Boroacetylation of Carbohydrates. Correlations between Structure and Mass Spectral Behavior in Monoacetylhexose Cyclic Boronic Esters[1]

Jacek Wiecko and William R. Sherman*

Contribution from the Departments of Psychiatry and Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110.
Received February 13, 1976

Abstract: The interaction of an acetyl moiety with boron accompanied by the loss of alkyl radical is shown to occur following electron beam ionization in the mass spectrometer. This is similar to the interaction of phosphate with boron previously shown to occur in alkaneboronic esters of certain sugar phosphate dimethyl esters. In the case of acetates, the interacting moiety can frequently be introduced into a hexose in an equilibrium reaction in which treatment with an alkaneboronic acid is followed by acetylation of the remaining hydroxyl. The interaction, following ionization, is shown to be localized to the adjacent cyclic boronate in the case of D-glucose and D-galactose alkaneboronate acetates. The use of this interaction as a stereochemically sensitive probe of structure is demonstrated. In the course of this work, the cyclic bis(alkaneboronates) of monoacetylated D-glucose, D-galactose, D-mannose, D-idose, D-allose, D-altrose, D-talose, D-glucose, D-fructose, D-sorbose, D-tagatose, and D-psicose were examined by mass spectrometry. High-resolution data, as well as specific deuterium labeling of the six carbon atoms of glucose, reveal the origin of characteristic classes of ions.

The preparation of a substance for mass spectral analysis by gas chromatography–mass spectrometry (GC–MS) is an opportunity to exercise some control over the fragmentation process of the ionized molecule. Cyclic boronic esters of carbohydrates are useful because they have steric requirements for their formation and thus confer an additional degree of specificity on stereoisomers. In this respect they resemble isopropylidene derivatives. The boronic esters, however, are rapidly formed in reactions which give quantitative conversions with many sugars.

The isopropylidene ketals[2] have a further similarity to the alkaneboronates in that both are capable, on electron beam ionization, of losing an alkyl side chain from the derivative moiety to produce a high abundance even electron ion. This similarity is only a formal one, however, because of the different electronic structures of boron and carbon. In the isopropylidene case, the radical loss from carbon produces an ion that is readily stabilized by the 1,3-dioxolane structure. The situation with boron is very different in that the cyclic boronate is incapable of stabilizing the charge left by the departing radical without the intervention of another functionality. We have reported on this interaction in the case of cyclic alkaneboronates of sugar phosphates.[3] In the latter the phosphate is the interacting moiety which, when favored by proximity, supports the formation of high abundance $[M - alkyl]^+$ ions.

The principal subject of the present paper is the nature of this process in monoacetates of sugar alkaneboronates. The interaction of the acetate with boron appears weaker than that of phosphate, thus more stereoselective, and therefore a more useful probe of structure. Furthermore, the single acetyl moiety is easily introduced in equilibrium reactions with sugars and alkaneboronic acids. The reactions give products whose structures are often predictable from the stereochemistry of the parent sugars. The esterification of the remaining hydroxyl of aldo- and ketohexoses also improves the gas chromatographic (GC) separation characteristics of the sugar boronates.

The trimethylsilyl (Me_3Si) group has also been reported as a coderivatizing functionality of carbohydrate alkaneboronates.[4] The mass spectra of these compounds are dominated by processes related to the Me_3Si group.

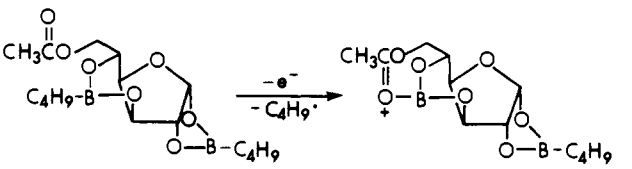
In addition to their usefulness as structurally informative derivatives, the acetate boronates are valuable in the measurement of stable isotope labels, for example, in glucose.[5] Part of this advantage results from the character of the natural composition of boron in which the minor abundance isotope is lighter. Thus, heavy isotope labels are measured against smaller contributions from endogenous species in the sample. Furthermore, particularly in the case of the acetate butaneboronate of glucose, a large portion of the ion current is carried by $[M - C_4H_9]^+$ which retains all of the skeletal carbon and hydrogen atoms.

Results and Discussion

The acetate boronates fall into several structurally related groups and these are discussed in turn.

The supporting data for all the ion structures in this study include high-resolution spectra and, in the case of D-glucose, spectra of the cyclic diboronates of the 1-, 2-, 3-, 4-, [5-$^2H_1$]-, [6,6-$^2H_2$]-, and [$^2H_7$]hexose. No metastable ions were found using the LKB-9000 mass spectrometer.

Aldofuranose 6-Acetates. When a solution of D-glucose anomers in pyridine is treated with butaneboronic acid followed by the addition of acetic anhydride, a rapid and quantitative conversion to α-D-glucofuranose cyclic 1,2:3,5-bis(butaneboronate) 6-acetate (**1**) takes place. The structure of **1** is uniquely defined by the mass spectra of its individual deuterium-labeled analogues, by arguments presented previously,[1,3] and by the fact that **1** is formed from D-glucose 6-acetate (as prepared by the method of Duff[6]) when the latter is treated with butaneboronic acid in pyridine.



1 *m/e* 297

The second most abundant ion in the mass spectrum of **1** (Figure 1) is that at *m/e* 297, formed by expulsion of a butyl radical and charge stabilization via interaction of acetate with the boron atom of the 3,5-cyclic boronate. The extent of this interaction and the abundance of the $[M - alkyl]^+$ ion can be modified by stereochemistry, structural isomerism, and the size of the departing radical. That the interaction occurs is supported by several arguments already presented,[1,3] among them

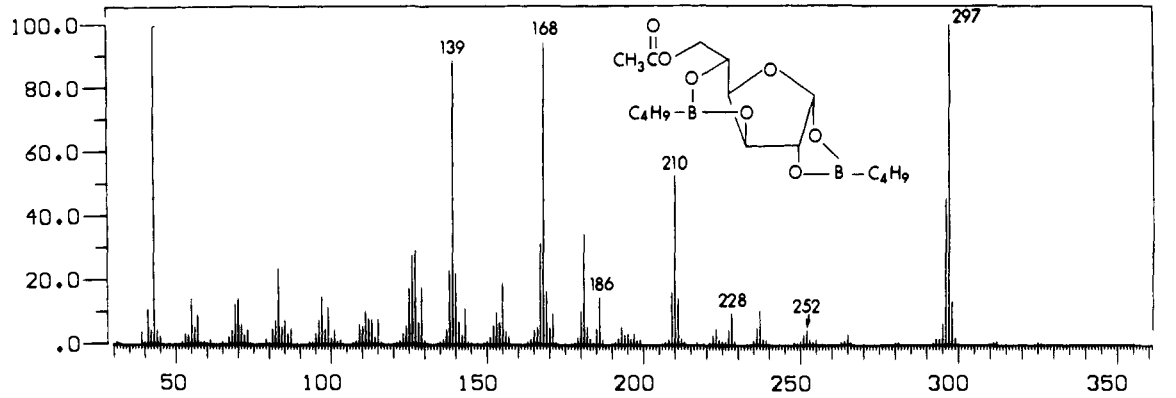


Figure 1. The 70-eV mass spectrum of α-D-glucofuranose cyclic 1,2:3,5-bis(butaneboronate) 6-acetate (**1**) normalized to *m/e* 297.

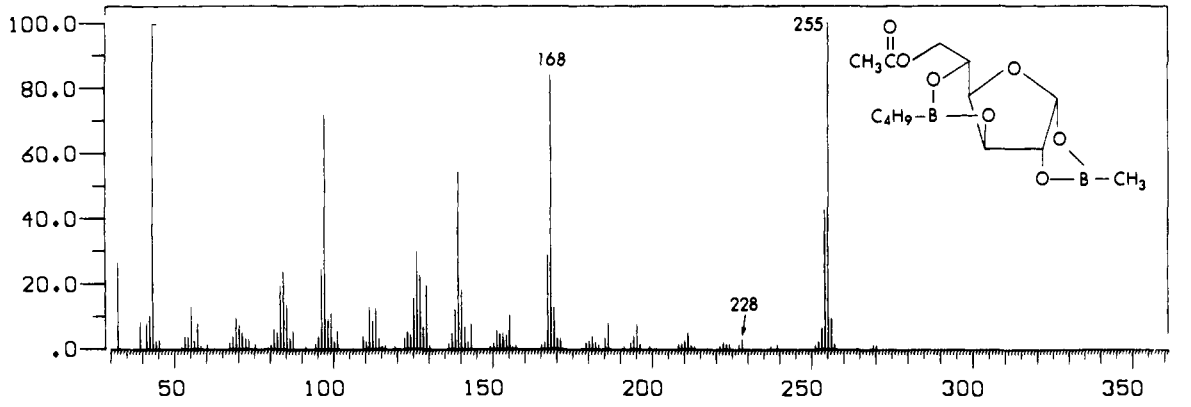


Figure 2. The 70-eV mass spectrum of α-D-glucofuranose cyclic (1,2-methaneboronate 3,5-butaneboronate) 6-acetate (**3**) normalized to *m/e* 255.

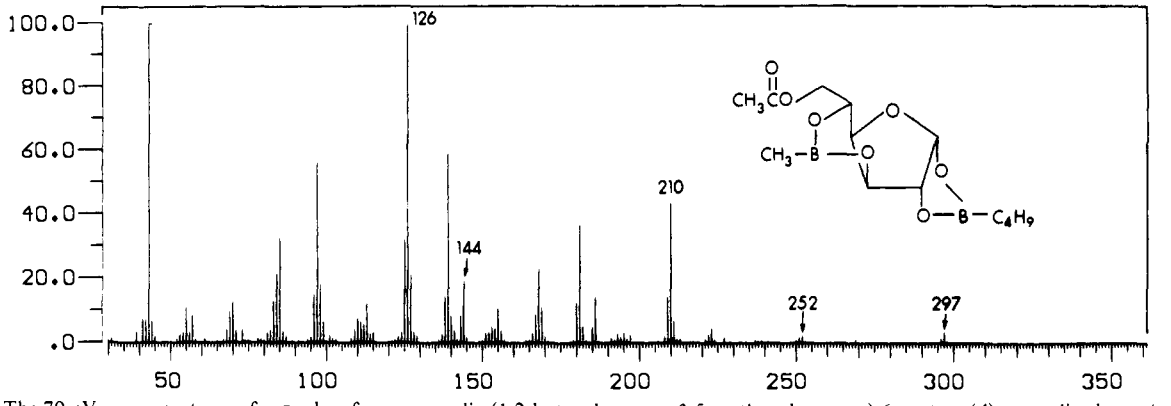


Figure 3. The 70-eV mass spectrum of α-D-glucofuranose cyclic (1,2-butaneboronate 3,5-methaneboronate) 6-acetate (**4**) normalized to *m/e* 126.

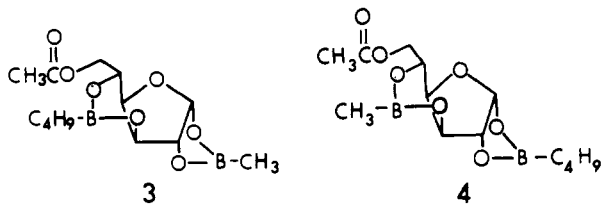
the fact that cyclic borenium ions are unlikely in the gaseous state[7] and that, in the mass spectra of phosphate-boronates of sugars, ions having P-O-B bonds are observed whereas these bonds are not known to occur in the ground-state molecules.

Additional evidence will now be presented that the interaction illustrated by the structure of the ion at *m/e* 297 occurs as depicted.

When methaneboronic acid and acetic anhydride react with D-glucose in pyridine, the methaneboronate **2** analogous to **1** is produced. GC-MS of **2** affords a spectrum with an $[M - CH_3]^+$ ion at *m/e* 255 of low abundance when compared with $[M - C_4H_9]^+$ in Figure 1 (Σ_{40} *m/e* 297, 6.9% in **1**; Σ_{40} *m/e* 255, 0.39% in **2**). The enhanced intensity of the $[M - R]^+$ ion in the butaneboronate mass spectrum may be due to stabilization of the departing radical by rearrangement to the *tert*-butyl species, a view supported by the similar high abundance of $[M - R]^+$ in the spectrum of the analogous bis(octaneboronate) of D-glucose 6-acetate.[8]

We were able to put this difference to use in an experiment showing that the acetyl-boron interaction is confined to only one of the two cyclic boronic ester groups in D-glucose. When D-glucose was reacted with a tenfold molar excess of an equimolar mixture of methane- and butaneboronic acids both α-D-glucofuranose cyclic (1,2-methaneboronate 3,5-butaneboronate) 6-acetate (**3**) and (1,2-butaneboronate 3,5-methaneboronate) 6-acetate (**4**) were formed. These compounds were found to separate by gas chromatography to a degree that made possible the acquisition of a spectrum of each isomer uncontaminated by the other (Figures 2 and 3).

In the spectra of **3** and **4** the ion which corresponds to $[M - C_4H_9]^+$ is *m/e* 255 while $[M - CH_3]^+$ occurs at *m/e* 297. When the spectra are compared, *m/e* 255 is found in higher



3 4

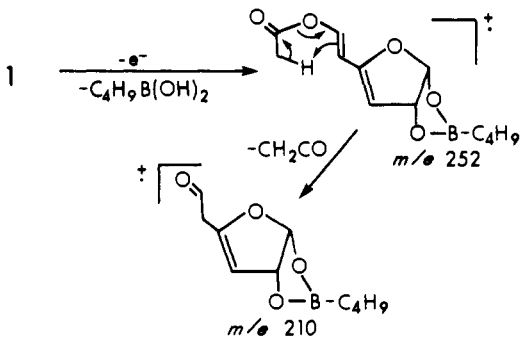
abundance in the earlier eluting species which, logically, would have the structure **3**. In the second GC peak *m/e* 297 is of low intensity. This is consistent with what is observed in the spectra of the homogeneous derivatives **1** and **2**, that is, when butyl radical is lost, a larger abundance ion is produced than when methyl radical is lost.

More importantly, the loss of $C_4H_9\cdot$ is not random; it occurs in only one of the two ester positional isomers, as does the loss of $CH_3\cdot$, and thus only one of the cyclic boronates is accessible to the acetate. Not only do the structures of **3** and **4** require the $M - C_4H_9\cdot$ loss to occur uniquely from **3** but this conclusion is supported by the intensities of other ions in the spectra. It will be shown that ions at *m/e* 228 (parent molecule: the bis(butaneboronate)) as well as at 144 and 126 (parent molecule: the bis(methaneboronate)) carry the cyclic 3,5-boronate moiety. The ion at *m/e* 210 (bis(butaneboronate) series) retains the cyclic 1,2-boronate residue. Figures 2 and 3 show these species to occur in abundances which are consistent with the structures assigned to **3** and **4**.

From the above it is apparent that the interaction of the 6-acetate is only with the adjacent cyclic 3,5-boronate and thus can be considered to have potential as a probe of geometry and substitution at that position.

The spectra of **1, 2, 3,** and **4** contain two families of odd-electron fragments that are of interest both because they retain discrete portions of the parent sugar and because they uniquely describe the structure of the diboronate acetate of D-glucose.

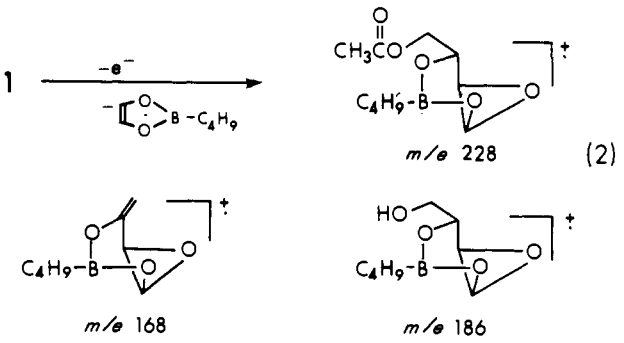
The ions at *m/e* 210 and 252 are particularly remarkable in that their structures, and therefore that of **1**, are so strongly indicated by deuterium labeling. Using the individually labeled glucose species, one 6-D and 80% of 4-D are found to be lost from the above ions; the rest of the labels are retained. With CD_3 acetate only one deuterium is retained in *m/e* 210. Barring extensive rearrangement, this behavior can only be explained by the process shown in eq 1.



(1)

The second odd-electron series which supports the 1,2:3,5-diboronate structure of **1** is comprised of ions at *m/e* 228, 168, and 186 (eq 2).

A source of confusion occurs in the comparison of ions retaining a single boronate alkyl moiety. This results partly from two different processes which occur in the fragmentation of alkaneboronate acetates resulting in a loss of 42 amu. These are the loss of ketene from ions containing the acetate group, and of C_3H_6 from ions containing the butaneboronate moiety. In addition, the difference in mass between methyl and butyl of 42 amu adds a further complication. Thus, caution must be

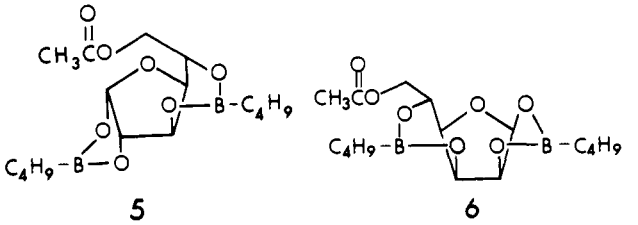


(2)

used when comparing the low-resolution spectra, especially those of the mixed methane- and butaneboronates. This is seen in a listing of equivalent ions of the two series, e.g., *m/e* 210 (butaneboronate series) ≡ *m/e* 168 (methaneboronate series); 252 ≡ 210; 228 ≡ 186; 186 ≡ 144; 168 ≡ 126.

The odd-electron ions in the two alkaneboronate series have comparable intensities, unlike the even-electron ions resulting from the loss of an alkyl radical, i.e., ions at *m/e* 297 and 255. Thus, the likelihood of losing a neutral species such as alkaneboronic acid (e.g., the 3,5-boronate expulsion in eq 1) or of the loss of the cyclic boronate with C-1 and -2 of glucose (as in eq 2) is independent of the alkyl group size. This adds support to our suggestion that the enhanced abundance of *m/e* 297 in the spectrum of **1** as compared to *m/e* 255 in the spectrum of **2** is a result of a process unique to the radical loss, such as stabilization of the butyl radical by rearrangement.

Mention has been made of the use of the acetate–boronate interaction as a probe of structure in the "mixed" derivatives **3** and **4** above. Another example of this is found when comparing the spectra of the 1,2:3,5-bis(butaneboronate) 6-acetate of D-glucose and of D-idose, enantiomers except at C-5. The spectrum of α-D-idofuranose cyclic 1,2:3,5-bis(butaneboronate) 6-acetate (**5**) differs from that of **1** in a dramatic reduction of *m/e* 297 $[M - C_4H_9]^+$ (Σ_{40} 0.61%). Consideration of molecular models of the two isomers shows that the furanose ring would sterically interfere with the seven-membered ring arising from the carbonyl–boron interaction in the case of idose but not in glucose. D-Mannofuranose cyclic 1,2:3,5-bis(butaneboronate) 6-acetate (**6**) affords an example of a related type



5 6

of hindrance affecting the mass spectrum (cf. the case of the corresponding 6-phosphate[3]). The C-1,2 boronate bridge is cisoid with respect to both the 3,5-cyclic boronate and the 6-acetate (in **1** and **5** it is transoid). This appears to make the interaction of acetate with the 3,5-cyclic boronate more difficult (Σ_{40} *m/e* 297, 0.37%) without a compensatory acetate–1,2-boronate interaction.

Aldopyranose 6-Acetates. Under the equilibrium boroacetylation conditions (see the section below dealing with preparative aspects of boroacetylation) galactose yields a single product: the 1,2:3,4-diester of 6-acetylgalactopyranose (**7**). The mass spectrum of the 6-acetylgalactose diboronate is, as with glucose, analogous to that of the corresponding sugar phosphate[3] in that the boronate–acetate interaction is weak (*m/e* 297, Σ_{40} 0.52%), perhaps because it can be realized only through an eight-membered ring. However, the presence and stereospecificity of this interaction, even in this limited case, were demonstrated by mixed boroacetylation of galactose with

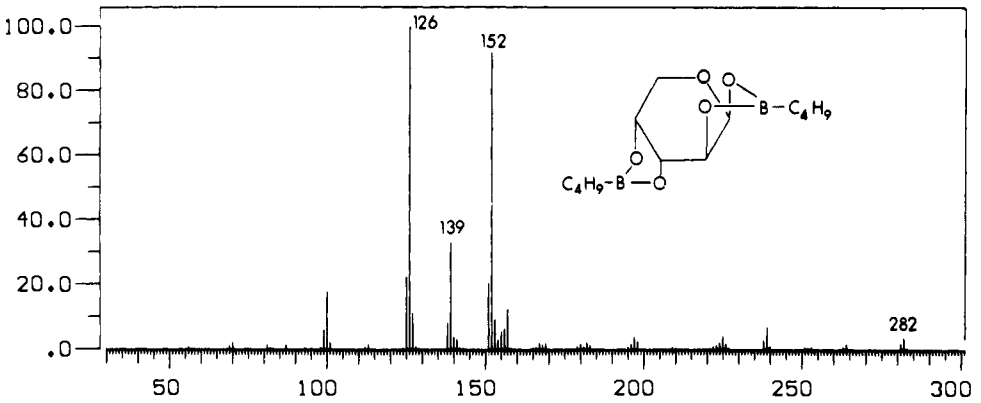


Figure 4. The 15-eV mass spectrum of β-D-arabinopyranose cyclic 1,2:3,4-bis(butaneboronate) normalized to *m/e* 126.

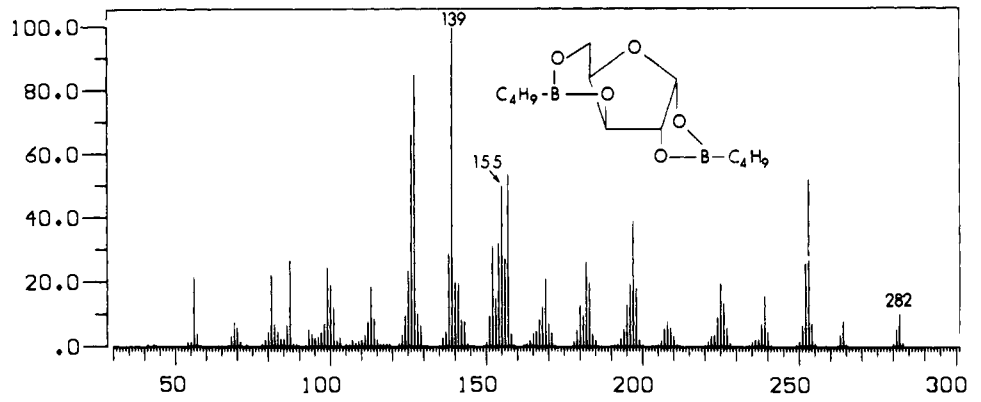
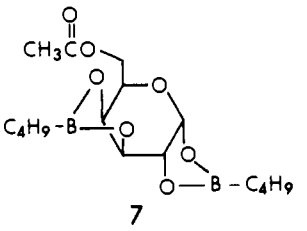


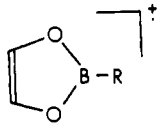
Figure 5. The 15-eV mass spectrum of α-D-xylofuranose cyclic 1,2:3,5-bis(butaneboronate) normalized to *m/e* 139.



7

both methane- and butaneboronic acids. The presence of two isomers, analogous to **3** and **4** above, was demonstrated by GC–MS. Only the later eluting species showed a detectable $[M - R]^+$ peak (at *m/e* 255), requiring that the C-3,4 boronic ester bear the butyl moiety in that isomer (the bis(methaneboronate) of 6-acetylgalactose shows no detectable $[M - CH_3]^+$ peak in its mass spectrum).

The base peak in the spectrum of **7** is *m/e* 126 (a) (Σ_{40} 10.8%) accompanied by *m/e* 70, the third largest peak in the spectrum (Σ_{40} 5.4%), formed by the loss of C_4H_8 from the *m/e*

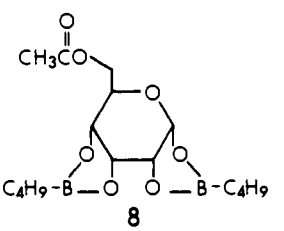


a, R = C_4H_9, *m/e* 126
R = CH_3, *m/e* 84
R = H, *m/e* 70

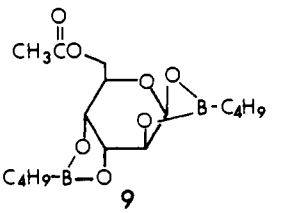
126 ion. In the corresponding methaneboronate derivative *m/e* 84 completely dominates the spectrum (Σ_{40} 35.0%). It is interesting to compare the spectrum of **7** with that of the bis(butaneboronate) of D-fucose (6-deoxygalactose), essentially **7** with a hydrogen atom replacing the acetoxy group.[9] Here the fragmentation is again simplified, with the expected two ions dominating the spectrum: *m/e* 126 (Σ_{40} 40.0%) and 70 (Σ_{40} 9.6%).

The spectrum of another simple pyranose diboronate, D-arabinopyranose 1,2:3,4-bis(butaneboronate),[10] also shows these ions in high abundance (Σ_{40} *m/e* 126, 17.7%; *m/e* 70, 7.7%). These ions are of lesser prominence in the spectra of furanose diboronates and it seems to be a general rule that aldopyranose structures extrude the fragment a more readily than do aldofuranose diboronates. This effect is more clearly seen at reduced ionization energy. As an example, compare the simplicity of fragmentation behavior of arabinose bis(butaneboronate) with the complex mass spectrum of the 1,2:3,5-xylofuranose cyclic diester, both at 15 eV (Figures 4 and 5).

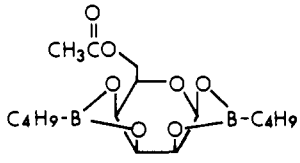
In order to obtain several monoacetylhexose diboronates not formed under the equilibrium boroacetylation conditions, a procedure in which acetylation[6] precedes boronation was used. This simple method (see Boroacetylation of Carbohydrates and the Experimental Section) made possible the acquisition of the spectra of 6-acetylallo-, 6-acetylaltro-, and 6-acetyltalopyranose 1,2:3,4-bis(butaneboronates) (compounds **8, 9,** and **10,** respectively).



8

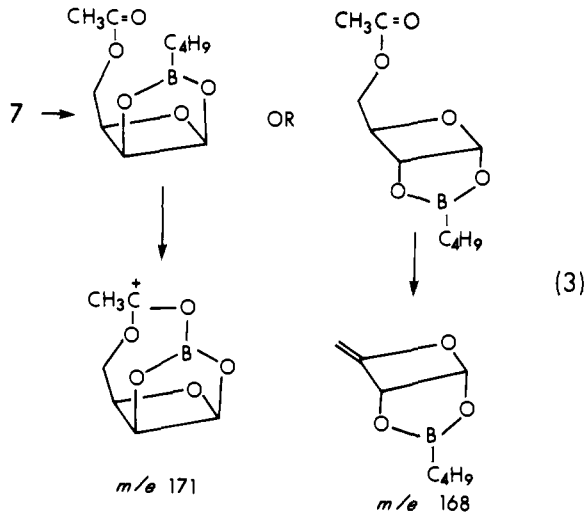


9



10

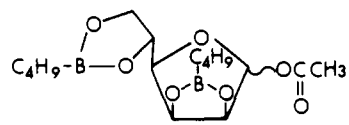
The spectra of the pyranose 6-acetates **7, 8, 9,** and **10** furnish another example of how the boron–acetyl interaction and its dependence on stereochemical factors can affect the fragmentation process. The 6-acetylpyranose molecule, after loss of the neutral fragment corresponding to the fragment a bearing C-1 and -2, or C-3 and -4, can, among other possible processes, eliminate acetic acid, giving a fragment ion at *m/e* 168, or, alternatively, can eject the butyl radical if interaction between the acetyl group and boron is stereochemically favored, giving rise to a peak at *m/e* 171. Equation 3 depicts this



(3)

process for **7.** It is clear that in molecules **7** and **9** either process is possible depending on which carbons are involved in the initial extrusion of the neutral fragment corresponding to a. For **8,** the formation of *m/e* 171 is not favored, as both cyclic boronate bridges are transoid to the acetyl group, whereas in **10** this situation is reversed. The intensity ratio of *m/e* 171 to 168 reflects a degree of this stereochemical control: it is 1.82, 0.13, 0.79, and 2.5 for **7, 8, 9** and **10,** respectively. It is noteworthy that the largest ratio is obtained when both cyclic boronate moieties are on the same side of the pyranose ring as the acetate (**10**).

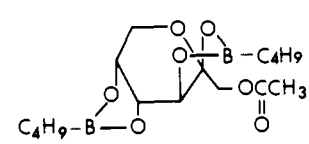
Aldose 1-Acetates. Under the equilibrium boroacetylation conditions D-mannose reacts to produce a 2,3:5,6-diboronate furanose 1-acetate (**11**). D-Gulose also reacts in this way. The spectra of these compounds are quite simple. There is no acetate–boronate interaction (*m/e* 297 absent). The peak at *m/e* 127, resulting from the scission of the C-4–C-5 bond, is the third most intense in these spectra (in the spectrum of **11,** Σ_{40} 4.4%; in the spectrum of the gulose derivative **12,** Σ_{40} 7.1%), and an ion not seen before, *m/e* 227, is found. This is the larger fragment resulting from the C-4–C-5 cleavage. The *m/e* 228, 186, and 168 odd-electron ion series is notably absent in these spectra, due to domination of the C-4–C-5 cleavage process.



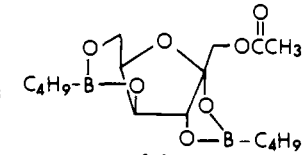
11

Ketoses. In the spectrum of the equilibrium boroacetylation product of D-fructose (**13**), *m/e* 281 is the base peak (Σ_{40} 12.2%; at 15 eV, 31.6%). This ion results from the loss of the C-1 acetoxymethyl radical and stabilization of charge by the two oxygen atoms this carbon is linked to. Most of the other ions unique to this structure appear to be derived from *m/e* 281, e.g., *m/e* 225 (loss of C_4H_8 from *m/e* 281), *m/e* 197 (loss of C_4H_9BO), and *m/e* 179 (loss of $C_4H_9B[OH]_2$).

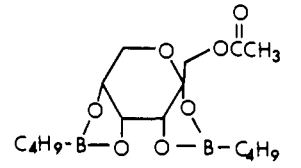
The spectrum of the product formed from the reaction of D-sorbose with acetic anhydride and butaneboronic acid suggests structure **14** for this molecule, differing from D-fructose in forming a furanose 1-acetate rather than a pyranose. The effect of this structural difference on the mass spectrum is notable, and explicable in terms of the carbohydrate ring size. It results in a reduction of the intensity of *m/e* 281, and an increase in the complexity of the spectrum of **14** in comparison to that of **13** particularly noticeable at reduced ionization energy (see Figures 6 and 7 for spectra taken at 15 eV). The reduction of the *m/e* 281 intensity (Σ_{40} 1.27% in the spectrum of **14**) probably results from the relatively greater difficulty of stabilization of this ion in the furanose **14** with fused five-membered rings which give rise to a more "buckled" structure than is the case with the pyranose (fructose) system of **13.** The achievement of a planar ion is easier in **13,** resulting in the more intense *m/e* 281. One of the boroacetylation products of D-psicose has a spectrum nearly identical with that of **13,** suggesting structure **15** for that species. One of the principal products of equilibrium boroacetylation of D-tagatose has a mass spectrum much like that of the D-sorbose butaneboronate **14** and is thus assigned structure **16.**



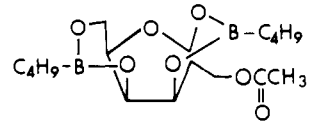
13



14

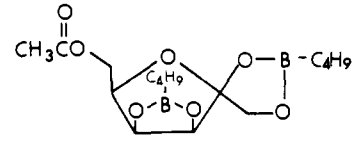


15



16

Under the equilibrium conditions D-tagatose also forms a bis(butaneboronate) which appears to be a 6-acetate. A spiro structure for this isomer (**17**) is consistent with the appearance



17

in its mass spectrum of two intense ions which probably arise from symmetrically related fragmentation pathways. In one case *m/e* 252 (Σ_{40} 4.9%), $C_{12}H_{22}B_2O_4$, seems to be produced by the loss of the elements of acetylglycolaldehyde (acetate, C-6, C-5, and the furan oxygen) from M^+. This ion is not observed in other spectra studied in this series of sugars. The second ion is *m/e* 155 (Σ_{40} 2.6%) $C_6H_8BO_4$, logically derived from $[M - C_4H_9\cdot]^+$ by further loss of the spiro moiety including C-1, C-2, and the furan oxygen. In the low-resolution spectrum of **17** *m/e* 155 is shared with the multiorigin ion $C_7H_{12}BO_3$ (vide infra) which is present in equal abundance and is distinguished from the ion unique to tagatose by not shifting in the CD_3-acetate analogue.

Common Multiorigin Mass Spectral Ions. The tricyclic carbohydrate–boronate systems formed in condensations of aldoses and ketoses with alkaneboronic acids consistently show,

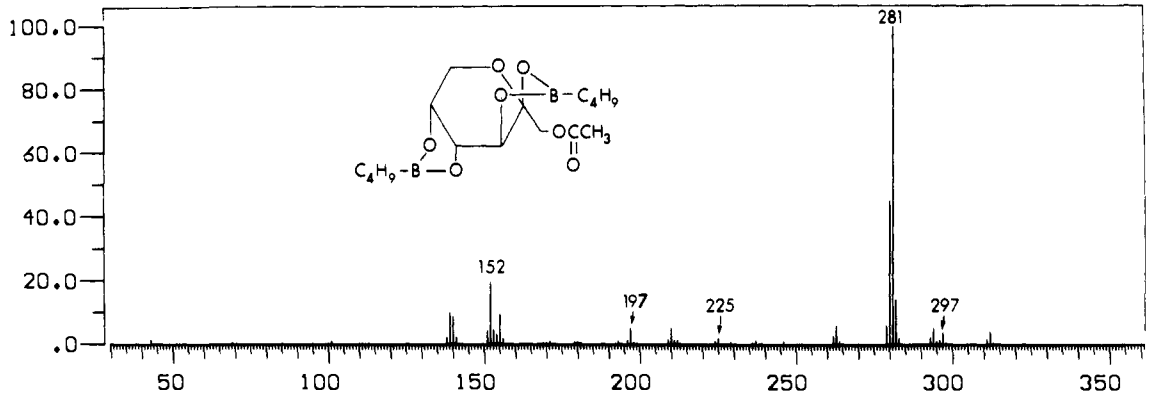


Figure 6. The 15-eV mass spectrum of β-D-fructopyranose cyclic 2,3:4,5-bis(butaneboronate) 1-acetate (**13**) normalized to *m/e* 281.

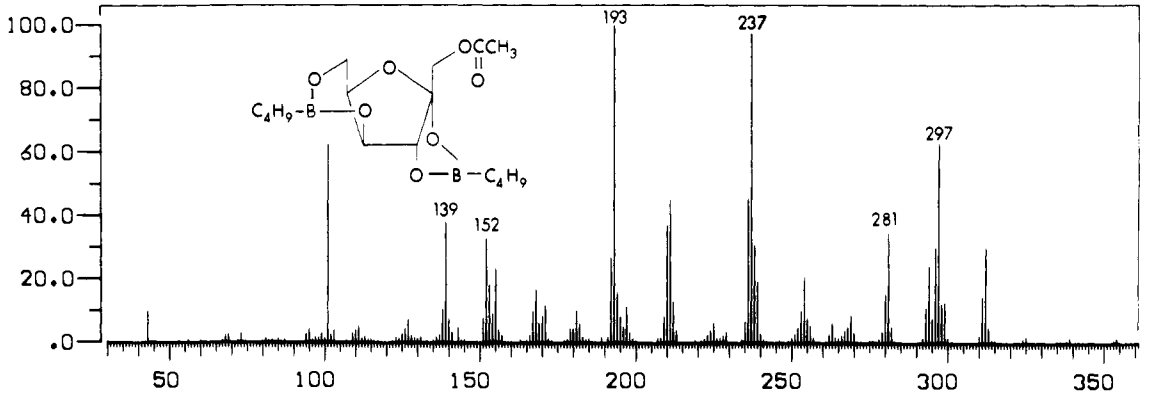


Figure 7. The 15-eV mass spectrum of α-D-sorbofuranose cyclic 2,3:4,6-bis(butaneboronate) 1-acetate (**14**) normalized to *m/e* 193.

in their mass spectra, a remarkable series of ions of nondiscrete origin (as shown by their label-shift behavior in the spectrum of **1**, for example). All the ions in this group have one unique characteristic in common: all can be formally represented as cyclic structures possessing a continuous overlapping system of π orbitals, with the ring sizes ranging from 5 to 11 atoms. In mass spectra of butaneboronates, the lightest ions belonging to this group are *m/e* 126 (fragment a) and *m/e* 139 $[C_7H_{12}BO_2]^+$, which is also present in the spectra of sugar phosphate-boronates.[3] Expansion of these two cyclic ions by one or more of three moieties can formally account for all the remaining ions in the series. The three moieties are: -CH=CH- (b, 26 amu), -CH=O- (c, 29 amu), and -(C_4H_9)BO- (d, 84 amu). With a as the starting point, *m/e* 152 is obtained upon insertion of b, and *m/e* 181 upon the additional insertion of c, which in turn yields *m/e* 265 after the insertion of d. Combination of a and c yields *m/e* 155 and the combination of *m/e* 139 and d accounts for *m/e* 223. The lighter members of the series (*m/e* 126, 139, and 155) usually have counterpart ions resulting from loss of C_3H_6 or C_4H_8 from the butyl side chain appearing in the spectra. It seems possible that these are fluctional, valence isomerizing species.

Boroacetylation of Carbohydrates. In order to prepare the cyclic boronic esters of hexoses in solution (usually in pyridine) so that they could be examined by gas chromatography or GC-MS, two different procedures were used. The first method, referred to earlier as the equilibrium boroacetylation, consists of first having the hexose react with the alkaneboronic acid so that any anomeric or isomeric (pyranose vs. furanose) forms of the free hexose are converted into one or more isomeric cyclic diboronate derivatives. Once equilibrium is reached (after about 2 h at room temperature), acetic anhydride is added in order to acetylate the remaining free hydroxyl group(s). Under these conditions, glucose, galactose, mannose, fructose, and sorbose give single products with butaneboronic acid (structures **1, 7, 11, 13,** and **14**). The GC retention times of these products are given in Table I. All other hexoses examined in this study give rise to two or more products under the equilibrium boroacetylation conditions. Further details are included in the Experimental Section.

Table I. Gas Chromatographic Properties of Monoacetylhexose Cyclic Boronates[a]

	Retention time, min	
	Bis(butaneboronate)[b]	Bis(methaneboronate)[c]
6-Acetylglucofuranose	8.3	10.5
6-Acetylgalactopyranose	8.0	9.7
1-Acetylfructopyranose	7.1	8.2
1-Acetylsorbofuranose	7.7	9.6
1-Acetylmannofuranose	9.9	

[a] Separations were carried out on 3% OV-17 coated on 100/120 mesh Gas-Chrom Q using 0.25 in o.d. × 6 ft silanized glass columns with He carrier flow of 40 ml/min. [b] Column temperature 200 °C. [c] Column temperature 150 °C.

The second procedure, permitting access to boronate acetates not formed under the equilibrium conditions, takes advantage of the fact that primary hydroxyl groups of free hexoses are acetylated more rapidly than the secondary ones in 50% acetic acid at 100 °C.[6] Typically, an aldohexose is acetylated in the 6 position to the extent of 20–30% after 12 h under these conditions, with the remaining material mainly consisting of unacetylated starting hexose. After boronation an aliquot of this mixture gives the 6-acetyl diboronate having the structure expected from stereochemistry of the parent sugar,

though other products may also be obtained. Additional data are given in the Experimental Section.

Experimental Section

Derivatization. Samples were usually prepared for gas chromatography as follows: 2 mg (0.01 mmol) of hexose was suspended in 1 ml of dry pyridine and 10 mg (0.1 mmol) of butaneboronic acid (Ventron-Alfa Inorganics) was added. After agitation on a shaker for 2 h the clear solution was treated with 50 μl (0.5 mmol) of acetic anhydride. One hour later the solution was ready for gas chromatography. The methaneboronates were prepared in the same way using methaneboronic acid. The hexoses D-glucose, D-galactose, D-mannose, D-fructose, and D-sorbose gave rise to a single product by GC analysis under these conditions. The following sugars gave rise to mixtures of two or more products, by GC analysis, with butanebonic acid. In the following list the name of each hexose is followed by retention times of the GC peaks, with the relative intensities of the peaks and the identifying numbers of the compounds discussed in the text given in parentheses. Chromatographic conditions were 3% OV-17 coated on 100/120 mesh Gas-Chrom Q in 6 ft × 0.25 in. o.d. glass columns operated at 210 °C with a carrier flow of 30 ml of He/min: D-idose, peaks at 6.4 min (relative peak height of 5, **5**) and 7.5 min (1); D-gulose, peaks at 5.4 min (1), 6.2 min (1), and 6.9 min (4, **12**); D-psicose, peaks at 3.9 min (10), 4.5 min (3), 5.3 min (2), and 5.9 min (1, **15**); D-tagatose, peaks at 5.7 min (8, **17**), 6.4 min (2, **16**), 8.9 min (1), and 9.8 min (1).

The acetate boronate derivatives of common hexoses, i.e., **1, 7, 11, 13,** and **14,** could be obtained in larger quantities (10–100 mg) sufficient for spectral examination by, for example, NMR, by boiling the hexose with a 10% excess of a boronic acid in dry dioxane.[11] This was followed by evaporation of the solvent and acetylation of the residue, dissolved in pyridine, using an excess of acetic anhydride. The butaneboronates of sugars are not crystalline solids, unlike the corresponding benzeneboronate derivatives (cf. the observations of Wood and Siddiqui[9]).

Partial acetylation of hexoses following the procedure of Duff,[6] followed by boronation, was accomplished as follows: a dilute (5 mg in 1 ml) solution of the hexose in 50% acetic acid was heated at 100 °C for 12 h. At this time an aliquot of the solution was taken to dryness under N_2 and taken up in pyridine. A five- to tenfold molar excess of butaneboronic acid (assuming a 25% yield of acetylation of the primary hydroxyl group of the hexose) was added and the mixture was then examined by GC–MS. The product formed from D-glucose by this method was identical with **1** by GC. In the following list, the names of hexoses are followed by data on the GC peaks they gave rise to when subjected to this procedure. The relative intensities are given in parentheses. The species discussed in the text are identified by their numbers whenever applicable. The GC conditions were identical with those given above for the equilibrium boroacetylation products, except that the column temperature was 215 °C: D-mannose, peaks at 2.7 min (1), 4.8 min (5, **6**) and 6.4 min (1); D-allose, peaks at 5.2 min (1), 5.7 min (2), and 6.4 min (6, **8**); D-altrose, peaks at 4.1 min (1), 5.2 min (2), and 6.3 min (10, **9**); D-talose, peaks at 3.8 min (7), 6.3 min (8), 7.4 min (6), 9.9 min (3, **10**), and 14.7 min (6).

Labeled Derivatives. D-Glucose-*1*-d_1, -*6,6*-d_2, and -d_7, as well as D-galactose-d_7 were purchased from Merck, Sharp and Dohme, Canada. D-Glucose-*2*-d_1 was obtained by introduction of one deuterium at C-2 of D-glucose 6-phosphate using phosphoglucose isomerase in D_2O[12] followed by dephosphorylation with bacterial (*Escherichia coli*) alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.). Glucose-*3*-d_1 and -*4*-d_1 were obtained from Professor A. S. Perlin. Glucose-*5*-d_1 was prepared by the procedure of Mackie and Perlin.[13] In all the labeled samples the extent of deuteration exceeded 95%, except in glucose-*4*-d_1, where it was 80 ± 5%.

Gas Chromatography–Mass Spectrometry. An LKB-9000 gas chromatograph–mass spectrometer–PDP-12 computer system was used to obtain low-resolution spectra.[14] The GC conditions were those given in the Derivatization section. The mass spectrometer was operated with 70-eV electron beam ionization energy, while the source and separator temperatures were 270 and 240 °C, respectively. The high-resolution spectrum of compound **1** (which was obtained by preparative GC) was carried out at the Battelle Memorial Institute Mass Spectrometry Laboratory with an A.E.I. MS-9 instrument operated at 500-eV ionizing potential,[15] source temperature 150–200 °C, direct probe 100–200 °C, a nominal resolution of 10 000, and an accuracy of at least 8 ppm for the measured masses of individual peaks matched to atomic compositions. High-resolution GC–MS of **7, 11, 13, 14, 16,** and **17** was carried out by Shrader Analytical and Consulting Laboratories, Detroit, Mich., with an A.E.I. MS-30 instrument operated at 70-eV ionizing voltage and nominal resolution of 10 000, with the separator and source temperatures at 200 °C.

Acknowledgments. This work was supported by National Institutes of Health Grants NS-05159, AM-17904, and RR-00954. We wish to thank Professor A. S. Perlin of McGill University, Montreal, Canada for the gift of glucose-*3*-d_1 and -*4*-d_1, Professor H. S. Isbell of American University for the gift of D-talose and D-altrose, and Dr. Robert Schaffer of the National Bureau of Standards for the gift of D-idose and D-psicose.

References and Notes

(1) J. Wiecko and W. R. Sherman, *Proc. Annu. Conf. Mass Spectrom. Allied Top., 22d,* 323 (1974).
(2) D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86,** 67 (1964).
(3) J. Wiecko and W. R. Sherman, *Org. Mass Spectrom.*, **10,** 1007 (1975).
(4) V. N. Reinhold, F. Wirtz-Peitz, and K. Biemann, *Carbohydr. Res.*, **37,** 203 (1974); P. J. Wood, I. R. Siddiqui, and J. Weisz, *ibid.*, **42,** 1 (1975).
(5) D. M. Bier, W. R. Sherman, W. H. Holland, and D. M. Kipnis, *Proc. Int. Conf. Stable Isot. Chem., Biol., Med., 1st,* 397 (1973); D. M. Bier, R. D. Leake, L. D. Gruenke, and M. A. Sperling, *Proc. Int. Conf. Stable Isot. Chem., Biol., Med., 2d,* A-4 (1975).
(6) R. B. Duff, *J. Chem. Soc.*, 4730 (1957); R. B. Duff and V. C. Farmer, *Biochem. J.*, **70,** 515 (1958).
(7) J. C. Kotz, R. J. V. Zanden, and R. G. Cooks, *Chem. Commun.*, 923 (1970).
(8) J. Wiecko and W. R. Sherman, *Proc. Annu. Conf. Mass Spectrom. Allied Top., 24th,* in press (1976).
(9) P. J. Wood and I. R. Siddiqui, *Carbohydr. Res.*, **36,** 247 (1974).
(10) P. J. Wood and I. R. Siddiqui, *Carbohydr. Res.*, **32,** 97 (1974).
(11) R. J. Ferrier, *J. Chem. Soc.*, 2325 (1961).
(12) I. M. Lowenstein, *Methods Enzymol.*, **6,** 878 (1963).
(13) W. Mackie and A. S. Perlin, *Can. J. Chem.*, **43,** 2921 (1965).
(14) W. R. Holmes, W. H. Holland, and J. A. Parker, *Anal. Chem.*, **43,** 1806 (1971).
(15) In a personal communication, Dr. R. L. Foltz described the spectra obtained at 500 eV with the Battelle combined electron ionization–chemical ionization source as not significantly different from spectra obtained with the conventional MS-9 operated at 70 eV.