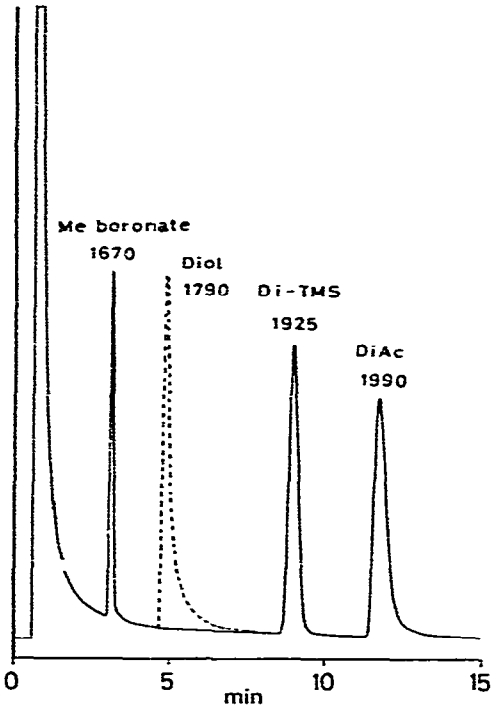

Fig. 2. Gas chromatographic separation of the methaneboronate (6c) di-TMS ether (6b) and diacetate (6a) of acenaphthene-*cis*-1,2-diol (with the peak obtained separately for the free diol superimposed). Column, 1% OV-1 (1.8 m × 4 mm I.D.); column temperature, 150°C; nitrogen flow-rate, 40 ml/min.

lustrated by the gas chromatogram in Fig. 2: the sequence of elution of the di-TMS ether and diacetate derivatives is typical of the whole group of diols.

Some salient features of the mass spectra of diols 1–7 and their derivatives are summarised in Table II. It is clear that the di-TMS ethers, which are the most generally useful derivatives for GLC, yield satisfactory molecular ions only for the indane and acenaphthene diols, while only one of the diacetates in the group affords an abundant molecular ion, because of the preponderance of eliminations of acetic acid and ketene. The methaneboronates yield abundant molecular ions, which are a little less prominent in butaneboronates: here the larger alkyl group offers additional pathways of fragmentation, and also appears to promote readier loss of the elements of alkaneboronic acid —a process affording the base peaks of m/z ($M-100$) from the butaneboronates of indanediol[13] and acenaphthenediol. The fragmentations of typical derivatives are further illustrated with respect to acenaphthene-*cis*-1,2-diol (6) in Figs. 3 and 4. Each spectrum contains a clear molecular ion (preponderant only in the methaneboronate) and an ion of m/z 168 formally representing the acenaphthenone or epoxyacenaphthene ion. A fragment of m/z 152 (acenaphthylene ion) resulting from loss of both oxygen substituents is prominent only in the free diol spectrum. The relatively lower abundance of molecular ions in the spectra of the diacetate and di-TMS ether of the *trans*-diol (7) (*cf.* Table II) is of diagnostic and mechanistic interest: both the loss of Me_3SiOH by *cis*-elimination to give ($M-90$) and, more unexpect-

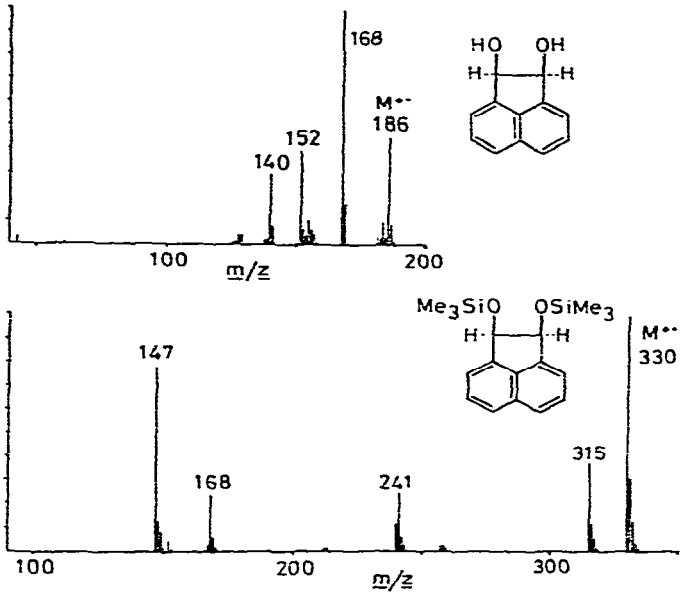

Fig. 3. Mass spectra (20 eV) of acenaphthene-*cis*-1,2-diol (6) and di-TMS ether (6b) measured on an LKB 9000 gas chromatograph–mass spectrometer. Column, 1% OV-1 (1.8 m × 4 mm I.D.); column temperatures as in Table I; helium flow-rate, 30 ml/min; accelerating voltage, 3.5 kV; source temperature, 260°C; trap current, 60 μA.

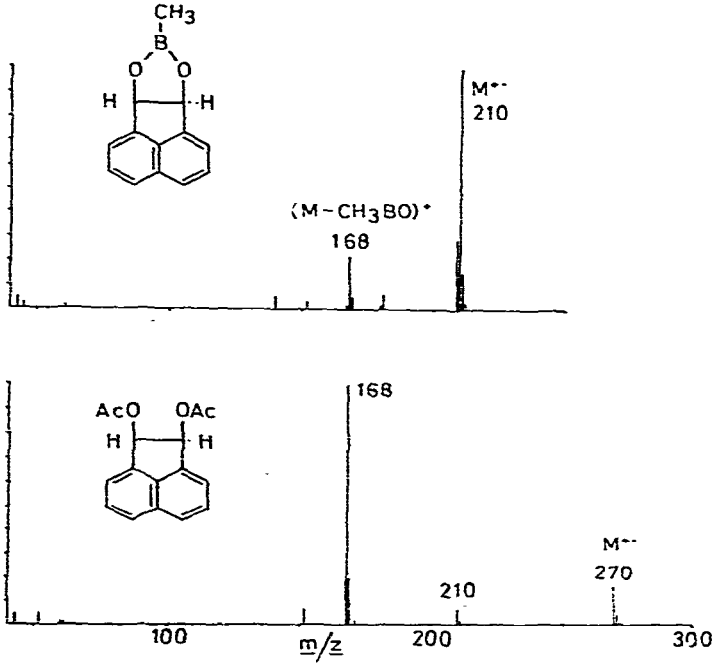

Fig. 4. Mass spectra (20 eV) of acenaphthene-*cis*-1,2-diol methaneboronate (6c) and diacetate (6a). GC–MS conditions as in Fig. 3.

TABLE II

BASE PEAKS AND RELATIVE ABUNDANCE OF MOLECULAR IONS IN THE MASS SPECTRA OF INDANEDIOLS, TETRAHYDRONAPHTHALENEDIOLS, ACENAPHTHENEDIOLS AND THEIR DERIVATIVES

Diol	*Free*			*Diacetate*			*Di-TMS ether*			*Me boronate*			*Bu boronate*		
	M		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*
	m/z	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*
1	150	5	104 ($M-46$)	234	0	132 ($M-102$)	294	62	147*	174	100	174 (M)	216	7	116 ($M-100$)
2	150	6	104 ($M-46$)	234	0	132 ($M-102$)	294	17	147*	Not formed			Not formed		
3	164	0	146 ($M-18$)	248	0	146 ($M-102$)	308	0	192 ($M-116$)	188	95	128 ($M-60$)	230	20	128 ($M-102$)
4	164	5	104 ($M-60$)	248	0	146 ($M-102$)	308	0	192 ($M-116$)	188	29	160 ($M-28$)	230	25	202 ($M-28$)
5	164	24	146 ($M-18$)	248	0	128 ($M-120$)	308	1	218 ($M-90$)	188	100	188 (M)	230	40	139 ($M-91$)
6	186	60	168 ($M-18$)	270	15	168 ($M-102$)	330	100	330 (M)	210	100	210 (M)	252	50	152 ($M-100$)
7	186	7	168 ($M-18$)	270	0.2	168 ($M-102$)	330	26	147*	Not formed			Not formed		

* Ion $Me_2Si{=}\dot{O}SiMe_3$, typical of diol di-TMS ethers.

edly, the loss of $Me_3SiOSiMe_3$ to give $(M-162)$ appear to be enhanced in the *trans*-diol derivative as compared with the *cis*-isomer.

Figs. 5 and 6 provide a simple demonstration of the complementary value of di-TMS ethers and cyclic boronate esters in structural diagnosis. Both derivatives easily distinguish the tetrahydronaphthalene *cis*-1,2- and *trans*-2,3-isomers; however, for the former isomer (3) the *retro*-Diels–Alder fragment of *m/z* 192, strongly promoted by the dual α-cleavage of the di-TMS ether, is characteristic. The cyclic boronate group bridging the 1,2-positions suppresses the *retro*-Diels–Alder fragmentation. Conversely, the parent 2,3-diol structure (5) is indicated much more clearly from its methaneboronate spectrum (Fig. 6), by a pair of ions of *m/z* 91 ($C_7H_7^+$) and 97 ($C_4H_6BO_2^+$) formed by cleavage of the tetrahydro ring. The *retro*-Diels–Alder ion of *m/z* 104 is also more prominent in the latter spectrum than in that of the corresponding di-TMS ether.

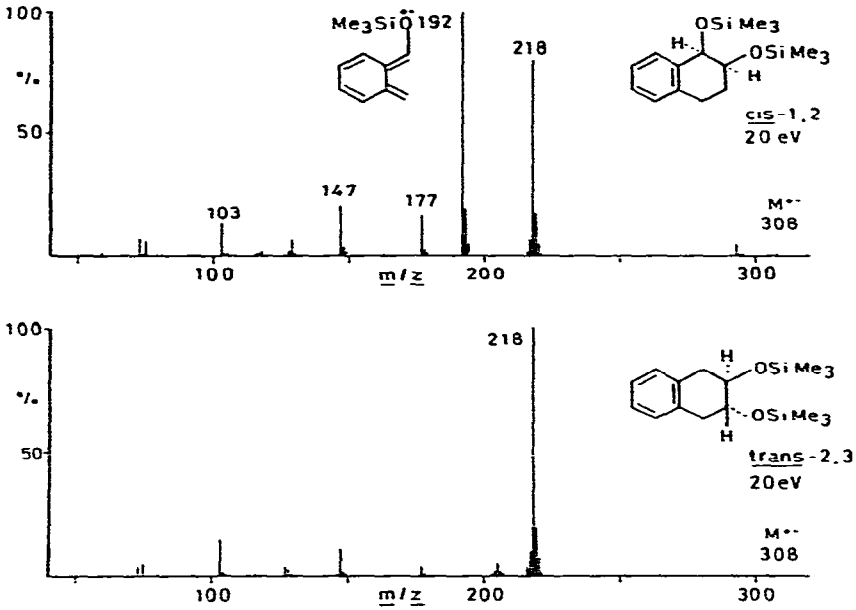

Fig. 5. Mass spectra (20 eV) of 1,2,3,4-tetrahydronaphthalene-*cis*-1,2-diol di-TMS ether (3b) and 1,2,3,4-tetrahydronaphthalene-*trans*-2,3-diol di-TMS ether (5b). GC–MS conditions as in Fig. 3.

Tetrahydroanthracenediols and a dihydroanthracenediol (compounds 8–10)

The tetrahydroanthracene-*cis*- and *trans*-1,2-diols, like their naphthalene analogues, both formed cyclic boronates, the *trans*-esters being produced incompletely even with a considerable excess of reagent. It proved impracticable to form a cyclic boronate from the cyclohexadienediol grouping in the anthracene metabolite, 1,2-dihydroanthracene-*trans*-1,2-diol (10). Inspection of molecular models suggests that such a cyclic ester would be highly strained (*cf.* however, the boronates of compound 18, described below).

In the tetrahydro series, the methaneboronates have the shortest retention times on OV-1 phase, as illustrated for the *cis*-diol in Fig. 7. [The additional small

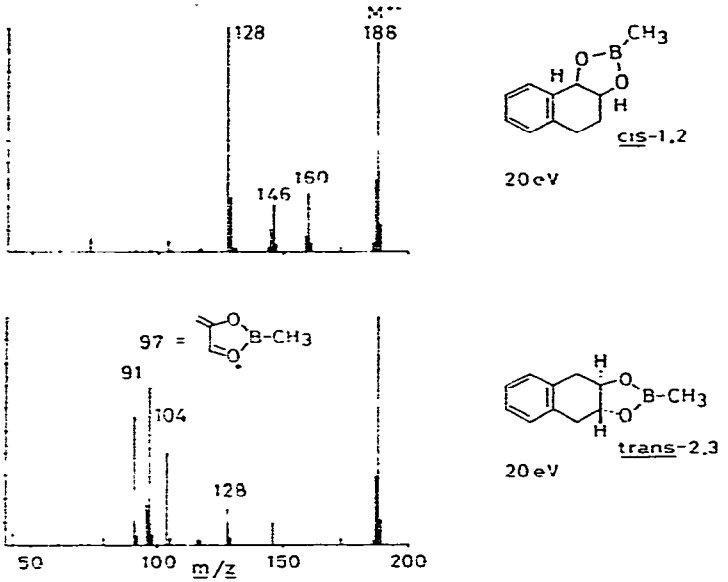

Fig. 6. Mass spectra (20 eV) of 1,2,3,4-tetrahydronaphthalene-*cis*-1,2-diol methaneboronate (3c) and 1,2,3,4-tetrahydronaphthalene-*trans*-2,3-diol methaneboronate (5c). GC–MS conditions as in Fig. 3.

peaks appear to have arisen from an impurity of octahydroanthracenediol present in the parent diol.] GLC data summarised in Table III show that the cyclic boronates afford the largest differences in retention index between the *cis*- and *trans*-tetrahydrodiols, an effect attributable to the fixation of distinctive conformations by ring formation. The stability of the metabolic diol, 1,2-dihydroanthracene-*trans*-1,2-diol, towards GLC was confirmed by comparison with its dehydration product, 2-anthrol (11) and their derivatives. The data included for tetrahydroanthracen-2-ol (13) show

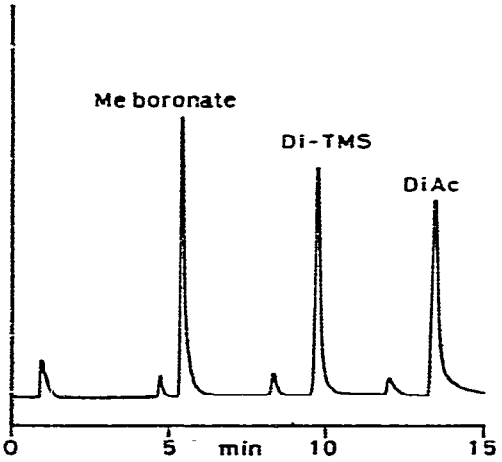

Fig. 7. Gas chromatographic separation of the methaneboronate (8c), di-TMS ether (8b) and diacetate (8a) of 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol. Column, WCOT OV-1 (12 m × 0.25 mm I.D.); column temperature, 190°C; helium flow-rate, 2 ml/min.

TABLE III

KOVÁTS RETENTION INDICES (*I*) FOR TETRAHYDRO- AND DIHYDROANTHRACENEDIOLS AND DERIVATIVES, AND RELATED COMPOUNDS

Stationary phase	*Derivative*	*Compound no.*		
		*8***	*9***	*10****
OV-1	Diol	2085	2095	2135
	Diacetate (a)*	2250****	2250****	2255
	Di-TMS (b)	2150	2140	2215
	Me boronate (c)	1960	1990	–
	Bu boronate (d)	2235	2290	–
OV-17	Diol	2540§	2550§	§§§
	Diacetate (a)	2700§§	2690§§	§§§
	Di-TMS (b)	2340§	2330§	§§§
	Me boronate (c)	2290	2310	–
	Bu boronate (d)	2570	2615	–
		Compound no.		
		*11***	*12*†*	*13***
OV-1	Parent compound	2100	1700	1905
	Acetate (e)	2165	–	2035
	TMS (f)	2135	–	2000
OV-17	Parent compound	2485	1955	2300††
	Acetate (e)	2520	–	2410††
	TMS (f)	2390	–	2230††

* Letters denote derivative types.
** 175°C.
*** 200°C.
§ 210°C.
§§ 230°C.
§§§ Not examined.
† 150°C.
†† 190°C.

that the retention increments for introduction of the 1-hydroxy group are of the expected order ($\Delta I \approx 180$ on OV-1 phase).

The base peaks and molecular ion abundances in the mass spectra of diols 8–10 and their derivatives are summarised in Table IV. Mass spectra of the tetrahydroanthracenediol series, shown in Figs. 8–11, are of limited value in differentiating between the *cis*- and *trans*-isomers. The free diols (Fig. 8) give strong $[M]^{+\cdot}$ and $([M-H_2O]^{+\cdot})$ ions, together with $[M-44]^{+\cdot}$ (loss of vinyl alcohol by a *retro*-Diels–Adler process) and $[M-60]^{+\cdot}$ (m/z 154.0785 from diol 8) resulting from elimination of ethenediol (C-1 + C-2). The mass spectra of the diacetates (Fig. 9) are dominated by ions resulting from losses of acetic acid and ketene. In both the free diols and diacetates, the relative intensities of the common ions, though different for the *cis*- and *trans*-isomers, are of little discriminatory value in view of the similar retention times.

TABLE IV

BASE PEAKS AND RELATIVE ABUNDANCE OF MOLECULAR IONS IN THE MASS SPECTRA OF DIHYDRO- AND TETRAHYDROANTHRACENEDIOLS AND THEIR DERIVATIVES

Diol	*Free*			*Diacetate*			*Di-TMS ether*			*Me boronate*			*Bu boronate*		
	M		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*
	m/z	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*
8	214	95	196 ($M - 18$)	298	13	178 ($M - 120$)	358	10	268 ($M - 90$)	238	75	178 ($M - 60$)	280	47	178 ($M - 102$)
9	214	100	214 (M)	298	3	196 ($M - 102$)	358	11	268 ($M - 90$)	238	100	238 (M)	280	100	280 (M)
10	212	1	194 ($M - 18$)	296	0.2	194 ($M - 102$)	356	7	191 ($M - 165$)	Not formed			Not formed		

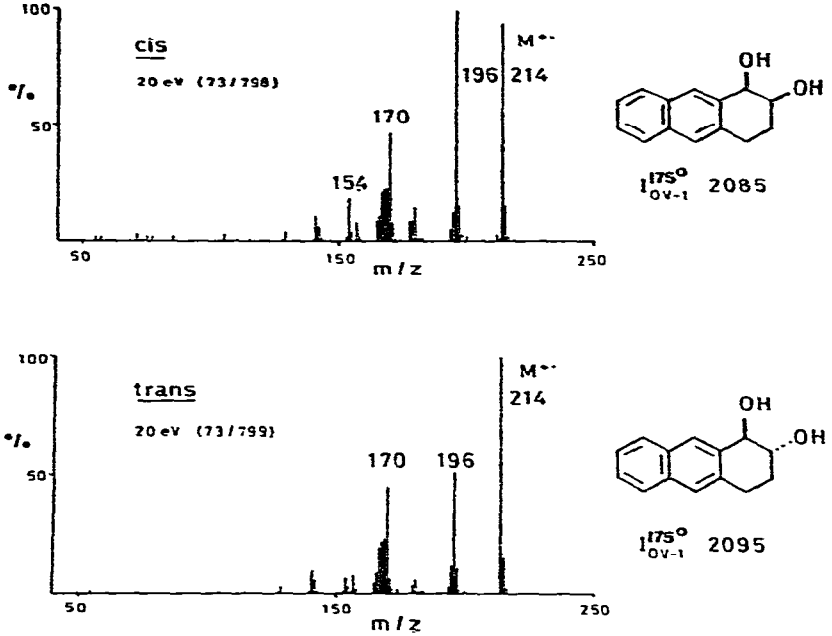

Fig. 8. Mass spectra (20 eV) of 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol (8) and -*trans*-1,2-diol (9). GC–MS conditions as in Fig. 3, except that column temperatures were as in Table III.

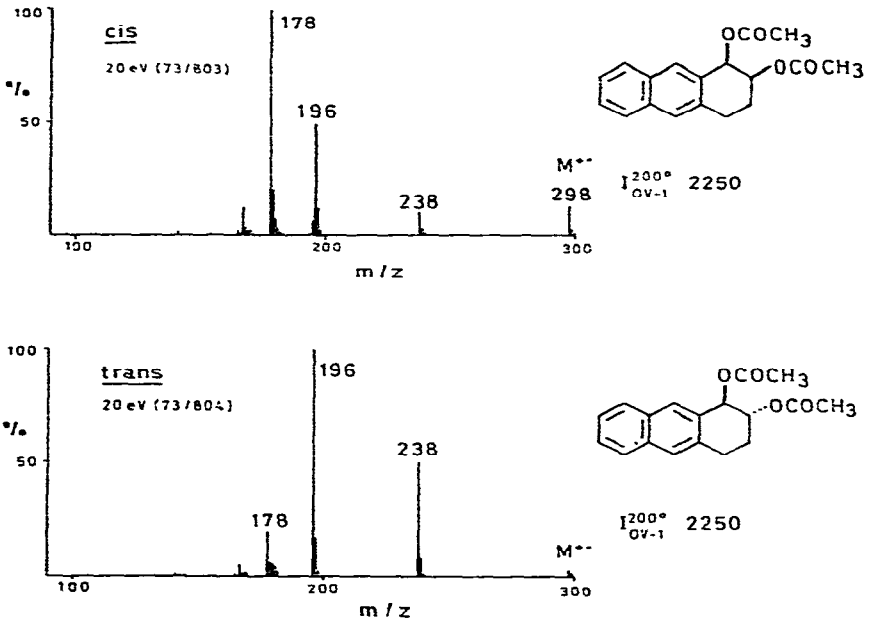

Fig. 9. Mass spectra (20 eV) of 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol diacetate (8a) and -*trans*-1,2-diol diacetate (9a). GC–MS conditions as in Fig. 8.

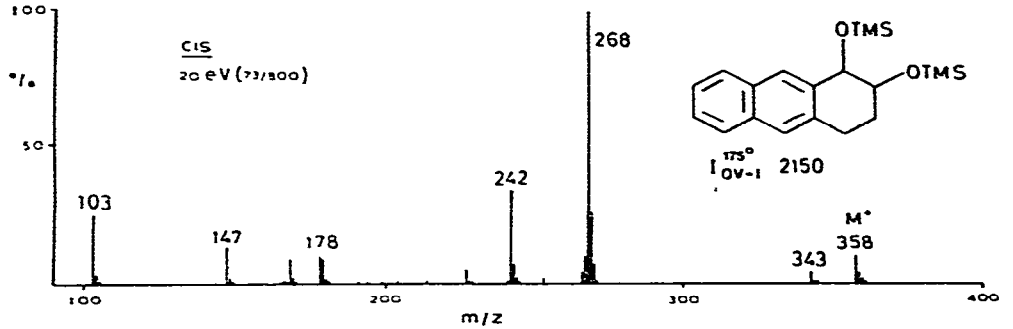

Fig. 10. Mass spectrum (20 eV) of 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol di-TMS ether (8b). GC–MS conditions as in Fig. 8.

In the case of the di-TMS ethers, the mass spectra are virtually identical, and that of the *cis*-diol derivative (Fig. 10) is sufficiently depictive of both. As in the naphthalene analogue (Fig. 5), major peaks are due to a *retro*-Diels–Alder process and to the elimination of trimethylsilanol moieties, while "rearrangement" ions of *m/z* 147 and 103 appear in the lower mass range. Finally, the spectra of the methaneboronates, shown in Fig. 11, are notable for the ions $[M-28]^{+\cdot}$ which are distinctly more abundant in the *trans*-isomer spectrum then in the *cis*: this regularity holds too for the butaneboronates, and is even more marked for the corresponding tetrahydronaphthalenediols (*cf.* Table II). Accurate mass measurement shows the ion $[M-28]^{+\cdot}$ to result from loss of C_2H_4, doubtless from C-3 + C-4: presumably the greater strain in the tetrahydro rings of cyclic boronates derived from *trans*-diols leads to a readier elimination of ethene resulting formally in a benzo- or naphthocyclobutenediol bo-

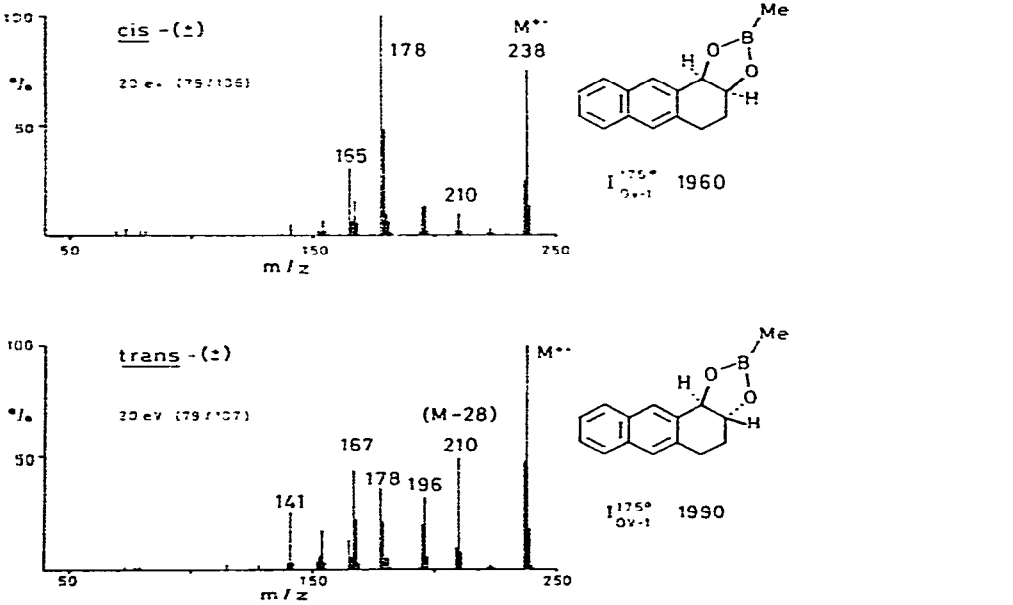

Fig. 11. Mass spectra (20 eV) of 1,2,3,4-tetrahydroanthracene-*cis*-1,2-diol methaneboronate (8c) and -*trans*-1,2-diol methaneboronate (9c). GC–MS conditions as in Fig. 8.

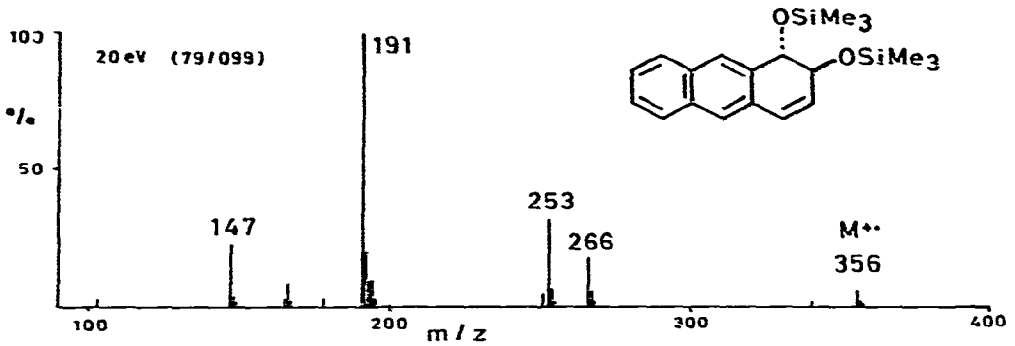

Fig. 12. Mass spectrum (20 eV) of 1,2-dihydroanthracene-*trans*-1,2-diol di-TMS ether (10b). GC–MS conditions as in Fig. 8.

ronate ion. A second regular distinction between the *cis*- and *trans*-tetrahydrodiol boronate spectra is the greater abundance of $[M-RBO_2H_2]^{+\cdot}$ ions from the *cis*-isomers.

The mass spectrum of 1,2-dihydroanthracene-*trans*-1,2-diol di-TMS ether (10b) shown in Fig. 12 includes clear, though small, $[M]^{+\cdot}$ and $[M-15]^{+\cdot}$ ions, together with the typical rearrangement ions of *m/z* 147 ($Me_3SiO{=}SiMe_2$), 191 ($MeSiOCH{=}OSiMe_3$) and 253 ($[M-CH_2OSiMe_3]^+$).

Dihydrophenanthrenediols (compounds 14–18)

Preparation of derivatives, including cyclic boronates, from the four *cis*-diols

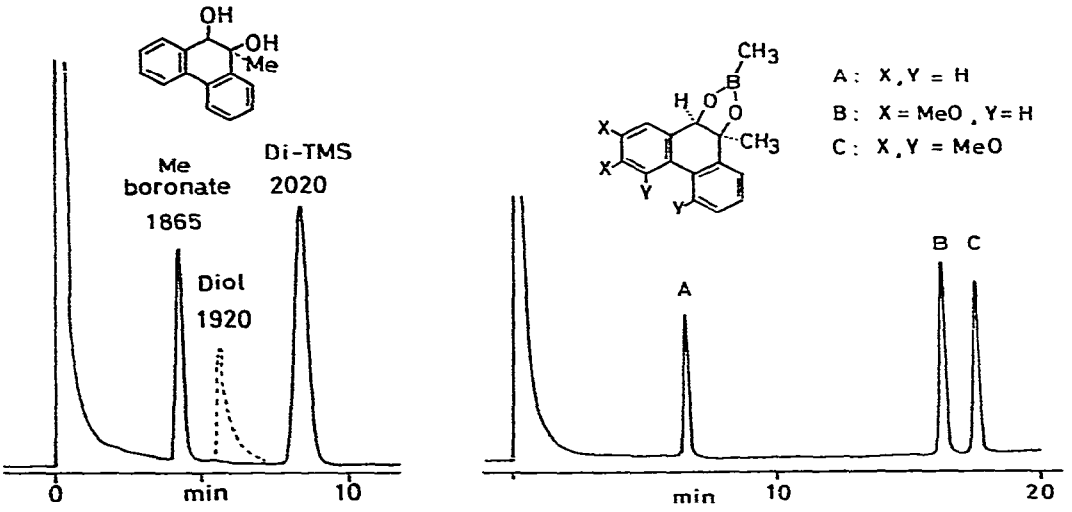

Fig. 13. Gas chromatographic separation of the methaneboronate (14c) and di-TMS ether (14b) of 9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol (with the peak obtained separately for the free diol superimposed). Column, 1% OV-1 (1.5 m × 4 mm I.D.); column temperature, 180°C; nitrogen flow-rate, 40 ml/min.

Fig. 14. Gas chromatographic separation of methaneboronates (14c, 15c and 16c, respectively) of 9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol and its 2,3-dimethoxy- and 2,3,4,5-tetramethoxy-derivatives. Column, 1% OV-17 (1.8 m × 4 mm I.D.); column temperature, programmed from 165 to 250°C at 4°C/min; nitrogen flow-rate, 40 ml/min.

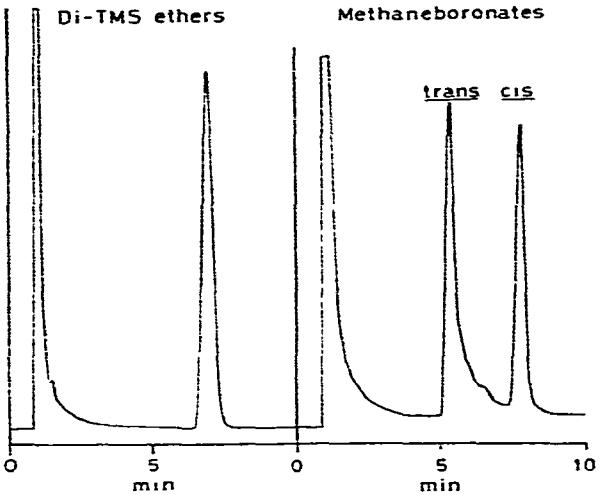

Fig. 15. Gas chromatograms of derivatives of 9,10-dihydrophenanthrene-*cis*- and -*trans*-9,10-diol: (i), di-TMS ethers (17b, 18b); (ii), methaneboronates (17c, 18c). Column, 1% OV-17 (1.8 m × 4 mm I.D.); column temperature, 180°C; nitrogen flow-rate, 40 ml/min.

(14–17), proceeded without difficulty. Formation of cyclic boronates from the *trans*-diol (18) required the use of 2.2 molar proportions of reagent (this diol was early reported not to yield an acetonide under conditions suitable for the corresponding derivative of the *cis*-diol[17]).

Gas chromatograms (Figs. 13–15) exemplify the utility of the methaneboronates in this series. The invariably lower retention indices (on OV-1) of these deriva-

TABLE V

KOVÁTS RETENTION INDICES (*I*) FOR DIHYDROPHENANTHRENE *cis*- AND *trans*-DIOLS AND DERIVATIVES

Stationary phase	*Derivative*	*Compound no.*				
		*14***	*15****	*16****	*17***	*18***
OV-1	Diol	1920	2350	2460	1980	2020
	Diacetate (a)*	2200	2630	2735	2120	2115
	Di-TMS (b)	2020	2360	2440	2015	2020
	Me boronate (c)	1865	2285	2340	1920	1880
	Bu boronate (d)	2125****	2540§	2575§	2180	2165
OV-17	Diol	2275	2815	2930	2365	2430
	Diacetate (a)	2570	3100	3245	2525	2535
	Di-TMS (b)	2195	2625	2725	2225	2230
	Me boronate (c)	2170	2710	2780	2260	2155
	Bu boronate (d)	2455****	2985§	3045§	2525	2450

* Letters denote derivative types.
** 180°C.
*** 200°C.
§ 220°C.

tives than those of the parent diols are illustrated for the 9-methyldiol in Fig. 13. The analogous 2,3-dimethoxy- and 2,3,4,5-tetramethoxy-9-methyldiols are equally conveniently studied as methaneboronates, which give very satisfactory peaks, even on a packed column (Fig. 14). With respect to the distinction between *cis*- and *trans*-isomers, Fig. 15 shows that the di-TMS ether peaks are unresolved under conditions whereby the methaneboronates are clearly separated, the *trans*-isomer being eluted first. The greater degree of tailing of the *trans*-diol ester peak reflects the strained nature of the cyclohexadienediol boronate ring, which probably undergoes partial cleavage and reclosure during GLC: the small additional peak may be due to a pyroboronate derivative, but the mass spectrum provided no evidence thereof. Gas chromatographic retention indices for all the derivatives of diols 14–18 on OV-1 and OV-17 phases are listed in Table V.

The mass spectra of all the 9,10-dihydrophenanthrene-9,10-diols and derivatives studied (Table VI) are noteworthy for the general occurrence of molecular ions (absent only for the diacetates of compounds 14 and 15) so that, for example, the di-TMS ethers of this group of diols are quite satisfactory for defining molecular masses. The base peaks from all the diacetates correspond to the phenanthrol fragments arising from elimination of acetic acid and ketene ($[M-102]^{+\cdot}$). Nine out of the ten cyclic boronates listed yield molecular ions as base peaks: in the exceptional case (compound 14d) the abundance of $[M]^{+\cdot}$ is 76% of a base peak ascribable to the fluorenyl ion (*m/z* 165) resulting from rearrangements and loss of the boronate group together with a two-carbon fragment.

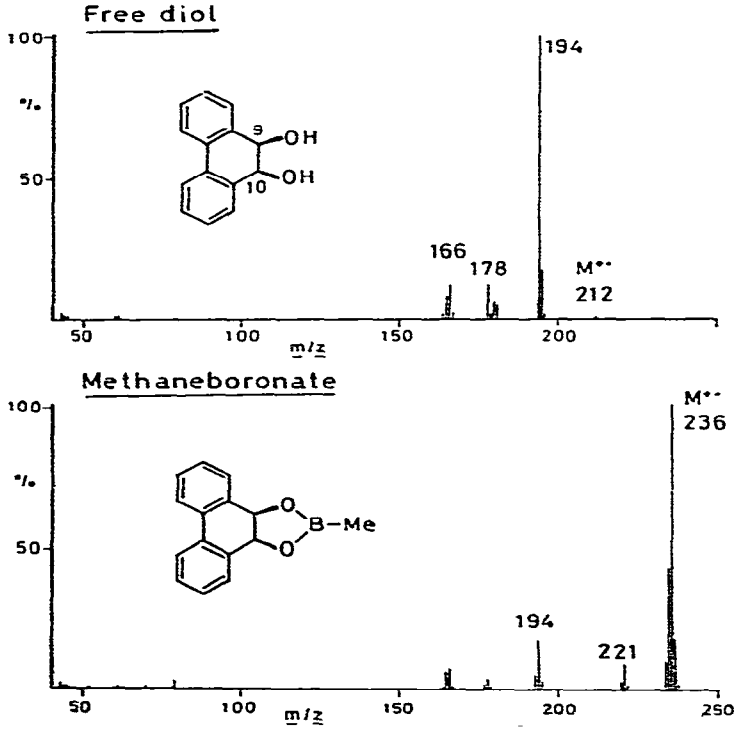

Fig. 16. Mass spectra (20 eV) of 9,10-dihydrophenanthrene-*cis*-9,10-diol (17) and its methaneboronate (17c). GC–MS conditions as in Fig. 3, except that column temperature was 180°C.

TABLE VI

BASE PEAKS AND RELATIVE ABUNDANCE OF MOLECULAR IONS IN THE MASS SPECTRA OF 9,10-DIHYDROPHENANTHRENEDIOLS AND THEIR DERIVATIVES

Diol	*Free*			*Diacetate*			*Di-TMS ether*			*Me boronate*			*Bu boronate*		
	M		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*	*M*		*Base peak*
	m/z	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*	*m/z*	%	*m/z*
14	226	100	226 (*M*)	310	0	208 (*M* − 102)	370	67	267 (*M* − 103)	250	100	250 (*M*)	292	76	165 (*M* − 127)
15	286	26	268 (*M* − 18)	370	0	268 (*M* − 102)	430	100	430 (*M*)	310	100	310 (*M*)	352	100	352 (*M*)
16	346	22	328 (*M* − 18)	430	5	328 (*M* − 102)	490	100	490 (*M*)	370	100	370 (*M*)	412	100	412 (*M*)
17	212	1	194 (*M* − 18)	296	5	194 (*M* − 102)	356	22	147*	236	100	236 (*M*)	278	100	278 (*M*)
18	212	6	196 (*M* − 18)	296	0.5	194 (*M* − 102)	356	28	147*	236	100	236 (*M*)	278	100	278 (*M*)

* Ion $Me_2Si{=}\overset{+}{O}SiMe_3$.

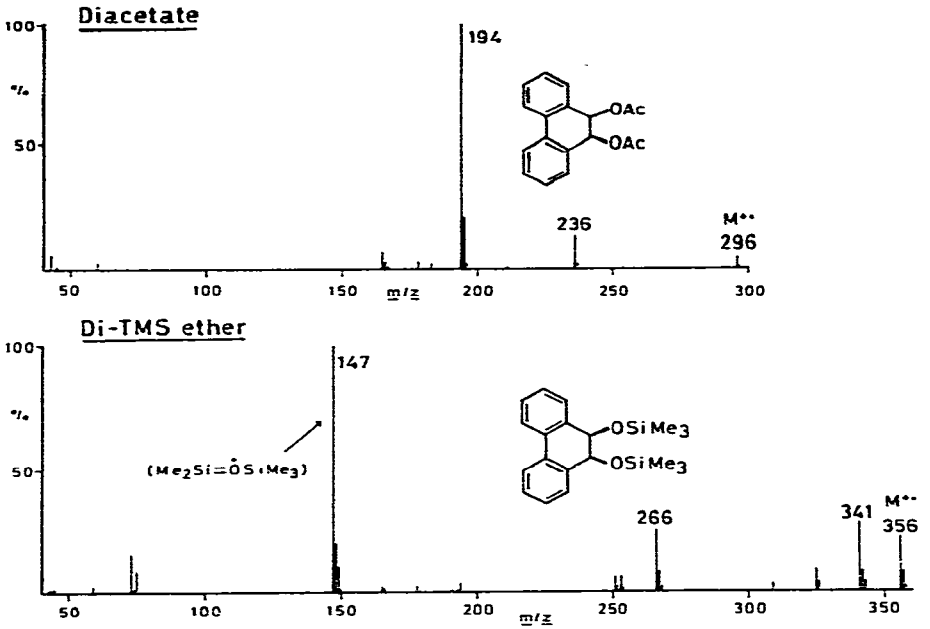

Fig. 17. Mass spectra (20 eV) of 9,10-dihydrophenanthrene-*cis*-9,10-diol diacetate (17a) and di-TMS ether (17b). GC–MS conditions as in Fig. 16.

Full mass spectra are shown in Figs. 16 and 17 for 9,10-dihydrophenanthrene-*cis*-9,10-diol (17) and three of its derivatives. [The spectra of the *trans*-series are very similar, emphasizing the importance of a chromatographic distinction as noted above.] Elimination of H_2O_2 to give a phenanthrene ion (*m/z* 178) is marked in the free diol spectrum, which also contains *m/z* 194 ($[M-H_2O]^{+\cdot}$) as base peak, and ions of fluorene and fluorenyl character (*m/z* 166, 165). In the methaneboronate (Fig. 16) the preferred fragmentation via loss of MeBO again yields a phenanthrol-type ion of *m/z* 194. Fig. 17 shows the preponderance of the similar ion in the diacetate spectrum, and the occurrence of the corresponding phenanthrol TMS fragment (*m/z* 266) in the mass spectrum of the di-TMS ether: the latter spectrum is, however, dominated by the rearrangement ion of *m/z* 147.

Nadolol (Fig. 18)

During the course of our work, the compound Nadolol (19) was introduced by E. R. Squibb & Sons as an antihypertensive agent. As it was unusual among drugs in

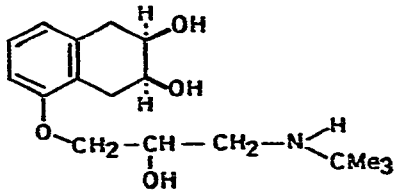

Fig. 18. Structure of Nadolol [5-(3-*tert.*-butylamino-2-hydroxypropoxy)-1,2,3,4-tetrahydronaphthalene-*cis*-2,3-diol] (19).

TABLE VII

KOVÁTS RETENTION INDICES (*I*) AND MASS SPECTROMETRIC DATA (20 eV) FOR NADOLOL (19) DERIVATIVES

Derivative	$I^{OV\text{-}1}_{225°C}$	$I^{OV\text{-}17}_{225°C}$	*M*	*Base peak*	*Other principal ions: m/z (intensities relative to base peak in parentheses)*						
Tetraacetate	3030	3005	477 (-)	98	420 (2)	417 (4)	403 (7)	361 (7)	343 (3)	301 (67)	259 (13)
					241 (67)	227 (9)	214 (52)	200 (81)	183 (20)	160 (52)	158 (91)
					144 (95)	128 (14)	100 (25)	84 (24)	72 (25)	56 (91)	
Tri-TMS ether	2505	2605	525 (0.3)	86	510 (1)	409 (1)	309 (0.3)	307 (0.2)	283 (0.2)	250 (0.3)	232 (0.3)
					217 (0.2)	203 (0.3)	184 (0.5)	144 (0.5)	128 (0.5)	116 (0.3)	112 (0.3)
					107 (0.3)	103 (0.6)					
Bismethaneboronate	2355	2680	357 (9)	342	300 (3)	289 (1)	246 (2)	244 (1)	217 (2)	204 (12)	162 (2)
					154 (3)	144 (5)	138 (5)	120 (20)	112 (7)	98 (8)	96 (5)
					86 (40)	70 (9)	57 (20)	56 (22)			

possessing a tetrahydronaphthalene-*cis*-2,3-diol group, some derivatives were examined as outlined below. Acid-catalysed acetylation afforded the N,O,O,O-tetraacetate, whereas the product of trimethylsilylation appeared to be the O,O,O-tri-TMS ether, the sterically hindered NH-*tert.*-Bu group remaining unreacted. As expected, the reaction with methaneboronic acid, which is hardly subject to steric hindrance, occurred with both the 2,3-*cis*-diol and side-chain β-hydroxyamine groups, although the unfavourable equilibria known to prevail in the formation of 1,3,2-oxazaborolidines made it necessary to use 2,2-dimethoxypropane as a scavenger of water produced in the reaction.

Gas chromatographic and mass spectrometric data for the three Nadolol derivatives are collected in Table VII. The tetraacetate had inconveniently long retention times, but the bismethaneboronate and tri-TMS ether were both satisfactory for GLC, as exemplified in Fig. 19.

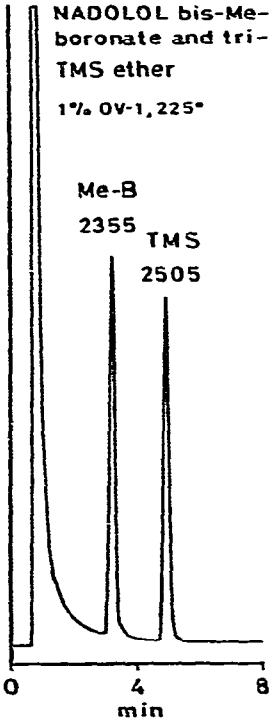

Fig. 19. Gas chromatographic separation of Nadolol bismethaneboronate (19c) and tri-TMS ether (19b). Column, 1% OV-1 (1.8 m × 4 mm I.D.). Column temperature, 225°C; nitrogen flow-rate, 40 ml/min.

Strikingly different mass spectra were observed for the three Nadolol derivatives. The tri-TMS ether spectrum (Table VII)[28] contained as base peak the ion from α-cleavage, $CH_2=NH$-*tert.*-Bu (*m/z* 86), with no other fragment ions at more than 1% relative abundance. The ion of *m/z* 86 has been used in the determination of serum Nadolol[28]. The bismethaneboronate and tetraacetate spectra are depicted in Fig. 20. The former spectrum shows as base peak the ion of *m/z* 342 ($[M-CH_3]^+$), which carries 20% of the total ion current, and could be of value in selective ion monitoring;

the only other prominent high-mass ions are $[M]^{+\cdot}$ and m/z 204 (tetrahydronaphthalene-2,3,5-triol methaneboronate fragment). In contrast, the tetraacetate spectrum shows no detectable molecular ion, but includes many fragment ions in substantially similar abundance, the origins of which are largely apparent. The tetraacetate is thus a very suitable derivative for characterisation of isolated samples of Nadolol, by virtue of its structurally informative mass spectrum.

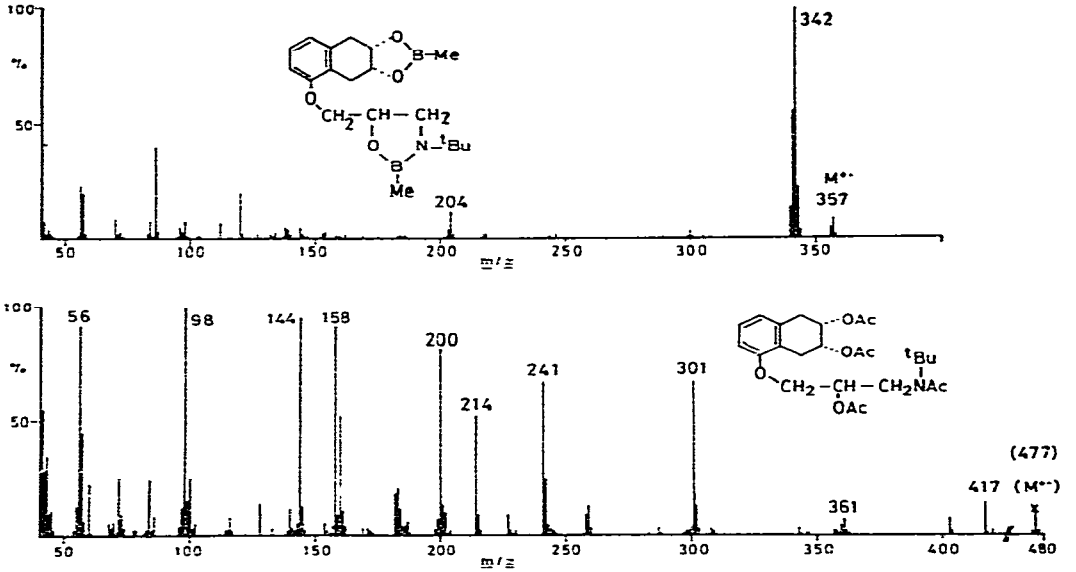

Fig. 20. Mass spectra (20 eV) of Nadolol bismethaneboronate (19c) and tetraacetate (19a). GC–MS conditions as in Fig. 3, except that column temperature was 225°C.

DISCUSSION

Attention is focused here principally upon those dihydrodiols that have been isolated as metabolites, and also (in the naphthalene and anthracene groups) upon the corresponding tetrahydrodiols. The latter compounds have not only been greatly used in the indirect characterisation of the less stable dihydrodiols, but they are also related to metabolic tetrahydrodiols such as the 1,2,3,4-(hydroxy/methylthio) derivatives of tetrahydronaphthalene recently identified as urinary metabolites of naphthalene in the rat[11,18]. One of these, a 1,3-di(methylthio)-1,2,3,4-tetrahydronaphthalene-2,4-diol, as its di-TMS ether, gave the informative *retro*-Diels–Alder fragment[11,18] analogous to that in Fig. 5; but the methaneboronate of the 2,4-diol grouping (apart from indicating it to be a *cis*-diol) did not yield a particularly useful mass spectrum except for an abundant molecular ion[11]. In their early work on the biogenesis of 1,2-naphthalene oxide and its conversion into the dihydrodiol, Jerina *et al.*[19] noted that mass spectra of the dihydrodiol did not yield evidence as to the location of ^{18}O in the diol group: accordingly, the diol was dehydrated to a mixture of 1- and 2-naphthol for mass spectrometry. It is clear that in 1,2,3,4-tetrahydroarene-1,2-diols (and 1,3-diols[11]) obtained as metabolites or by hydrogenation of dihy-

drodiols, distinction between the oxygen atoms is readily made via the *retro*-Diels–Alder fragmentation: although this is often prominent in the mass spectra of the free diols[20] (*cf.* Fig. 8), the higher mass of the ions from di-TMS ethers is often advantageous. The mass spectra of tetrahydronaphthalene-*cis*-1,2-diol and some related compounds were largely interpreted in an earlier investigation[20], though the fragmentation mode affording the ion at m/z $(M-60)$, mentioned above for the tetrahydroanthracenediols, was not discussed.

In the characterization of metabolic dihydrodiols and dihydrodiol epoxides derived from arenes of higher molecular weight such as benzpyrenes, mass spectrometry has been used mainly via direct probe sampling of fractions isolated by liquid chromatography[21,22]. However, as mentioned in the Introduction, GC–MS has been applied in studies of the *trans*-5,6-dihydrodiol and *trans*-8,9-dihydrodiol of 7,12-dimethylbenzanthracene[12]: the mass spectrum of the di-TMS ether of the 5,6-diol was characterised by the prominent rearrangement ions of m/z 191 and $(M-103)$ analogous to those from the 1,2-dihydroanthracenediol derivative (Fig. 12). Similar results have been reported in a study by GC–MS of the metabolites of phenanthrene in the coalfish[23]. The major dihydrodiol produced in that species, in contrast with the rat, was found to be a 1,2-diol, with very minor amounts of the 9,10-diol: the mass spectral data for the di-TMS ether of the latter isomer (presumably a *trans*-diol) are in good agreement with our observations on the reference samples (Table VI and Fig. 17). Brief data on the mass spectra of 1,2-dihydrophenanthrene-*trans*-1,2-diol, and of the corresponding *cis*-1,2-diol (as diacetate) have been reported[24].

One group of dihydrodiols to which GC–MS has been more extensively applied are those arising from metabolic oxidation of the olefinic bond in drugs derived from dibenzocycloheptene, dibenzoazepine[14] and related tricyclic structures. The flexibility of the seven-membered ring in many of these metabolites allows the formation of certain cyclic derivatives from both *cis*- and *trans*-diols (just as in the case of the 9,10-dihydrophenanthrene-9,10-diol groupings), and butaneboronates have been employed in the characterisation by GC–MS of 10,11-dihydro-10,11-diols among the metabolites of protriptyline[25], dibenzocycloheptadiene[26], carbamazepine[27] and related tricyclic drugs.

In the light of the above survey, it is clear that the range of derivatives studied in this paper includes the most generally useful types for the study of metabolic dihydrodiols and tetrahydrodiols by GC–MS. For those compounds which are not amenable to GC, the derivatives are still of value in mass spectrometry. The principal conclusions that can be drawn from our results, in combination with other published work, are briefly summarised below.

Gas chromatography

1. Trimethylsilyl ethers and acetates are both effective derivatives for stabilising dihydrodiols towards GC, but the TMS ethers have the advantage of shorter retention times, especially on the more polar OV-17 phase.

2. Cyclic alkaneboronates represent a selective type of derivative. *cis*-Dihydrodiols of rigid conformation and narrow dihedral angle, such as indane-*cis*-1,2-diol and acenaphthene-*cis*-1,2-diol, form especially stable cyclic boronates, while the equally rigid *trans*-isomers cannot form comparable derivatives. In other instances, where the diol groupings have enough conformational flexibility, cyclic boronates can

be formed from both *cis*- and *trans*-diols. We have observed that these derivatives provide the most satisfactory means of distinguishing between the *cis*- and *trans*-isomers by virtue of their different retention times. This is an important feature, because the di-TMS ethers and diacetates usually afford little distinction between retention data for *cis*- and *trans*-diols: moreover, mass spectra do not distinguish the isomers satisfactorily.

3. Cyclic methaneboronates are of special value in yielding retention times markedly shorter than those of the free diols and (on OV-1) those of any of the other derivatives studied. This allows diols of comparatively higher molecular weight, such as 2,3,4,5-tetramethoxy-9-methyl-9,10-dihydrophenanthrene-*cis*-9,10-diol (16) (Figs. 1 and 14) to be studied by GC–MS without the need for excessively high column temperatures or long retention times. A similar advantage arises for compounds such as Nadolol where four functional groups can be protected as a bismethaneboronate (Fig. 19).

Mass spectrometry

1. For the determination of molecular ions, the di-TMS ethers were effective for all but two of fifteen diols examined, whereas only seven of the diacetates gave detectable molecular ions. In those instances (twelve diols) where cyclic boronates could be formed they invariably afforded molecular ions at least 10% as abundant as the base peak, and usually constituting the base peak (Table VIII). In the particular case of Nadolol, the bismethaneboronate was the best derivative for establishing the molecular ion (the peak intensity at $M-1$ conforming with the presence of two boron atoms).

TABLE VIII

MOLECULAR ION ABUNDANCES FOR DIOLS AND DERIVATIVES RELATIVE TO RESPECTIVE BASE PEAKS (20 eV EI): DATA FROM TABLES II, IV AND VI

Derivative	*Number of examples*			
	0% abundance	*< 10% abundance*	*10–99% abundance*	*100% abundance*
Diols	1	6	6	2
Diacetates	8	5	2	0
Di-TMS ethers	2	3	7	3
Methaneboronates*	0	0	3	9
Butaneboronates*	0	0	4	8

* Three of the *trans*-diols did not yield boronates.

2. None of the particular derivative types examined was uniformly the best for the elucidation of structure from fragmentation modes. On the whole, the diacetates were the least useful in this respect, because of the prevalence of elimination of acetate and ketene moieties: however, in the exceptional case of Nadolol (Fig. 20) the moderating effect of the N-acetyl group on the α-cleavage reaction led to a good balance of informative fragmentations. More generally, the complementary use of di-TMS ethers and methaneboronates was found to be of great value because of the distinctive

differences in their modes of fragmentation, outlined in this paper and exemplified in Figs. 3/4, 5/6, 10/11 and 16/17. One such difference is the tendency for the cyclic esters to yield ions retaining the boronate group, which may also be accompanied by complementary fragment ions (*cf.* Fig. 6 in which the latter is a tropylium ion).

3. While high-resolution mass spectrometry is the most appropriate technique for establishing ion composition and for deducing fragmentation pathways, it is nevertheless possible to secure useful provisional data from low-resolution measurements. In this respect, the comparison of closely related derivatives (such as TMS and $[^2H_9]$TMS ethers) is a well established procedure. For the cyclic boronates, deuterium-labelled reagents are not conveniently accessible, but the comparative use of methane- and butaneboronates is practicable, as the latter derivatives (for a single diol grouping) have only slightly longer retention times than the di-TMS ethers and their mass spectrometric fragmentations largely parallel those of methaneboronates.

The mass spectrometric characteristics of the derivatives studied in this paper will be reported in more detail elsewhere.

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REFERENCES

1 E. Boyland and A. A. Levi, *Biochem. J.*, 29 (1935) 2679.
2 L. Young, *Biochem. J.*, 41 (1947) 417.
3 E. Boyland and G. Wolf, *Biochem. J.*, 47 (1950) 64.
4 J. Booth and E. Boyland, *Biochem. J.*, 44 (1949) 361.
5 J. W. Cook, J. D. Loudon and W. F. Williamson, *J. Chem. Soc.*, (1950) 911.
6 E. Boyland, *Symp. Biochem. Soc.*, No. 5 (1950) 40.
7 D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *J. Amer. Chem. Soc.*, 90 (1968) 6525.
8 D. T. Gibson, *CRC Crit. Rev. Microbiol.*, 1 (1971) 199.
9 R. V. Smith and J. P. Rosazza, *J. Pharm. Sci.*, 64 (1975) 1737.
10 C. J. W. Brooks and L. Young, *Biochem. J.*, 63 (1956) 264.
11 W. G. Stillwell, O. J. Bouwsma, J.-P. Thenot, M. G. Horning, G. W. Griffin, K. Ishikawa and M. Takaku, *Res. Commun. Chem. Pathol. Pharmacol.*, 20 (1978) 509.
12 Lan K. Wong, Chih-Lueh A. Wang and F. B. Daniel, *Biomed. Mass Spectrom.*, 6 (1979) 305.
13 C. J. W. Brooks and J. Watson, *Chem. Commun.*, (1967) 952.
14 C. Pantarotto, L. Cappellini, A. De Pascale and A. Frigerio, *J. Chromatogr.*, 134 (1977) 307.
15 R. P. Hopkins, C. J. W. Brooks and L. Young, *Biochem. J.*, 82 (1962) 457.
16 H. C. Brown and G. Zweifel, *J. Org. Chem.*, 27 (1962) 4708.
17 J. Booth, E. Boyland and E. E. Turner, *J. Chem. Soc.*, (1950) 1188.
18 W. G. Stillwell, O. J. Bouwsma and M. G. Horning, *Res. Commun. Chem. Pathol. Pharmacol.*, 22 (1978) 329.
19 D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *Biochemistry*, 9 (1970) 147.
20 P. Perros, J. P. Morizur, J. Kossanyi and A. M. Duffield, *Bull. Soc. Chim. Fr.*, (1973) 2105.

21 K. M. Straub, T. Meehan, A. L. Burlingame and M. Calvin, *Proc. Nat. Acad. Sci. U.S.*, 74 (1977) 5285.
22 D. R. Thakker, W. Levin, A. W. Wood, A. H. Conney, T. A. Stoming and D. M. Jerina, *J. Amer. Chem. Soc.*, 100 (1978) 645.
23 J. E. Solbakken, K. H. Palmork, T. Neppelberg and R. R. Scheline, *Acta Pharm. Toxicol.*, 46 (1980) 127.
24 D. M. Jerina, H. Selander, H. Yagi, M. C. Wells, J. F. Davey, V. Mahadevan and D. T. Gibson, *J. Amer. Chem. Soc.*, 98 (1976) 5988.
25 V. Rovei, G. Belvedere, C. Pantarotto and A. Frigerio, *J. Pharm. Sci.*, 65 (1976) 810.
26 C. Pantarotto, L. Cappellini, P. Negrini and A. Frigerio, *J. Chromatogr.*, 131 (1977) 430.
27 G. Belvedere, C. Pantarotto and A. Frigerio, *Res. Commun. Chem. Pathol. Pharmacol.*, 11 (1975) 221.
28 P. T. Funke, M. F. Malley, E. Ivashkiv and A. I. Cohen, *J. Pharm. Sci.*, 67 (1978) 653.