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THE USE OF BUTANEBORONIC ESTERS IN THE GAS–LIQUID CHROMATOGRAPHY OF SOME CARBOHYDRATES*

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ABSTRACT

Gas–liquid chromatographic data for the butaneboronic esters and trimethylsilyl derivatives of some monosaccharides and alditols are described. Mass-spectral data for the trimethylsilylated butaneboronates of D-fructose, D-glucose, D-galactose, and D-mannose are also recorded. The butaneboronic esters are useful for the qualitative identification of some monosaccharides, and are of particular value in the quantitative analysis of mixtures of D-glucose and D-fructose. The low rate of trimethylsilylation of D-fructose is examined.

INTRODUCTION

G.l.c. of suitably volatile derivatives of carbohydrates, such as methyl ethers[1] and trimethylsilyl (Me_3Si) ethers[2], is complicated by multiple peaks arising from the various structural and anomeric forms of each sugar. This is not always a disadvantage, but may be avoided by conversion into an open-chain derivative such as the alditol[3], *O*-methyl oxime[4], or aldononitrile[5,6]. Of these methods, reduction to the alditol is the most commonly used, but destruction of the anomeric centre can lead to ambiguity in, for example, the analysis of mixtures of glucose and fructose.

The use of cyclic boronic esters for g.l.c. of compounds containing diol groups was described by Brooks and Maclean[7], and preliminary reports[8,9] indicated a potential for the analysis of carbohydrates as their butaneboronic esters. The difunctional nature of the butaneboronic acid [$BuB(OH)_2$] and the consequent steric requirements for reaction might lead to the preponderant, or exclusive, formation of one derivative from an anomeric mixture of sugars, thereby making possible a simplified g.l.c. analysis of some sugar mixtures. In addition, the formation of constitutional isomers from diastereomers might allow unequivocal identification by mass spectrometry[18].

Preliminary results[8] showed that L-fucose, L-arabinose, and D-xylose gave single peaks on g.l.c. following reaction with $BuB(OH)_2$. Trimethylsilylation of the

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reaction products from hexoses was necessary for formation of suitably volatile derivatives. D-Glucose and D-fructose gave single peaks on g.l.c., but D-galactose and D-mannose showed single, major peaks with at least one minor component. The present report extends these preliminary results.

RESULTS AND DISCUSSION

A number of the common, naturally occurring carbohydrates were treated with $BuB(OH)_2$ alone, $BuB(OH)_2$ followed by trimethylsilylation, or trimethylsilylation reagents alone, and the results are summarized in Table I. No satisfactory peaks were observed on g.l.c. (column 1) for the $BuB(OH)_2$ or $BuB(OH)_2/Me_3Si$ derivatives of D-galacturonic acid, D-glucuronic acid, D-glucurono-6,3-lactone, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-glucose.

As reported by Eisenberg[9,10], g.l.c. of the butaneboronates of the alditols showed single peaks, but the peaks from L-arabinitol and xylitol were broad and much retarded on the column. Following trimethylsilylation, sharp peaks were obtained, but all the alditols, with the exception of xylitol, showed the presence of more than one product.

Synthetic studies[11,12] have shown that the boronic esters of L-fucose, D-xylose, and L-arabinose contain no free hydroxyl groups and are thus unaltered by trimethylsilylation. With column 1, no g.l.c. peaks were observed for the product of reaction of the hexoses with $BuB(OH)_2$ alone. Attempts to synthesise the butaneboronate of D-glucose failed when high-vacuum distillation of the product gave a viscous, presumably polymeric, residue and a distillate which, after trimethylsilylation, showed two major peaks and some minor peaks on g.l.c. This may account for Eisenberg's observation[9,10] of two major peaks and one minor peak on g.l.c. of the butaneboronate of D-glucose.

Therefore, as esters containing free hydroxyl groups may be unstable at elevated temperatures, further studies were concentrated on the Me_3Si derivatives and restricted to the commonly occurring sugars L-fucose, L-arabinose, D-xylose, D-fructose, D-galactose, D-mannose, and D-glucose.

Heating in dry pyridine with excess $BuB(OH)_2$ at 100° for 10 min, followed by trimethylsilylation essentially as described by Sweeley *et al.*[2], gave the best results. At lower temperatures (*e.g.*, 30°), complete reaction with $BuB(OH)_2$ took 4 h or longer. Use of ethyl acetate as a solvent, 2,2-dimethoxypropane as a water scavenger, and alternative silylation procedures were of no advantage; water, which produces excessive formation of a white precipitate, must be excluded from the reaction mixture. The small amount of precipitate formed otherwise does not affect the analysis.

The effect of varying the $BuB(OH)_2$–sugar ratios is shown in Fig. 1. With the exception of D-mannose, the relative area of the major peak from each sugar was approximately constant over a wide range, although D-glucose showed a small

TABLE I

G.L.C. RETENTION TIMES[a]

Sugar	*Column 1*[b]			*Column 2*[b]	
	a	b	c	b	c
Erythritol	0.73	0.44(*s*),0.47(*s*), 0.69(*s*),0.78(*m*)	0.19(*m*) 1.33(*s*)	0.39,0.42(*m*), 0.52,0.56	0.22
L-Arabinitol	2.03(*b*)	0.61(*s*),0.95,1.11(*m*)	0.44	0.54(*s*),0.66(*s*), 0.70,0.78(*m*)	0.37
Xylitol	2.09(*b*)	1.06	0.41	0.52(*s*),0.75(*m*)	0.36(*m*),0.46(*s*)
D-Glucitol	1.97	1.93	0.78	0.80,0.84,1.05(*m*)	0.50
D-Mannitol	1.76	1.22,1.27,1.74(*m*)	0.77	0.67(*s*),0.77(*s*), 0.80(*s*),0.84,0.86(*m*)	0.49(*m*),0.58(*s*)
Galactitol	2.10	1.18(*m*),2.07	0.81	0.81(*m*),0.82(*s*),1.10	0.55(*m*),0.59(*s*)
2-Deoxy-D-*erythro*-pentose	n.p.	0.48(*m*),0.55,0.64	0.16,0.19(*m*),0.24	—	—
D-Ribose	1.40 (*sh*)	0.60(*m*),0.90	0.33(*m*),0.35	—	—
L-Arabinose	1.24	1.24	0.31(*m*),0.38,0.41	0.75	0.32,0.34,0.39(*m*)
D-Xylose	1.39	1.36	0.39,0.47(*m*)	0.80	0.37(*s*),0.42,0.47(*m*)
L-Fucose	1.07	1.07	0.29,0.32,0.41(*m*)	0.70	0.33,0.37,0.43(*m*)
L-Rhamnose	1.10(*s*),1.44(*m*)	0.45(*m*),1.43	0.29(*m*),0.39	—	—
D-Glucose	n.p.	1.56	0.64,0.85(*m*)	0.90	0.52,0.62(*m*)
D-Mannose	n.p.	1.34	0.50(*m*),0.69	0.62(*s*),0.78,0.86(*m*)	0.44(*m*),0.54,0.67(*s*)
D-Galactose	n.p.	1.28	0.59,0.63,0.72(*m*)	0.68(*s*),0.78(*s*),0.85(*m*)	0.47,0.50,0.56(*m*)
D-Fructose	n.p.	1.21	0.55(*m*),0.57,0.75(*s*)	0.61(*s*),0.81(*m*)	0.44(*m*),0.57

[a]Relative to methyl palmitate (column 1) and methyl arachidate (column 2) of reaction products with (*a*) $BuB(OH)_2$ alone, (*b*) $BuB(OH)_2$ followed by HMDS and Me_3SiCl, and (*c*) HMDS and Me_3SiCl. [b]n.p., No peak; *m*, main peak; *s*, small peak; *b*, broad peak; *sh*, peak with shoulder.

decrease, and D-fructose, and possibly L-arabinose, a slight increase in response for a 10-fold excess of $BuB(OH)_2$.

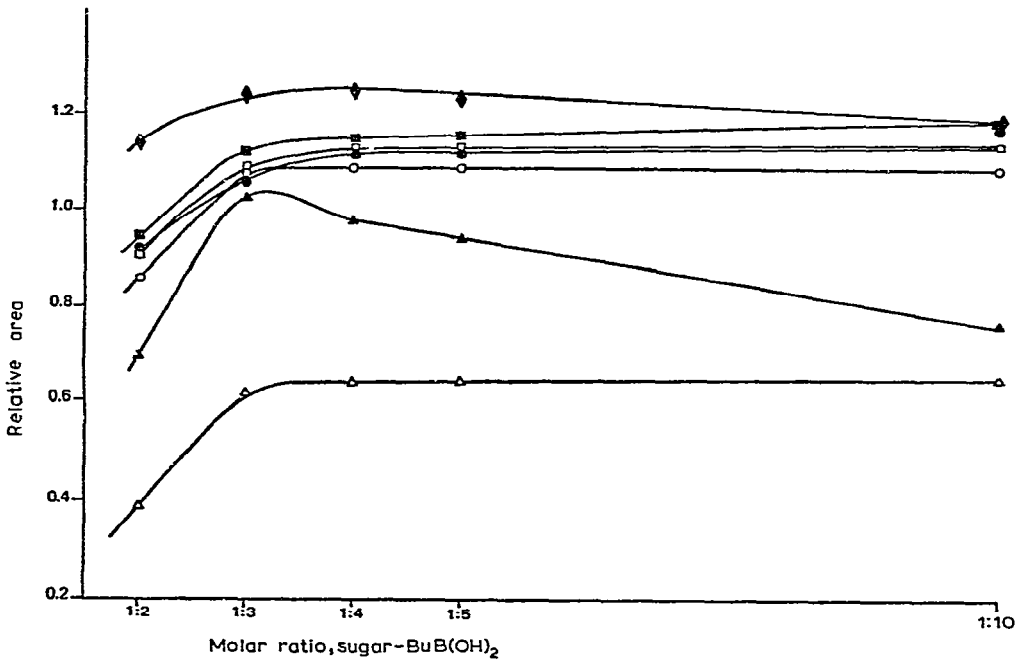

Fig. 1. Effect of varying the $BuB(OH)_2$–sugar ratio on area response (relative to methyl arachidate) of: ◆, D-glucose; ■, D-fructose; ●, L-arabinose; □, D-xylose; ○, L-fucose; ▲, D-mannose; △, D-galactose.

Methyl arachidate was an appropriate, inert, internal standard, for which the response of the flame-ionisation detector was linear in the range 0.5–10 μg. The response of the major peak from each monosaccharide was also linear from 0.1–10 μg, except for D-xylose which showed a small departure from linearity above 7.5 μg, and D-galactose, the minor peak of which increased at low concentrations at the expense of the major peak. The total area, however, showed a linear response.

The $BuB(OH)_2$–Me_3Si derivatives of all the sugars, except D-mannose, were stable for at least one week in the trimethylsilylation mixture. The main-peak area of D-mannose declined by 40% during that period.

Throughout these preliminary studies, difficulties with reproducibility were encountered. In some instances, numerous small and unidentified peaks appeared on the chromatograms. One factor contributing to this seemed to be variability in the butaneboronic acid samples. The dry reagent is extremely susceptible to breakdown, but use of the wet reagent should be avoided since moisture interferes with ester formation. Material that has been dried on a porous plate at room temperature for 1–3 h is therefore recommended. Excess reagent appeared as a peak

arising from tributylboroxine (identified by mass spectrometry) on the gas chromatogram (Fig. 4).

Quantitative measurements were first attempted with mixtures of D-glucose and D-fructose. It is perhaps not widely realised that the trimethylsilylation of fructose does not reach completion as rapidly as the reaction with glucose. As illustrated in Fig. 2 and Table II, using the method of Sweeley *et al.*[2], reaction is not complete at room temperature in less than 6 h. However, once the reaction is complete, the peak ratios and the total area remain constant for at least 2 days. Although this has received some mention in the literature[13,14,19], the quantitative consequences of the varying number and ratio of peaks does not seem to have been emphasised.

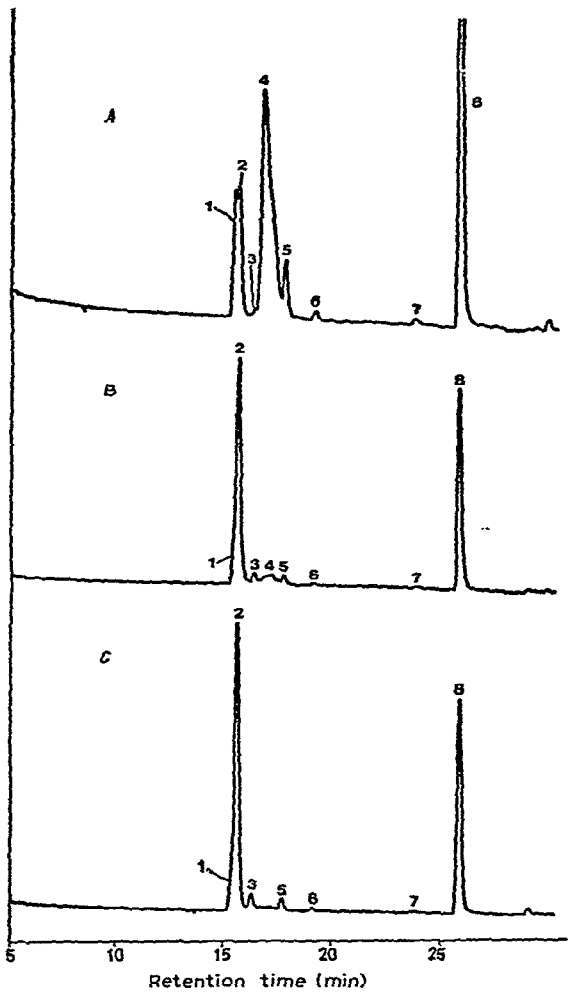

Fig. 2. Gas chromatogram (column 3) of trimethylsilyl ethers of D-fructose as formed at room temperature: *A*, 0.5-h reaction; *B*, 4-h reaction; *C*, 24-h reaction. Peaks 1–5, D-fructose; peaks 6 and 7, unknown; peak 8, methyl arachidate.

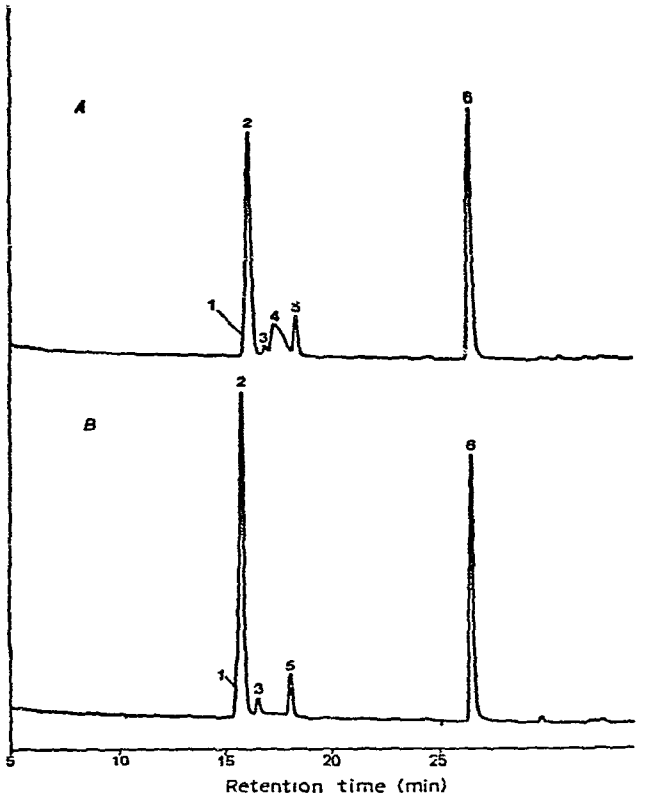

Fig. 3. Gas chromatogram (column 3) of trimethylsilyl ethers of D-fructose as formed at 61°: *A*, 0.5-h reaction; *B*, 4-h reaction. Peaks 1–5, D-fructose; peak 6, methyl arachidate.

Trimethylsilylation at elevated temperatures (60°) had the effect shown in Fig. 3. Again, both total and relative peak-areas changed with time, but more rapidly and towards a different end-product than for the reaction at room temperature, and peak 4 did not entirely disappear, causing difficulties in integration. The 4-h reaction mixture did not change on standing at room temperature overnight. Use of freshly prepared, dry pyridine did not affect the results; the effect was not due to solubility, as similar results were obtained when D-fructose was maintained for 1 h up to 25 h in pyridine at room temperature prior to trimethylsilylation. Varying the volume of pyridine, or amounts of hexamethyldisilazane and Me_3SiCl caused small changes in peak ratios. The trimethylsilylation of D-glucose, however, was complete within 0.5 h at room temperature. No significant change in relative-area response of D-glucose was observed over a period of 2–3 weeks.

Consequently, analysis of glucose and fructose as the Me_3Si ethers requires an overnight reaction period. In contrast, the butaneboronic ester–Me_3Si ether can be prepared for g.l.c. in 50 min, and since single peaks were obtained for each sugar (Fig. 4), determination of peak areas was simplified. Analysis of some mixtures are

TABLE II

THE EFFECT OF TIME ON THE TRIMETHYLSILYLATION OF D-FRUCTOSE AND D-GLUCOSE

Sugar and peak		T[a]	*Relative area*[b]				
			30 min	*1.25 h*	*4 h*	*1 day*	*2 days*
D-Fructose:	1	0.58	0.21				
	2	0.59	0.20	0.91	1.32	1.41	1.42
	3	0.62	0.01	0.05	0.10	0.12	0.13
	4	0.64	0.90	0.46	0.10		
	5	0.68	0.06	0.06	0.05	0.05	0.04
Total		—	1.37	1.49	1.57	1.58	1.59
D-Glucose	α	0.66	0.73	0.73	0.72	0.72	0.72
	β	0.74	0.94	0.94	0.93	0.92	0.93
Total		—	1.67	1.66	1.65	1.64	1.65

T, Retention time relative to methyl arachidate. [b]Area relative to methyl arachidate.

shown in Table III. Good accuracy, particularly for the glucose–fructose ratio, was obtained over a wide range. The method has been successfully applied to the analysis of honey[15].

Extension of quantitative analysis to other sugars has met with less success. The results for a number of mixtures of D-glucose, L-fucose, L-arabinose, and D-xylose are shown in Table IV. Small amounts of unreacted sugars appear as the Me_3Si ethers (*e.g.*, Fig. 4), but are well separated from the butaneboronate–Me_3Si ether peaks. The reaction mixtures of both D-xylose and L-arabinose, however,

TABLE III

DETERMINATION OF D-GLUCOSE AND D-FRUCTOSE BY G.L.C. OF THEIR TRIMETHYLSILYLATED BUTANEBORONIC ESTERS

Wt. of sugar used (mg)		*Recovery of sugar (%)*		*Calculated ratio*
D-*Glucose*	D-*Fructose*	D-*Glucose*	D-*Fructose*	D-*glucose*:D-*fructose*
1	1	97	97	1:1
1	2	100	100	1:2
2	1	97	97	2:1
0.5	4	100	101	1:8.1
4	0.5	101	98	8.2:1
1	10	103	103	1:10
10	1	103	103	10:1
0.5	10	102	102	1:20
10	0.5	103	100	21:1
0.25	10	87	95	1:43
10	0.25	97	98	39:1
0.1	10	104	113	1:108
10	0.1	106	100	106:1

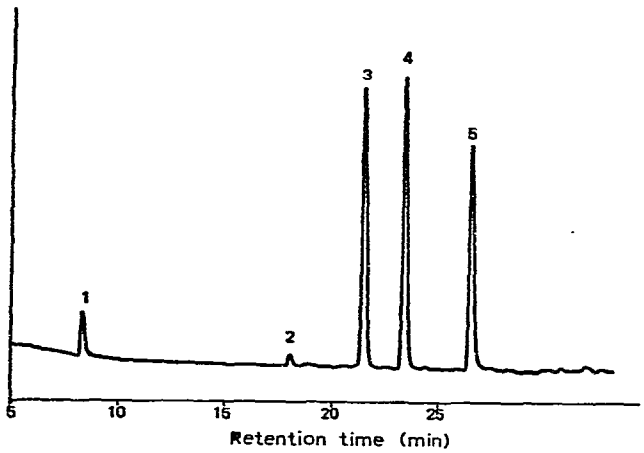

Fig. 4. Gas chromatogram (column 3) of $BuB(OH)_2$–Me_3Si derivatives of D-glucose and D-fructose: peak 1, tributylboroxine; peak 2, Me_3Si ether; peak 3, D-fructose; peak 4, D-glucose; peak 5, methyl arachidate.

TABLE IV

DETERMINATION OF SOME MONOSACCHARIDES BY G.L.C. OF THEIR TRIMETHYLSILYLATED BUTANEBORONIC ESTERS

Wt. of sugar used (mg)[a]				*Recovery of sugar (%)*			
Fuc	*Ara*	*Xyl*	*Glc*	*Fuc*	*Ara*	*Xyl*	*Glc*
1	1	1	1	99	103	106	104
4	2	3	1	103	102	104	96
3	1	2	4	99	100	99	99
1	3	2	4	94	103	102	103
0.5	0.5	0.5	10	84	94	94	102
0.25	0.25	0.25	10	78	92	91	99
0.25	10	0.25	0.25	98	106	109	97
0.25	0.25	10	0.25	98	100	111	107
10	0.25	0.25	0.25	104	106	96	112
0.25	1	0.5	0.5	95	102	102	99
0.25	5	0.5	1	92	106	105	103
0.25	1	5	0.5	97	102	109	99
0.5	0.5	1	0.25	93	99	107	98
0.25	1	0.5	5	87	98	96	105
5	0.5	1	0.25	102	103	103	106

showed minor, unidentified g.l.c. peaks, possibly arising from impurity in the sample or from other reaction products. Relative areas were corrected accordingly. Thus, the reaction product of D-xylose contained a g.l.c. peak (2% of the main xylose component) having the same retention time as the butaneboronate of L-fucose, and another peak (0.4% of the main xylose peak) having the same retention time as the

butaneboronate of L-arabinose. A very minor peak from the reaction product of L-arabinose (0.001% of main peak) interfered with the butaneboronate of L-fucose. Although unacceptably large errors are sometimes encountered, for example, when small amounts of fucose are estimated in the presence of large amounts of glucose, the overall, average errors in determinations were L-fucose, −5%; L-arabinose, +1%; D-xylose, +2%; D-glucose, +2%.

Columns 2 and 3 have been found to provide the best separations of the butaneboronic esters and their Me_3Si ethers. Relative response factors are shown in Table V. These are susceptible to variations of ±5% over a period of time and for quantitative work should be checked regularly.

TABLE V

RESPONSE FACTORS[a]

Sugar	*Response factor*[a]
L-Fucose	1.12
L-Arabinose	1.15
D-Xylose	1.15
D-Fructose	1.15
D-Glucose	1.20

[a]By weight, relative to methyl arachidate.

Mass spectra of the butaneboronic esters of L-fucose, L-arabinose, and D-xylose and the benzeneboronates of D-glucose and D-fructose have been reported [11,12]. The spectra from the major $BuB(OH)_2$–Me_3Si derivatives of D-glucose, D-fructose, D-galactose, and D-mannose are shown in Figs. 5 and 6. Although there are wide variations in peak intensity, the spectra are quite similar and show ions typical of both butaneboronic esters[11,12] (*m/e* 139, 127, 126), and trimethylsilyl ethers[16,17] (*m/e* 129, 117, 103, 73). The molecular ions (*m/e* 384) for the trimethyisilylated bis(butaneboronates) of these sugars are not visible in Figs. 5 and 6, but were observed at higher concentrations of sample. A distinctive M−15 peak (*m/e* 369) was easily observed in each spectrum, except that of the mannose derivative where, nevertheless, it was observed at higher concentrations of sample. The peak at m/e 370 shown in Fig. 6B was attributed to background; in all of these spectra, some of the higher mass peaks, shown at higher sensitivity, probably arise from background noise.

1-*O*-Trimethylsilyl-*β*-D-fructopyranose 2,3:4,5-bis(butaneboronate)[12] may be distinguished from the derivatives of the aldohexoses reported here by the peak for M−103 (*m/e* 281) which arises from the scission, characteristic of 2-ketohexoses[16], of the C-1–C-2 bond.

A clear distinction between the derivatives of glucose and galactose, on the basis of their mass spectra alone, is less straightforward. However the mannose derivative may be distinguished by the low intensity of the peak at *m/e* 117, which

is the base peak in the spectra of the glucose and galactose derivatives. Also, a prominent peak at *m/e* 228 further distinguishes the mannose from the galactose and glucose derivatives.

The $BuB(OH)_2$–Me_3Si reaction products of both galactose and mannose showed minor peaks on g.l.c. A distinctive mass spectrum was only obtained from the minor peak of the mannose reaction mixture, and indicated that this peak was from a tri-*O*-trimethylsilyl butaneboronate. The molecular ion (*m/e* 462) was not observed, but ions at M−15 and M−103 were. The base peak for the derivative was *m/e* 73, in keeping with the increased number of trimethylsilyl groups. This derivative had a retention time in g.l.c. between the values for bis(butaneboronates) and the conventional, fully trimethylsilylated monosaccharides, thus providing further evidence for a monoboronate structure.

This, and previous studies[11,12], show that some commonly occurring monosaccharides may be identified by g.l.c.–m.s. However, reliable quantitative analysis seems to be restricted to simple mixtures, in particular of glucose and fructose.

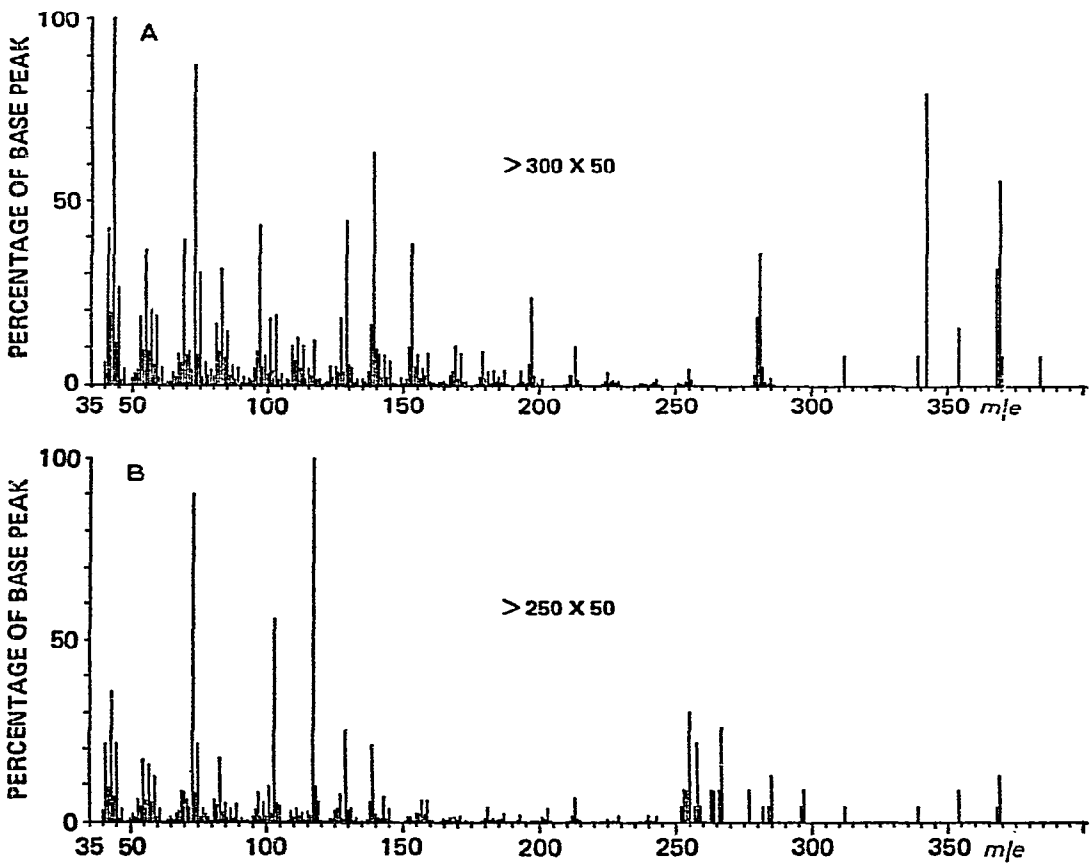

Fig. 5. Mass spectra of the $BuB(OH)_2$–Me_3Si derivatives of: A, D-fructose; B, D-glucose.

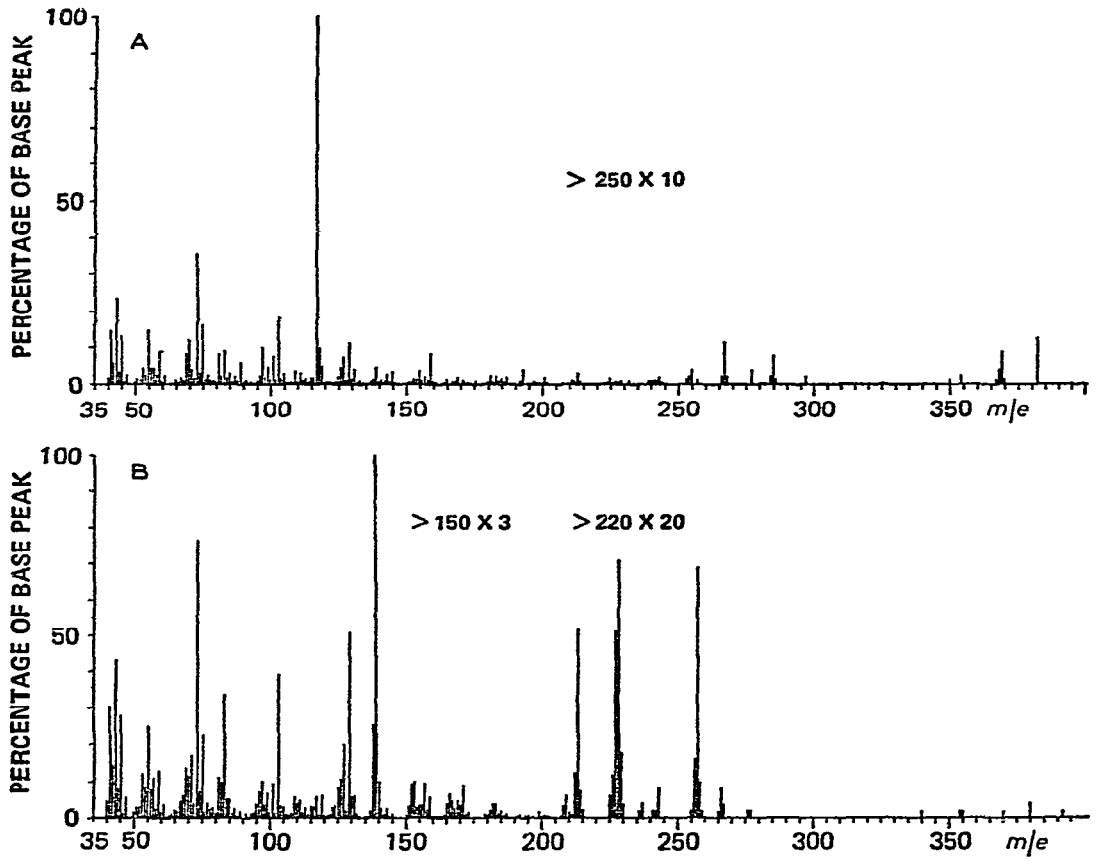

Fig. 6. Mass spectra of the $BuB(OH)_2$–Me_3Si derivatives of: A, D-galactose; B, D-mannose.

EXPERIMENTAL

General. — G.l.c. was carried out on a Pye 104 dual-column chromatograph with flame-ionisation detection and glass columns: (1) 7 ft × 0.25 in. containing 1% of ECNSS-M on 80–100 mesh Gas Chrom Q, injection at 115° with programming at 5°/min to 190°; (2) 5 ft × 0.25 in. containing 3% of OV-225 on 80–100 mesh Gas Chrom Q, injection at 100° with programming at 5°/min to 240°; (3) 5 ft × 0.25 in. containing 3% of OV-225 on 80–100 mesh Chromosorb W-HP, injection at 100° with programming at 5°/min to 270°. Nitrogen carrier-gas, at a flow rate of 45 ml/min, was used for each column. Peak areas were determined by measurement of height × (width at half-height) or by electronic integration (Hewlett Packard, Model 3373B or Columbia Scientific Industries Model CSI-208).

Butaneboronic acid was obtained from Applied Science Labs, Inc., and pyridine (silylation grade), hexamethyldisilazane (HMDS), and chlorotrimethylsilane from the Pierce Chemical Co. Sugars were obtained from the Fisher Scientific Co.

Ltd. and Pfanstiehl Laboratories Ltd., and were equilibrated in water for at least 18 h. Aliquots were evaporated, and pyridine was distilled 2–3 times from the residues before use. Evaporations were at 37° under reduced pressure.

Preparation of butaneboronic esters and their trimethylsilyl ethers. — For the general survey of sugars and alditols, samples (4 mg) were dissolved in pyridine (2 ml) containing an internal standard (2 mg; methyl palmitate or methyl arachidate). Aliquots (0.5 ml) were (*a*) heated with $BuB(OH)_2$ (5 mg) at 100° for 10 min; (*b*) heated with $BuB(OH)_2$ (5 mg) at 100° for 10 min, cooled to room temperature, and trimethylsilylated with HMDS (0.2 ml) and Me_3SiCl (0.1 ml); (*c*) trimethylsilylated with HMDS (0.2 ml) and Me_3SiCl (0.1 ml). Samples were then diluted with pyridine to 1 ml in a volumetric tube, and aliquots (1 μl) injected into the gas chromatograph.

Reaction at 30°. — Sugars were reacted with $BuB(OH)_2$ as described above, but at 30° for 5 min, 10 min, 20 min, 1 h, 4 h, and 20 h. Following trimethylsilylation, aliquots (1 μl) were injected into the gas chromatograph.

Alternative silylation procedures and use of 2,2-dimethoxypropane. — The butaneboronic ester of D-glucose was prepared as described above, and aliquots were treated with (*a*) Me_3SiCl (0.1 ml) followed by HMDS (0.2 ml); (*b*) *N,O*-bis(trimethylsilyl)acetamide (BSA) (0.1 ml; 12.5% v/v in pyridine); (*c*) BSA (0.1 ml; 12.5% v/v in pyridine) followed by Me_3SiCl (0.1 ml); (*d*) 2,2-dimethoxypropane (5 μl) prior to trimethylsilylation.

Effect of varying the $BuB(OH)_2$–sugar ratio. — Aliquots (0.5 ml) of sugars (8 mg) in pyridine (4 ml) containing methyl arachidate (8 mg) were heated at 100° for 10 min with 1:1, 2:1, 3:1, 4:1, 5:1, and 10:1 molar excess of $BuB(OH)_2$. Following trimethylsilylation, samples were analysed by g.l.c.

Linearity of response and response factors. — Response factors were determined relative to methyl arachidate, using at least two different amounts of sugar. Linearity of response was determined in the range 0.1 to 10 μg, without use of the internal standard. A weight ratio of 3:1 $BuB(OH)_2$–sugar was used. Regression analysis was used to determine linearity.

Quantitative analysis of sugar mixtures. — Each mixture (containing 0.1–10 μg of each sugar) was heated at 100° for 10 min in pyridine (0.5 ml) containing methyl arachidate (1 mg) and a three-fold excess by weight of $BuB(OH)_2$. Following trimethylsilylation, the sample was diluted with pyridine in a volumetric tube to 1 ml, and aliquots (1 μl) were injected into the gas chromatograph.

Trimethylsilylation of D-fructose and D-glucose. — D-Glucose (1 mg) or D-fructose (1 mg) in pyridine (~0.5 ml) containing methyl arachidate (1 mg) were trimethylsilylated by addition of HMDS (0.2 ml) and Me_3SiCl (0.1 ml). After dilution to 1 ml with pyridine, aliquots (1 μl) were injected into the gas chromatograph at intervals. Another sample of D-fructose was treated similarly, but the trimethylsilylation was carried out at 61° for 4 h followed by overnight reaction (21 h) at room temperature. Aliquots (1 μl) were injected into the gas chromatograph at intervals.

A further sample of D-fructose (10 mg) was dissolved in pyridine (1 ml),

aliquots (0.1 ml) were removed at intervals (0–25 h), and methyl arachidate (1 mg), HMDS (0.2 ml), and Me_3SiCl (0.1 ml) were added. After 0.5 h, the samples were diluted to 1 ml with pyridine, and aliquots (1 μl) were injected into the gas chromatograph.

G.l.c.–mass spectrometry. — Mass spectrometry was carried out on a combined Finnigan 3100D GC/MS equipped with a column (5 ft × 0.25 in.) of 3% of OV-17, using a program of 12°/min from 120° and helium carrier-gas. The separation temperature was 230°, analyser temperature 110°, and ionising electron energy 70 eV. The spectra were recorded as bar graphs by means of the Finnigan 6000 MS Data System.

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