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SYNTHESIS, GAS–LIQUID CHROMATOGRAPHY, AND MASS SPECTROMETRY OF PER-*O*-TRIMETHYLSILYL CARBOHYDRATE BORONATES*†

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ABSTRACT

The characterization by gas–liquid chromatography–mass spectrometry of benzene-, butane-, and methane-boronate trimethylsilyl ether derivatives of some common carbohydrates has been investigated by a combination of high- and low-resolution mass spectrometry. Preparation of stereospecific derivatives with alkane- or arene-boronic acids, followed by silylation and mass spectrometry, allows the identification of isomeric structures of pentoses, hexoses, 6-deoxyhexoses, and 2-acetamido-2-deoxyhexoses.

INTRODUCTION

The usefulness of mass spectrometry (m.s.) as a technique for the determination of complex organic molecules has been demonstrated and applied to a wide variety of compounds. The prior separation of complex mixtures by gas–liquid chromatography (g.l.c.), and the capability of handling data offered by on-line computers[1] and a library[2] of taped spectra have opened up new vistas in the structural determination of complex biological mixtures.

Partial and total hydrolysis of complex carbohydrate structures (*i.e.*, glycoproteins, glycolipids, mucins, cell walls, membranes, etc.), produces mixtures of compounds that require the masking of the polar functional groups for g.l.c.–m.s. analysis, a disadvantage that limits the usefulness of mass spectrometry for the study of many carbohydrates.

Trimethylsilyl derivatives of carbohydrates[3] and acetates of the corresponding glycitols[4] have been widely used for the gas-chromatographic separation of mono- and oligo-saccharides. Although the mass spectra of both types of derivatives give

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considerable structural information, they do not differentiate between stereoisomers, because cleavage of a single bond in the monocyclic ring system permits the resulting ion to rotate freely. Furthermore, the mass spectra of per(trimethylsilyl) derivatives yield many fragments that contain few carbon atoms of the parent sugar. Thus, it is difficult or impossible to assign structures to the common hexoses, or even to propose a sequence for a simple disaccharide based only on mass spectral data[5].

Formation of a five- or six-membered ring involving the hydroxyl groups of a sugar greatly enhances mass-spectral interpretation in two major ways. First, variations in the stereochemistry of a ring junction in bi- or oligo-cyclic systems lead to drastic differences in ion intensity, and secondly, the five- or six-membered rings are easily observed by the mass shift for the fragment containing that ring. Thus, cyclization of spatially suited hydroxyl groups of a furan or pyran ring ties the conformer with additional bonding that prevents free rotation upon single-bond cleavage and stabilizes an ensuing positive charge. An additional chemical specificity results from the steric requirements for the formation of small rings. Boronate derivatives were selected to form small rings. However, in many cases the polarity was too high for g.l.c. analysis; thus, the remaining free hydroxyl groups were substituted with trimethylsilyl residues.

RESULTS AND DISCUSSION

Brooks *et al.*[6–9] have characterized by g.l.c.–m.s. the boronates of a number of polar, bifunctional compounds, and have compared the butane-, cyclohexane-, and benzene-boronates. In 1971, two preliminary reports[10,11] described the g.l.c. of butane-boronates of carbohydrates.

The mass spectra of boronate silyl derivatives generally allow the determination of the number of carbon atoms (pentose or hexose), the ring size (furanose and pyranose), and the stereochemistry of the hydroxyl groups. The number of boronate groups indicate the number of pairs of hydroxyl groups present in a suitable spatial relationship for formation of a five- or six-membered ester grouping and the number of trimethylsilyl groups indicates the number of hydroxyl groups remaining after boronation. The m.s. of nonsilylated derivatives show that the cyclic boronate group generates characteristic ions from which the ring size and, thus, the relationship [2,3-, 4,6- (or both) cyclic boronate] of the hydroxyl groups involved can be deduced. However, the presence of the trimethylsilyl group completely obscures the appearance of fragments containing boron; in this case, the fragment ions bearing trimethylsilyl groups can identify the position and relationship of the hydroxyl groups not taking part in cyclic ester formation. For example, abundant ions at mass 204 and 218 indicate structural entities including two trimethylsilyl groups attached to two or three carbon atoms (see discussion of the spectra).

As determined by g.l.c. after silylation, formation of the cyclic boronate was quantitative, no side-products being detected. The nature of the alkane or arene substituent of the boronic acid had no detectable influence on the reaction and only five-

TABLE I

GAS–LIQUID CHROMATOGRAPHY OF METHYL GLYCOSIDE DERIVATIVES[a]

Methyl glycoside of	*Derivatives*							
	Per(trimethylsilyl)		*Butaneboronate*			*Methaneboronate*		*Boronate structure*[c]
	R_T[b]	*Mol. wt.*	R_T[b]	*Mol. wt.*		R_T[b]	*Mol. wt.*	
α-L-Rhamnopyranose	0.54	394		316	(22)	0.27	274	2,3-BL
α-L-Fucopyranose	0.57	394	0.61	316	(21)	0.30	274	3,4-BL
β-L-Fucopyranose	0.59	394		316		0.32	274	3,4-BL
α-D-Mannopyranose	0.78	482	1.24	326	(11)	0.50	242	2,3; 4,6-BL
β-D-Mannopyranose	0.81	482		326			242	2,3; 4,6-BL
α-D-Glucopyranose	0.94	482	1.05	404	(20)	0.72	362	4,6-BA
β-D-Glucopyranose	0.95	482	1.06	404			362	4,6-BA
α-D-Galactopyranose	0.87	482	0.96	404	(13)	0.66	363	3,4-BL
β-D-Galactopyranose	0.91	482	0.98	404		0.72	362	3,4-BL
α-D-Galactopyranose			1.04	404	(14)	0.76	362	4,6-BA
β-D-Galactopyranose			1.05	404		0.81	362	4,6-BA
2-Acetamido-2-deoxy-α-D-glucopyranose	1.37	451	1.45	373	(25)	1.18	331	4,6-BA
2-Acetamido-2-deoxy-β-D-glucopyranose	1.32	451		373			331	4,6-BA
2-Acetamido-2-deoxy-α-D-galactopyranose	1.33	451	1.54	373	(26)	1.28	331	4,6-BA
2-Acetamido-2-deoxy-β-D-galactopyranose	1.40	451		373			331	4,6-BA
2-Acetamido-2-deoxy-α-D-gulopyranose	1.31	451	1.42	373	(29)	1.32	331	4,6-BA

[a]0.05% OV-17 on 120–140 mesh glass beads; temperature programmed to increase at a rate of 8° per min starting at 80°. [b]Relative to eicosane (R_T 1.00). [c]Abbreviations: BL, dioxaboralane; BA, dioxaborane.

or six-membered (or both) cyclic esters were observed. The single derivative obtained from anomeric sugar mixtures, and the low temperatures of elution from g.l.c. columns suggest that this technique may be used for quantitative carbohydrate analysis.

All per(trimethylsilylated) boronate derivatives gave a single peak on g.l.c., with the exception of methyl D-galactosides (Table I). The methaneboronate derivatives were always eluted earlier than the per(trimethylsilylated) derivatives, whereas the benzene- and butane-boronate esters were generally eluted later.

As an example of the usefulness of the method, we can consider the molecular ion at *m/e* 300. This mass can be accounted for only by a hexose molecule containing two cyclic boronate groups and a single trimethylsilyl group (*i.e.* two pairs of hydroxyl groups in close proximity and a single hydroxyl group sterically unable to form a boronate cycle). In the naturally occurring D-hexoses, borosilylation results in the formation of a derivative that is fragmented specifically on electron impact; thus, a molecular ion at *m/e* 300 and a fragment ion at *m/e* 171 indicate mannose, whereas ions at *m/e* 117 and *m/e* 103 (in addition to *m/e* 300) indicate galactose and glucose, respectively (Figs. 18, 19, and 20).

Borosilylation of isomeric carbohydrates may form derivatives that have very different compositions: for example, in the pentose series, D-lyxose produces a molecular ion at *m/e* 318 (one cyclic boronate and two trimethylsilyl ether groups), and D-xylose at *m/e* 198 (two cyclic boronates) (Fig. 16); in the 6-deoxyhexose series, rhamnose a molecular ion at *m/e* 332 and fucose at *m/e* 212 (Fig. 15).

The usual derivatives (methyl, acetyl, and trimethylsilyl) do not provide fragmentation patterns having sufficient variations for structural interpretation, as illustrated by the mass spectrum of methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-α-D-mannopyranoside (**1**) (Fig. 1). The peak pattern and ion composition is the same as

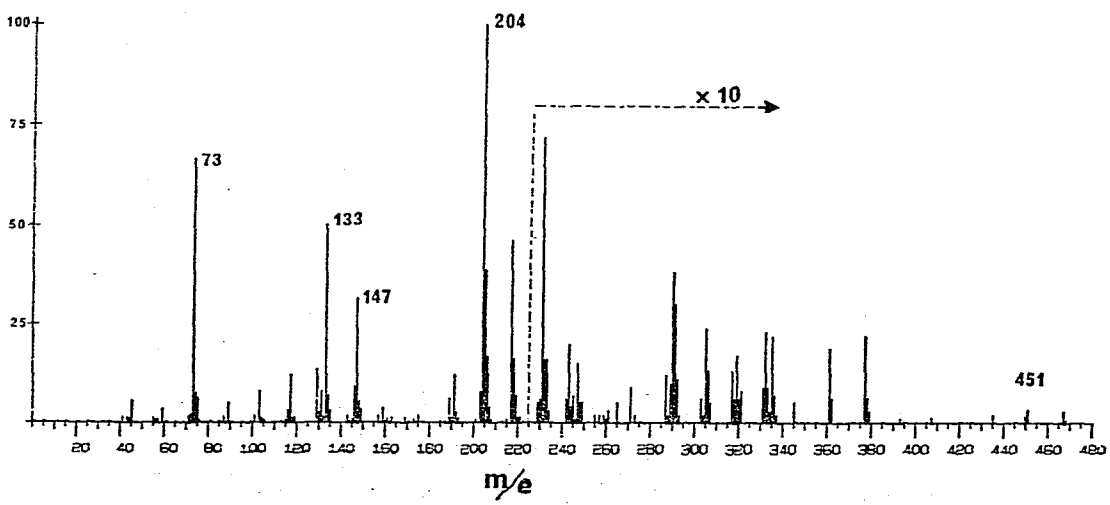
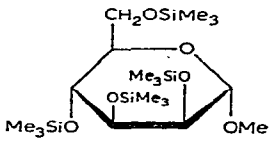


Fig. 1. Mass spectrum of methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-α-D-mannopyranoside (**1**).

that of the isomeric trimethylsilyl derivatives of methyl α-D-glucopyranoside[3] and methyl α-D-galactopyranoside[4]. The high molecular weight and the low intensity of the high-mass fragments are additional limitations.



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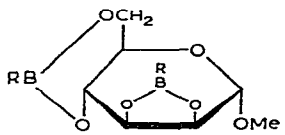


2 R = Ph
3 R = Bu
4 R = Me

Methyl α-D-mannopyranoside possesses two pairs of hydroxyl groups suitably spaced to form two boronate cyclic esters, a property shared by no other naturally-occurring hexose glycoside, and the benzene- (**2**), butane- (**3**), and methane (**4**) -boronates were prepared. Examination of the mass spectra (Figs. 2, 3, and 4) shows variations due primarily to the differences in mass, whereas fragmentation of the boronic acid substituent itself and rupture of carbohydrate ring bonds are identical. In most cases, the boron-containing ions showed greater stability than the corresponding per(trimethylsilylated) compounds, as shown by the relatively greater intensity of the high-mass fragments. The molecular ion was usually easily discernible and, in the case of methyl glycosides, the $(M-31)^+$ ion, which represents the loss of the methoxy group at C-1. As expected, the derivative prepared by borosilylation showed the absence of a trimethylsilyl group (lack of ions at *m/e* 73 and 75); thus, all hydroxyl groups were incorporated into boronate rings. The molecular ion (and ions obtained by loss of small fragments from the molecular ion) also agreed with a structure

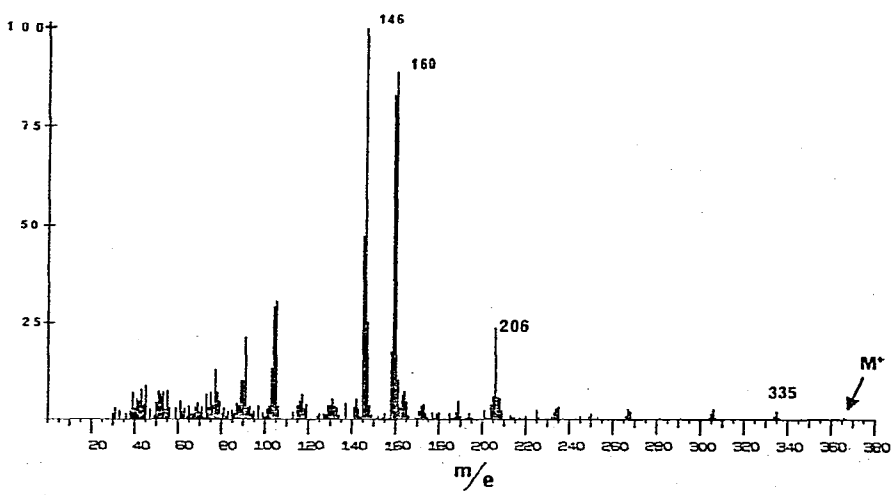


Fig. 2. Mass spectrum of methyl α-D-mannopyranoside 2,3:4,6-dibenzeneboronate (**2**).

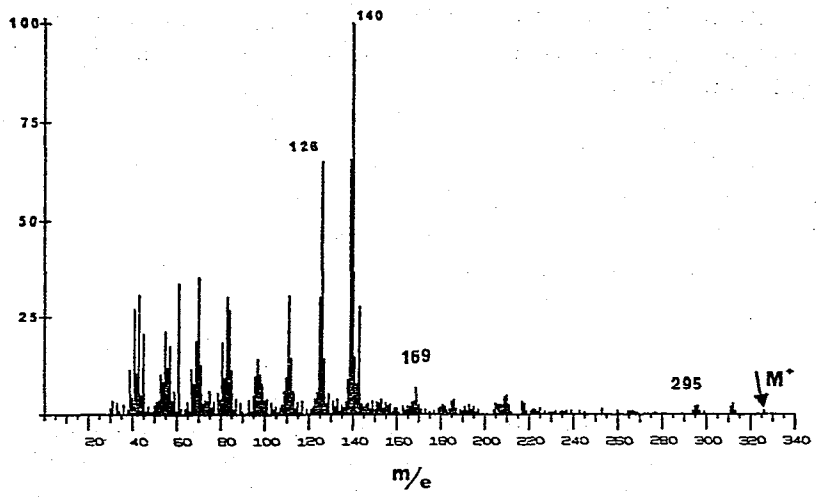


Fig. 3. Mass spectrum of methyl α-D-mannopyranoside 2,3:4,6-dibutaneboronate (3).

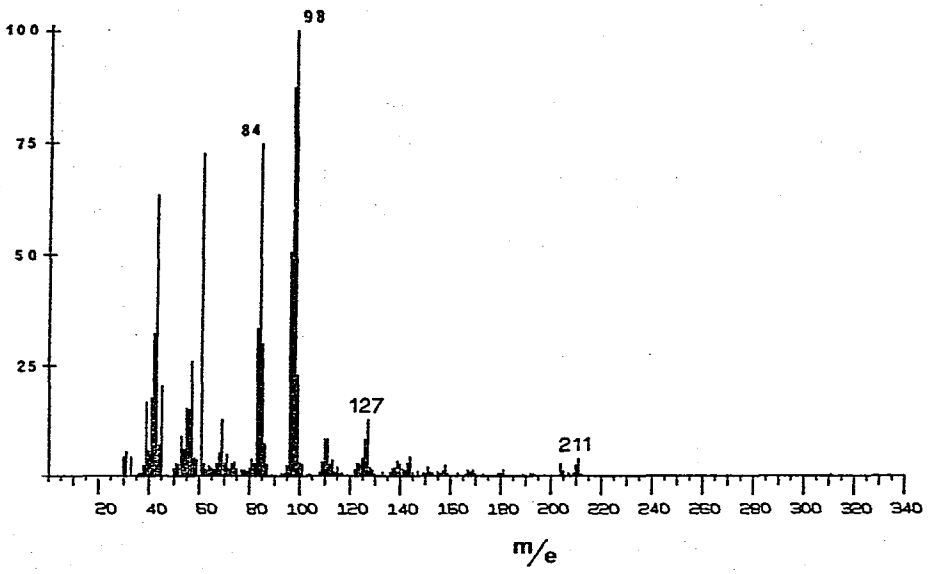
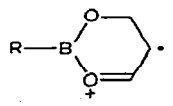
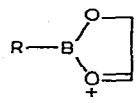


Fig. 4. Mass spectrum of methyl α-D-mannopyranoside 2,3:4,6-dimethaneboronate (4).

having two cyclic boronate groups. The cyclic boronate group shows considerable stability on electron bombardment and usually yields ions of major intensity. The ion composition indicates fragment structures containing six- (**5–7**) and five-membered (**8–10**) rings and are observed in the spectra of the benzene-, butane-, and methaneboronate derivatives, respectively, of methyl α-D-mannopyranoside (Figs. 2–4).

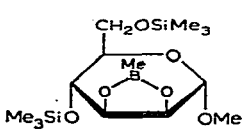




5 R = Ph *m/e* 160 ($C_9H_9BO_2$)
6 R = Bu *m/e* 140 ($C_7H_{13}BO_2$)
7 R = Me *m/e* 98 ($C_4H_7BO_2$)

8 R = Ph *m/e* 146 ($C_8H_7BO_2$)
9 R = Bu *m/e* 126 ($C_6H_{11}BO_2$)
10 R = Me *m/e* 84 ($C_3H_5BO_2$)

When one or more trimethylsilyl groups were introduced into the molecule, by treatment with less than one mol. equiv. of methaneboronic acid, to form the trimethylsilyl boronate derivatives **11** and **12**, the mass spectra (Figs. 5 and 6) showed



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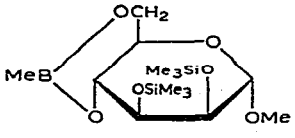

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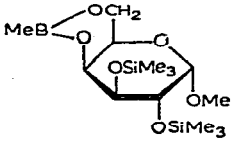

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fragmentation patterns characteristic of the trimethylsilyl group. The cyclic boronate ester group, however, markedly modified the patterns by increasing the intensity of the high-mass ions, even though the ion current carried by the boron ester group itself remained very small. Although molecular ions were not observed for these two derivatives **11** and **12**, the loss of a methyl group from one of the trimethylsilyl groups resulted in a ten- and fifty-fold increase in intensity of their respective $(M-15)^+$ ions,

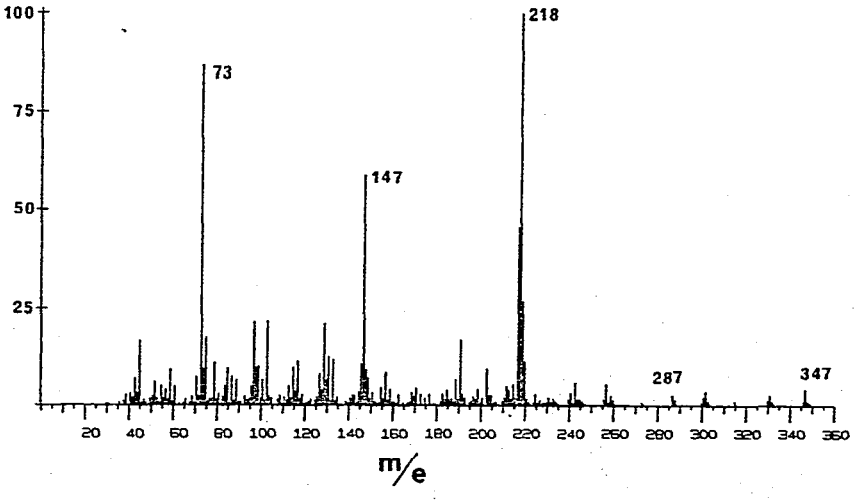


Fig. 5. Mass spectrum of methyl 4,6-di-*O*-(trimethylsilyl)-α-D-mannopyranoside 2,3-methaneboronate (**11**).

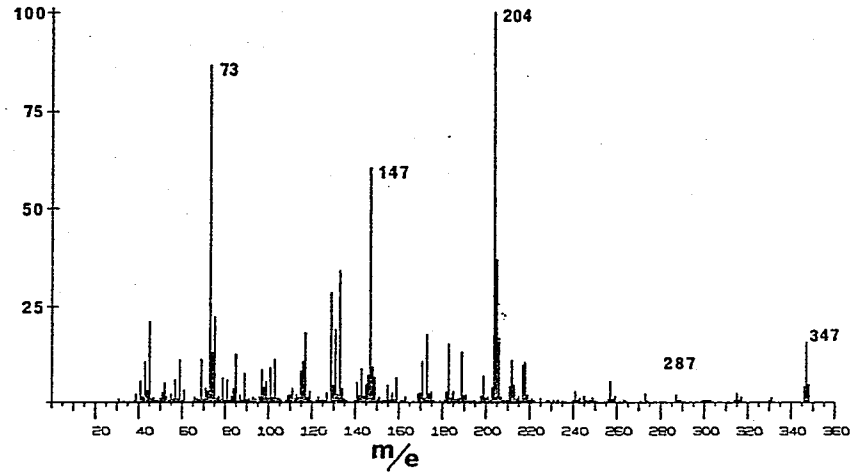


Fig. 6. Mass spectrum of methyl 2,3-di-*O*-(trimethylsilyl)-α-D-mannopyranoside 4,6-methaneboronate (**12**).

as compared to the derivative **1** lacking a cyclic boronate group (Fig. 1). This increased stability of boron-containing ions of high mass was also observed for the *m/e* 331, 315, and 287 ions, which represent a loss of 31, 47, and 75 atomic mass units from the molecular ion, processes that occur in the fragmentation of **1**, **11**, and **12**.

The trimethylsilyl boronate derivative of methyl α-D-galactopyranoside showed two separate peaks in g.l.c. Examination of molecular models of methyl α-D-galactopyranoside indicated O–O distances between the *cis* hydroxyl groups at C-3 and C-4 adequate for formation of a five-membered cyclic boronate or at C-4 and C-6 for a

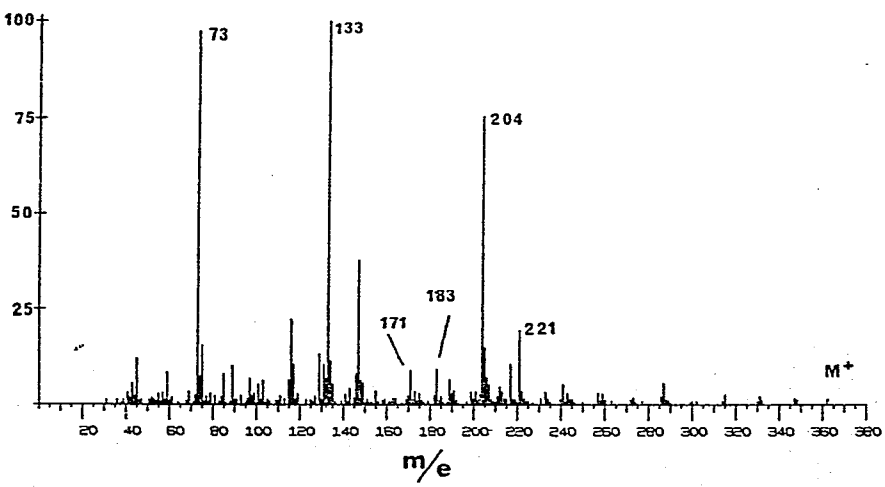


Fig. 7. Mass spectrum of methyl 2,3-di-*O*-(trimethylsilyl)-α-D-galactopyranoside 4,6-methaneboronate (**13**).

six-membered ring. The m.s. of the second peak (Fig. 7) was nearly identical to the m.s. of **12** (Fig. 6), indicating a 2,3-di-*O*-(trimethylsilyl) 4,6-methaneboronate derivative (**13**). In the m.s. of the first peak (Fig. 8) the ions at *m/e* 287 and 315 (representing losses of 75 and 47 from the molecular ion, as discussed earlier)

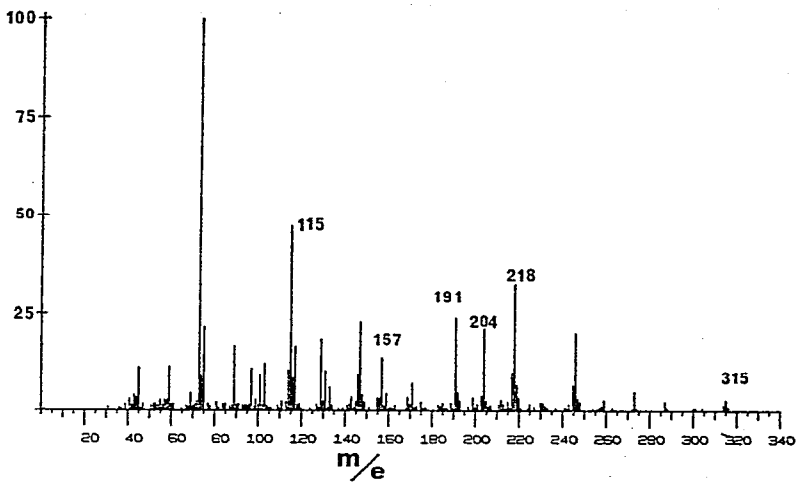
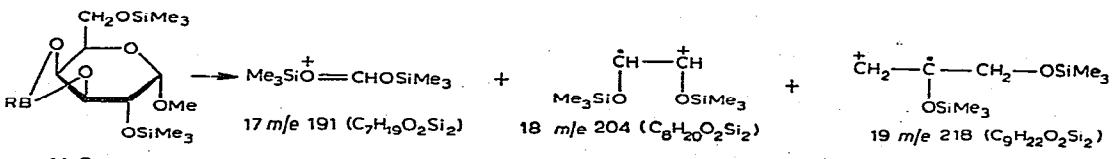


Fig. 8. Mass spectrum of methyl 2,6-di-*O*-(trimethylsilyl)-α-D-galactopyranoside 3,4-methaneboronate (**14**).

indicated a mol. wt. of 362, corresponding to one cyclic boronate and two trimethylsilyl groups. Only a 2,6-di-*O*-(trimethylsilyl) 3,4-methaneboronate structure (**14**) can account for this mol. wt. Further support, as discussed later, for this structure was obtained by comparison with the m.s. of the boronate silyl derivative of methyl L-fucopyranoside. The mass spectra of **14** and of the benzene and butane analogs showed major ion fragments at *m/e* 191, 204, and 218, which can be accounted by structures (**17–19**). This mechanism of fragmentation requires migration of a trimethylsilyl group across the carbohydrate ring, a rearrangement which appears to



14 R = Me
15 R = Bu
16 R = Ph

occur with equal ability for all three derivatives. The presence of the ions z^+ *m/e* 204, 217 in the m.s. of **14** would suggest a structure related to **11** or to **12** where similar fragments have been observed. However, an important difference in the m.s. of **14**

lies in the minor intensities of these fragments compared to their usual higher intensities (*c.f. m/e* 204 in Fig. 1 and Fig. 6; *m/e* 217 in Fig. 1 and Fig. 5).

The trimethylsilyl boronate derivative of methyl α-D-glucopyranoside gave, on g.l.c., a single peak. The molecular ion could be observed in the mass spectrum (Fig. 9) only with a large sample, as was also the case for the $(M-31)^+$ ion. However, the intensities of ions at *m/e* 347 and 315 usually varied around 5% of the base peak

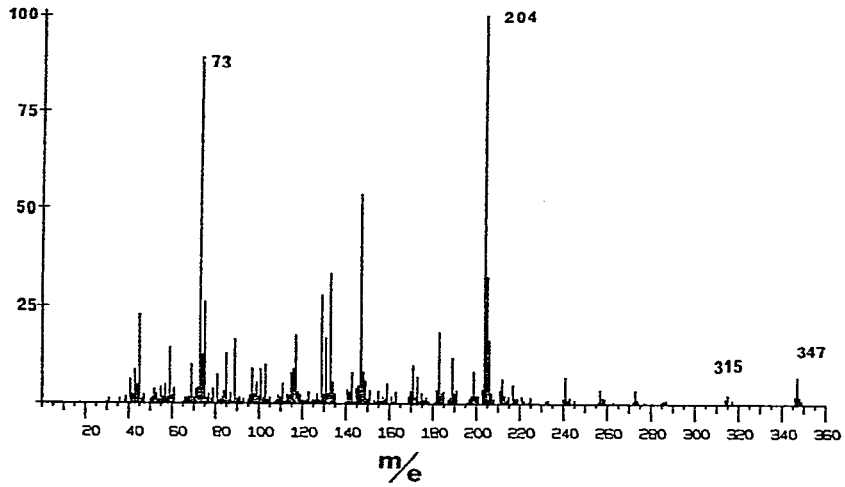


Fig. 9. Mass spectrum of methyl 2,3-di-*O*-(trimethylsilyl)-α-D-glucopyranoside 4,6-methaneboronate (**20**).

and their compositions indicated loss of a methyl group, and the combined loss of a methyl group and methanol from the molecular ion, respectively. These results suggest the formation of a monoboronate ester with two trimethylsilyl groups (mol. wt. 362). With the exception of small differences of intensity, the m.s. of this glucoside is identical with that of **12** (Fig. 6) and suggests structure **20**. The major fragments, *m/e* 73, 129, 131, 133, and 204, gave compositions corresponding with those obtained from **1**, **12**, **13**, and **14**.

The boronate trimethylsilyl derivatives of methyl α-L-rhamnopyranoside and methyl α-L-fucopyranoside gave the mass spectra presented in Figs. 10 and 11, respectively. The intensity of the molecular ion was less than 0.1% of that of the base peak in each derivative, but the $(M-15)^+$, $(M-31)^+$, and $(M-60)^+$ ions were all present in concentration adequate for easy determination of the molecular weight at 274. This value supports a monoboronate cyclic ester with one trimethylsilyl substituent in each of the derivatives, a structure that is supported by the presence of ions at *m/e* 73 and 75 and by the low intensity of the ion at *m/e* 147 (usually this ion is of high intensity whenever two or more trimethylsilyl groups occur in the molecule). The D-fucose (6-deoxy-D-galactose) and D-rhamnose (6-deoxy-D-mannose) molecules possess each only one pair of *cis* hydroxyl groups located at C-3–C-4 and C-2–C-3,

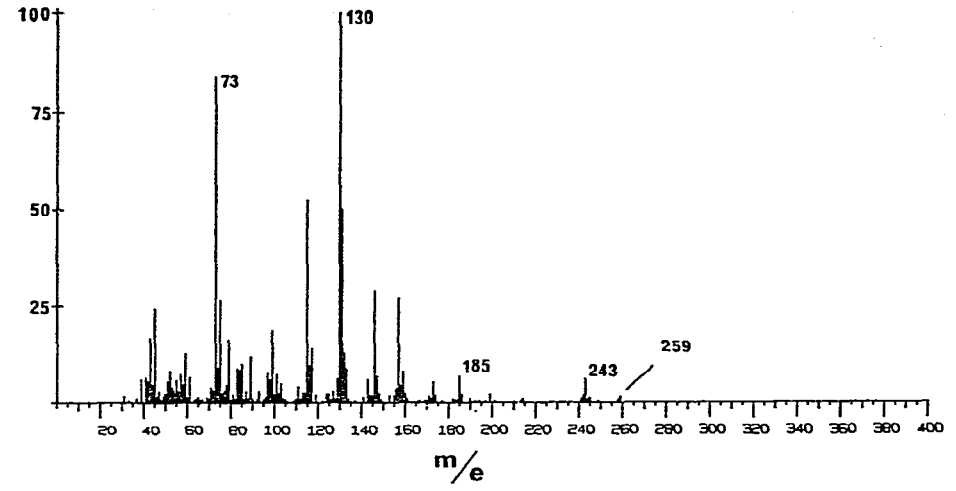


Fig. 10. Mass spectrum of methyl 4-*O*-(trimethylsilyl)-α-L-rhamnoside 2,3-methaneboronate (**22**).

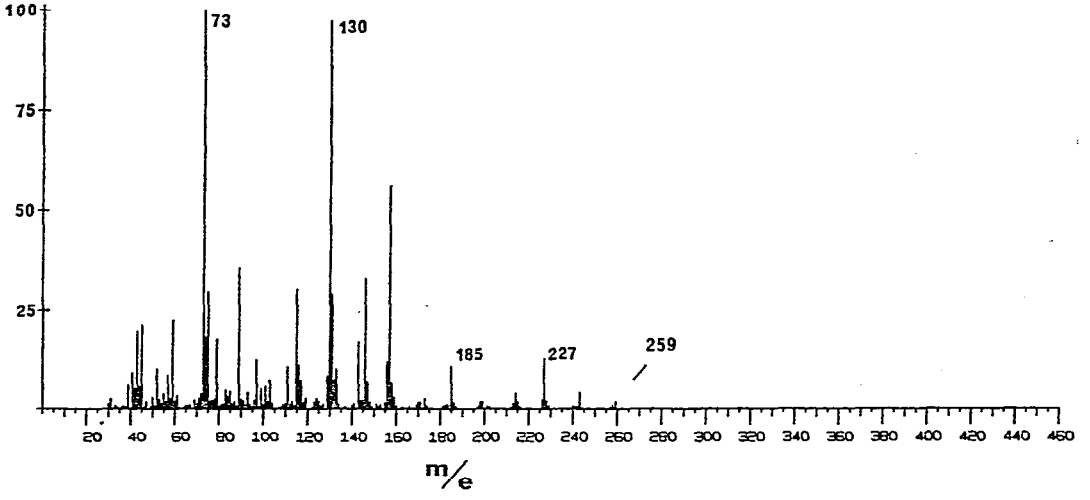
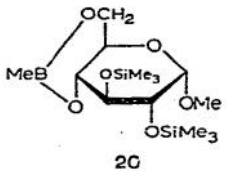


Fig. 11. Mass spectrum of methyl 2-*O*-(trimethylsilyl)-α-L-fucopyranoside 3,4-methaneboronate (**21**).

respectively. Thus, with the lack of an hydroxyl group at C-6 able to be esterified, a 3,4- (**21**) or 2,3-boronate (**22**) group, respectively, is expected. These structures were ascertained by comparison of the mass spectrum of the 3,4-boronate of methyl D-galactopyranoside (**14**) (Fig. 8) with that of the L-fucopyranoside derivative (**21**) (Fig. 11). The ions at *m/e* 73, 89, 115, 146, and 157 appear in both spectra, whereas the ions at *m/e* 273 and higher range appear 88 mass units lower in the fucoside m.s. The ion of high intensity at *m/e* 130 (**23**) probably derives from the C-4–C-6 portion of the molecule by a rearrangement analogous to that giving the ion at *m/e* 218 (**19**)

from the methyl α-D-galactopyranoside derivative (**14**) (Fig. 8). Comparison of the m.s. of **21** and **22** permits assessment of the structure of isomers containing a single five-membered cyclic boronate group. With one exception, differences were not observed in ion fragments, but the spectra show major variation in ion intensities



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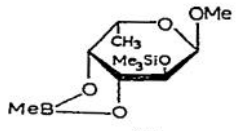

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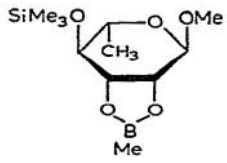

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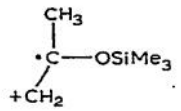
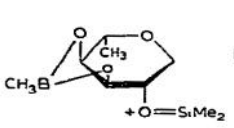
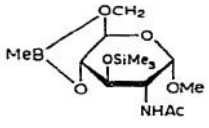


23 *m/e* 130 ($C_6H_{14}OSi$)

(Figs. 10 and 11). Most significant was the ion at *m/e* 227 (**24**) (Fig. 11), which indicated loss of a fragment equal to a methyl group and methanol from the molecular ion. The elimination of a methoxy group to produce ion **24** can occur only for the fucose derivative, and the presence of the substituent at C-2 may well account for the occurrence and stability of **24** in the mass spectrum of **21**.



24 *m/e* 227 ($C_9H_{16}BO_4Si$)



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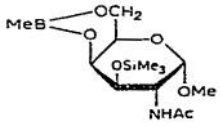

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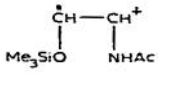
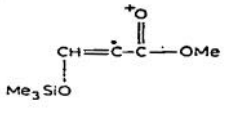


27 *m/e* 173 ($C_7H_{15}NO_2Si$)



28 *m/e* 173 ($C_7H_{13}O_3Si$)

Formation of the boronate trimethylsilyl derivative of methyl 2-acetamido-2-deoxy-α-D-hexopyranosides was complete under the conditions established for the other methyl glycosides, and the m.s. obtained for the D-gluco- (**25**) and D-galactoside (**26**) are shown in Figs. 12 and 13, respectively. The compositions of the ions at *m/e* 316, 299, and 284 of both **25** and **26** indicated the loss of a methyl group, methanol, and a methyl group plus methanol, respectively, from the molecular ion. This result indicates a mol. wt. of 331, corresponding to a single cyclic boronate and a trimethylsilyl group. The ions of high intensity at *m/e* 73 and 75 and the very small intensity of the ion at *m/e* 147 supports a structure with a single trimethylsilyl group. The peak at *m/e* 173 was found to be composed of two ions, one corresponding to two-thirds of the total intensity and having a composition and structure (**27**) identical with that of the *m/e* 173 ion for per(trimethylsilylated) methyl acetamido-2-deoxy-D-glucopyranoside previously reported[4]. The second ion had a composition and structure (**28**) identical with those of the ion obtained from **21** and **26**. The ion at *m/e* 131 (Figs. 12 and 13) had the composition $C_5H_{13}NOSi$ and may result from ketene elimination of the ion at *m/e* 173, as reported by De Jongh *et al.*[4]. The ions at *m/e* 241, 242, 271, and 272 observed in the m.s. of the methaneboronate trimethylsilyl derivatives of 2-acetamido-2-deoxy sugars are found to be 42 mass units higher in the

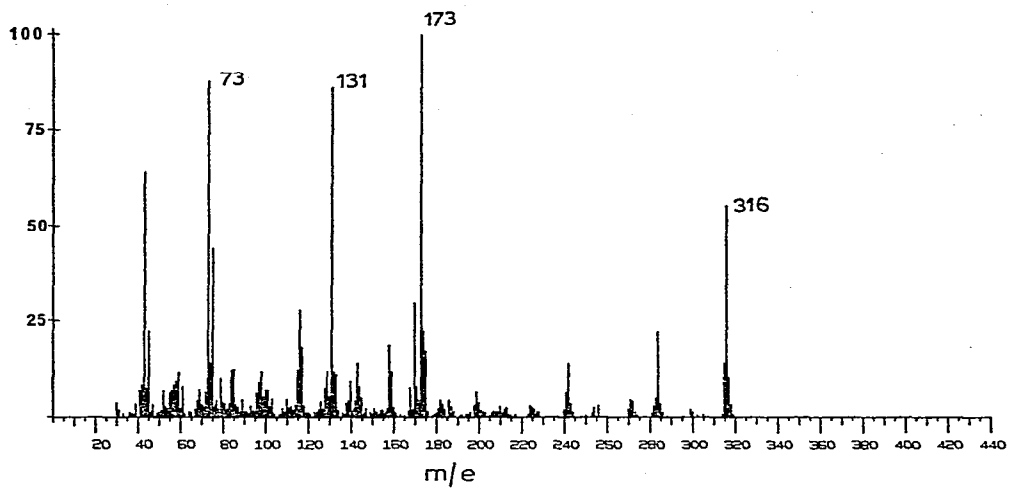


Fig. 12. Mass spectrum of methyl 2-acetamido-2-deoxy-3-*O*-(trimethylsilyl)-α-D-glucopyranoside 4,6-methaneboronate (25).

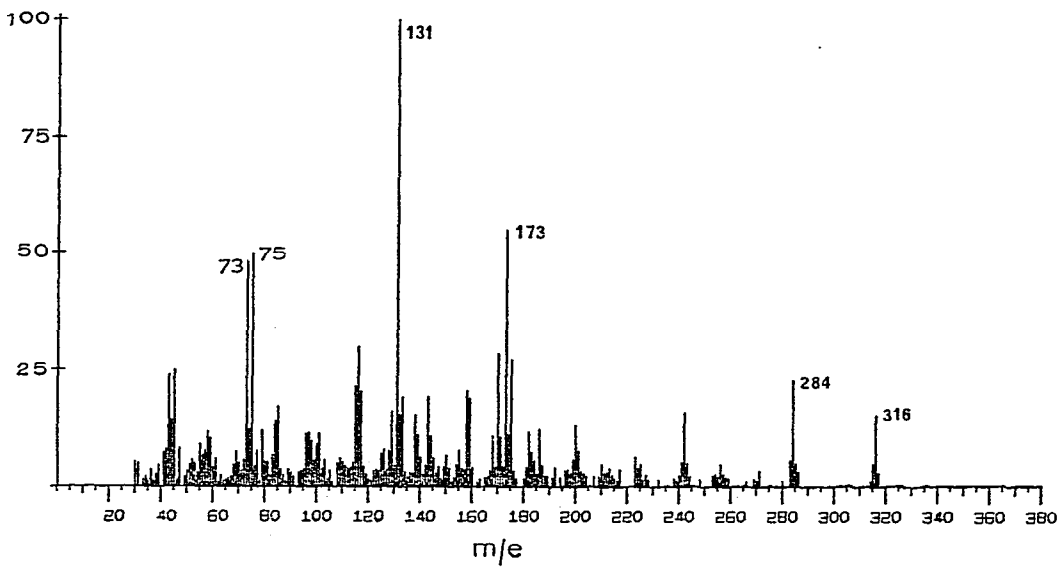
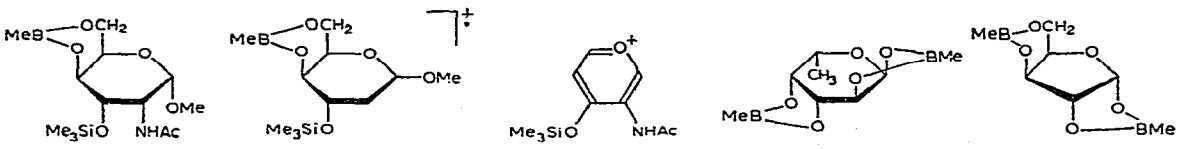


Fig. 13. Mass spectrum of methyl 2-acetamido-2-deoxy-3-*O*-(trimethylsilyl)-α-D-galactopyranoside 4,6-methaneboronate (26).

m.s. of the corresponding butyl boronate trimethylsilyl derivatives. The elemental compositions of these ions indicates a loss from the molecular ion equal to the mass of a trimethylsilyloxy group and of acetamide, respectively.

The derivatives of methyl 2-acetamido-2-deoxy-α- and β-D-galactopyranosides each gave a single peak when analyzed by g.l.c., an interesting contrast to the double

product composed of a five- and six-membered cyclic boronate ester obtained from methyl α- and β-D-galactopyranoside. The mass spectrum of the α anomer (**26**) is shown in Fig. 13, and although there was a marked increase in the intensity of the ion at *m/e* 131, the similarity of this pattern with that of Fig. 12 indicates that this derivative contains a single trimethylsilyloxy group at C-3 and a cyclic 4,6-boronate group.



The derivative of methyl 2-acetamido-2-deoxy-α-D-gulopyranoside (**29**) differs from the corresponding galactose derivative **26** by an axial instead of an equatorial trimethylsilyloxy group at C-3. Its m.s. (Fig. 14) shows, as most evident, ions at *m/e* 272 and 226, which have structures **30** and **31**, respectively.

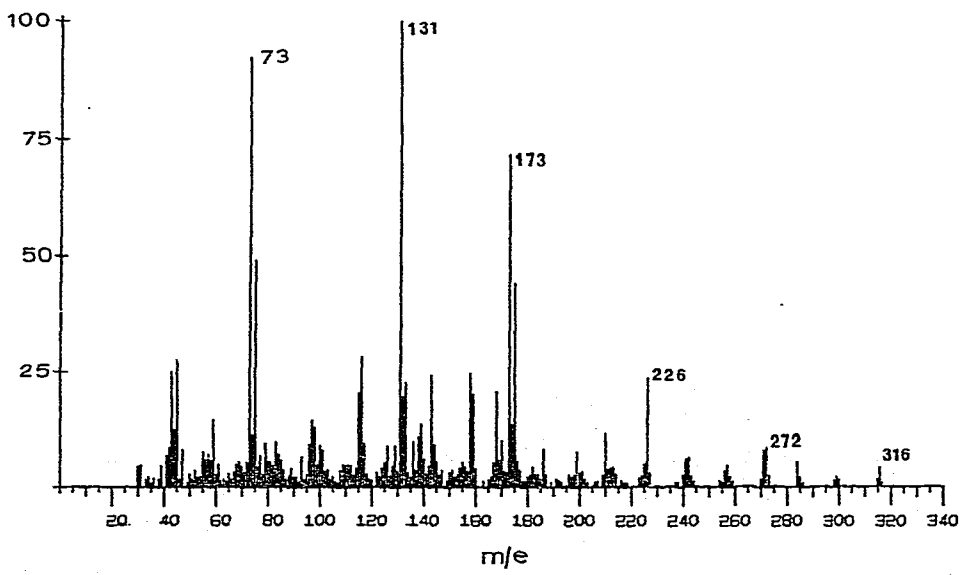


Fig. 14. Mass spectrum of methyl 2-acetamido-2-deoxy-3-*O*-(trimethylsilyl)-α-D-gulopyranoside 4,6-methaneboronate (**29**).

The free sugar or hemiacetal structure of a hexose or pentose may form a cyclic 1,2-boronate. In equilibrium mixtures, the conformer that has a vicinal *cis* relationship to the neighboring C-2 hydroxyl group would form a dioxaboralane ring which would remove this preferred anomer from an equilibrium mixture. Thus,

anomeric mixtures of individual sugars resulted in a single g.l.c. peak after boronate trimethylsilyl formation (Table II).

TABLE II

GAS–LIQUID CHROMATOGRAPHY OF SUGAR DERIVATIVES[a]

Sugar		*Methaneboronate*		*Boronate structure*[c]
		R_T[b]	*Mol. wt.*	
D-Xylofuranose	(33)	0.26	198	1,2-BL-3,5-BA
L-Fucopyranose	(32)	0.21	212	1,2-BL-3,4-BA
L-Arabinopyranose	(34)	0.22	198	1,2-BL-3,4-BA
D-Mannopyranose	(37)	0.62	300	2 3-BL-4,6-BA
D-Galactopyranose	(35)	0.59	300	1,2-BL-3,4-BA
D-Glucofuranose	(39)	0.65	300	1,2-BL-3,5-BA
2-Acetamido-2-deoxy-D-glucopyranose		0.58	389	4,6-BA
meso-Inositol		0.45	252	

[a]For conditions, see Table I. [b]Relative to eicosane (R_T 1.00). [c]Abbreviations: BL, dioxaboralane; BA, dioxaborane.

The derivative of fucose gave a mass spectrum (Fig. 15) showing the absence of trimethylsilyl groups and a molecular ion at *m/e* 212, which indicates the presence of two boronate groups. The intensity of the peak corresponding to the dioxaboralane fragment and the composition of the ion at *m/e* 168 ($C_6H_{10}B_2O_4$) suggests that the two boronate groups form adjacent, five-membered 1,2:3,4-cyclic esters (32). The derivatives of pentoses and 6-deoxyhexoses had a volatility sufficient that at low g.l.c.–column temperatures these carbohydrates could adequately be resolved from

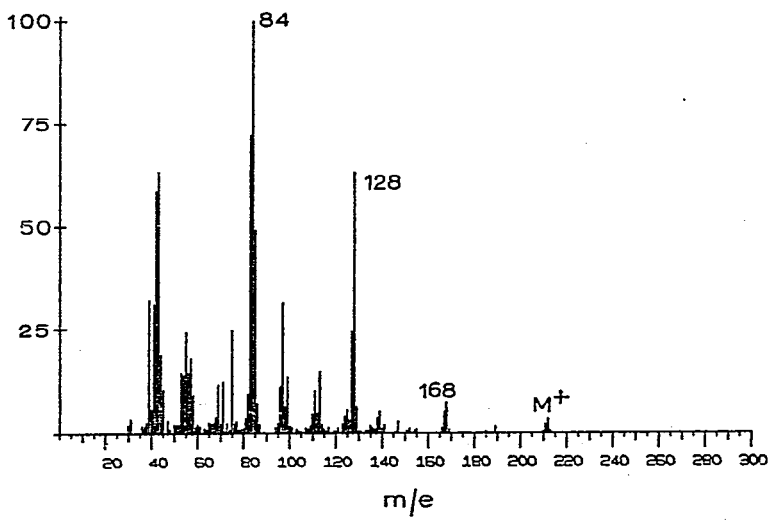


Fig. 15. Mass spectrum of α-L-fucopyranose 1,2:3,4-dimethaneboronate (32).

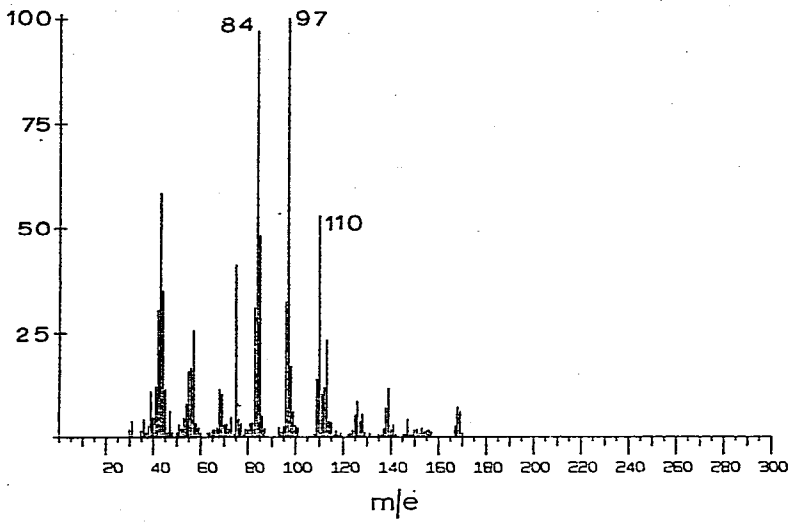
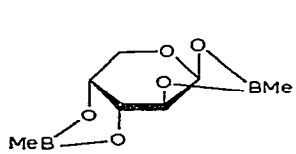


Fig. 16. Mass spectrum of α-D-xylofuranose 1,2:3,5-dimethaneboronate (33).

the solvent peak. The mass spectra of the D-xylose derivative (Fig. 16) indicates a mol. wt. of 198 and the absence of any trimethylsilyl group, suggesting a cyclic 1,2:3,5-diboronate with a furan ring (33).

As just discussed, the esterification with boronic acids of vicinal *cis* hydroxyl groups at C-1 and C-2 forms preferentially one anomer from an equilibrium mixture, the α-anomer for both L-fucose (32) and D-xylose (33), O-1 being incorporated into a five-membered boronate ester ring. Similarly, the β-anomer of D-arabinose has the vicinal *cis* configuration at C-1–C-2, and the mass spectrum of the derivative (Fig. 17) indicated the absence of trimethylsilyl groups and the presence of two boronate



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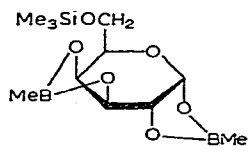

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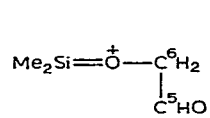
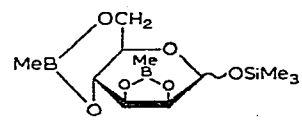


36 *m/e* 117 ($C_4H_9O_2Si$)



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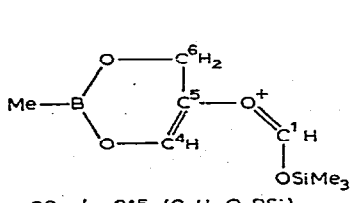
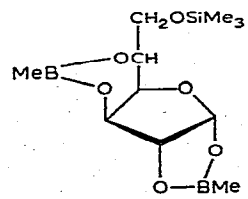


38 *m/e* 215 ($C_8H_{16}O_4BSi$)



39

groups, with a molecular ion at 198. These data suggest the formation of a cyclic 1,2:3,4-diboronate structure (**34**).

The borosilylation of D-galactose, D-mannose, and D-glucose resulted also in the formation of a single derivative for each compound, and the m.s. are presented in Figs. 18, 19, and 20, respectively. The $(M-15)^+$ ion at *m/e* 285 (mol. wt. 300), and the absence of an ion at *m/e* 147 indicate the presence of two cyclic boronate groups. The m.s. are markedly different from each other, owing to the different positions of

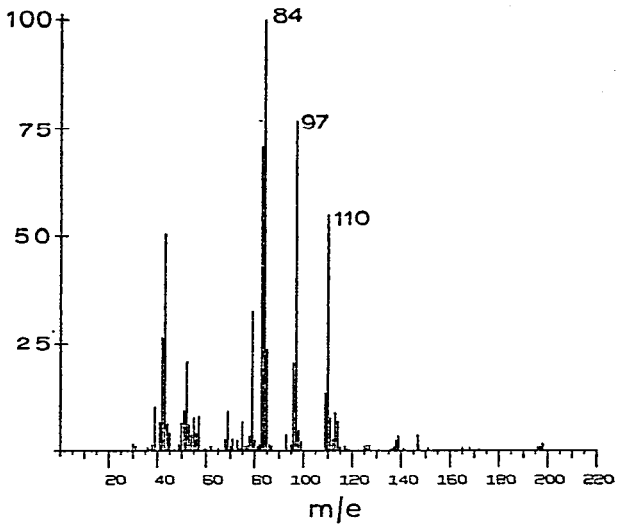


Fig. 17. Mass spectrum of β-D-arabinopyranose 1,2:3,4-dimethaneboronate (34).

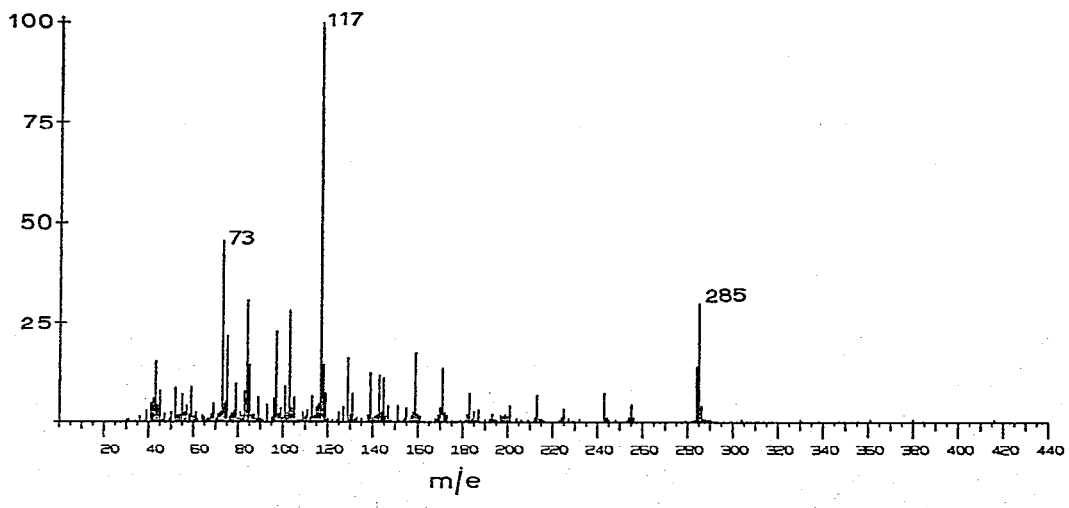


Fig. 18. Mass spectrum of 6-*O*-(trimethylsilyl)-α-D-galactopyranose 1,2:3,4-dimethaneboronate (35).

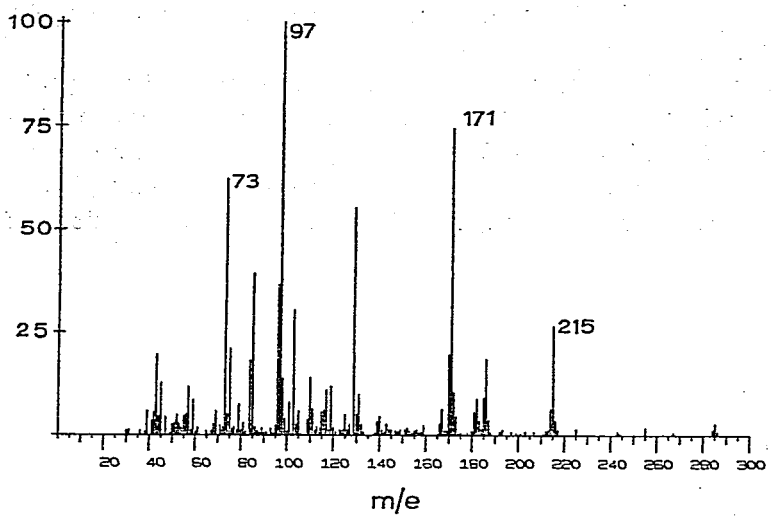


Fig. 19. Mass spectrum of trimethylsilyl α,β-D-mannopyranoside 2,3:4,6-dimethaneboronate (37).

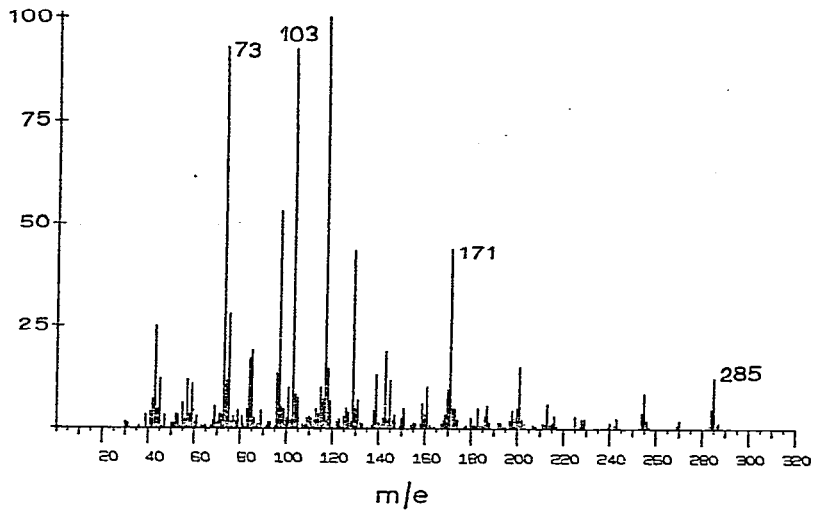


Fig. 20. Mass spectrum of 6-*O*-(trimethylsilyl)-α-D-glucofuranose 1,2:3,5-dimethaneboronate (39).

the dioxaboralane or dioxaborane ring. The high intensity of the ion at *m/e* 117 (36) in the mass spectrum of the D-galactose derivative (Fig. 18) indicates that the trimethylsilyloxy group is linked at C-6 (35). The mass spectrum of the D-mannose derivative (Fig. 19) indicates cyclic 2,3:4,6-diboronate groups with a trimethylsilyloxy group at C-1 (37), a structure supported by the ion at *m/e* 215 (38). The mass spectrum of the derivative of D-glucose (Fig. 20) shows an ion of high intensity at *m/e* 103, which suggests a furan ring and two 1,2:3,5-boronate groups (39).

EXPERIMENTAL

Chemicals. — Methane-, butane-, and benzene-boronic acids were purchased from Alpha Inorganics, Inc., Beverly, Massachusetts 01915. All carbohydrates were obtained from Pfanstiehl Laboratories, Inc., Waukegan, Illinois 60085, or from Professor R. W. Jeanloz, Harvard Medical School, Boston, Massachusetts 02139. Chlorotrimethylsilane and trifluorobis(trimethylsilyl)acetamide were purchased from Supelco, Inc., Bellefonte, Pennsylvania 16823, and, when mixed in equal quantities, were used as a silylating reagent.

Sample preparation. — A solution of carbohydrate (~200 μg) in a culture tube, equipped with a Teflon-lined screw-cap, was evaporated to dryness under a nitrogen stream, the last residues of water being removed as an azeotrope by addition of dichloromethane, followed by evaporation. The boronic acid (~20-molar excess) in pyridine, was added to the tube, which was closed, and kept for 30 min at 110° in a heating block. The silylation reagent [50 μl, containing equal parts of chlorotrimethylsilane and trifluorobis(trimethylsilyl)acetamide)] was added directly to the bottom of the culture tube, which was maintained at the heating-block temperature. The reaction tubes were sealed and, after 10 min, were removed. Aliquots of the clear solution were directly injected into the gas chromatograph.

Gas–liquid chromatography. — G.l.c. was performed with a Perkin–Elmer 900 chromatograph equipped with a stainless-steel column (0.3 mm × 1.5 m) of 0.05% OV-1 or OV-17 on glass beads (120–140 mesh). The temperature was programmed to increase at a rate of 8° per min, starting at 80°. Eicosane was used as the internal standard.

Mass spectrometry. — Mass spectra were recorded with a Perkin–Elmer 990 gas chromatograph, interfaced with a Perkin–Elmer–Hitachi RMU-6L mass spectrometer connected with an IBM 1800 computer. The ionizing voltage was 70 eV. The temperature of the ion source and manifold (including fritted-glass separator and valve) were held constant at 250°. High-resolution mass spectra were determined with a CEC-110, dual-focusing photoplate instrument (DuPont Instruments, Monrovia, California 91016).

REFERENCES

1 R. A. Hites and K. Biemann, *Anal. Chem.*, 42 (1970) 855–860.
2 H. S. Hertz, R. A. Hites, and K. Biemann, *Anal. Chem.*, 43 (1971) 681–691.
3 C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497–2507.
4 D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 91 (1969) 1728–1740.
5 V. N. Reinhold and K. Biemann, unpublished results.
6 C. J. W. Brooks and J. Watson, *J. Chem. Soc. Chem. Comm.*, (1967) 952–953.
7 C. J. W. Brooks and J. Watson, in C. L. A. Harbourn and R. Stock (Eds.), *Gas Chromatography*, Institute of Petroleum, London, 1968, pp. 129–141.
8 G. Anthony, C. Brooks, I. Maclean, and I. Sangster. *J. Chrom. Sci.*, 7 (1969) 623–631.
9 C. J. W. Brooks and I. Maclean *J. Chrom. Sci.*, 9 (1971) 18–24.
10 P. J. Wood and R. Siddiqui, *Carbohyd. Res.*, 19 (1971) 283–286.
11 F. Eisenberg, Jr., *Carbohyd. Res.*, 19 (1971) 135–138.