STRUCTURE AND SYNTHETIC USES OF CYCLIC BENZENEBORONATES OF POLYHYDROXY COMPOUNDS

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C O N T E N T S

CONTENTS

(continued)

ABSTRACT

1. Mass spectrometry has been employed to obtain information concerning the predominant structures of the cyclic benzeneboronates formed from two 1,6-dideoxy-hexitols and three 1-deoxy-pentitols.

2. The structures and relative abundances of the isomeric bisbenzeneboronates formed from each of seven pentitols have been elucidated through mass spectroscopic and methylation studies. The g.c.-m.s. analysis of the products obtained from methylation, partial hydrolysis and acetylation of the bisbenzeneboronates, showed that all of the pentitol boronates investigated contained more than one structural isomer.

3. The composition of the crude bisbenzeneboronate products have been rationalised from considerations of the possible conformations of the proposed structures.

4. The benzeneboronate of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was synthesised in high yield. The structure was determined through mass spectrometric and methylation studies.

5. Using this aminoglycoside benzeneboronate, a derivative of a biologically important disaccharide was synthesised in excellent yield, illustrating the use of the cyclic benzeneboronate entity as a protective group. The configuration of the glycosidic linkage of the synthesised disaccharide was determined through infrared, proton magnetic resonance and polarimetric studies.

6. The selective replacement of a 2-phenyl-1,3,2-dioxaborolane ring by a 2,2-dimethyl-1,3-dioxolane ring with acidified acetone, termed ketolysis, was investigated. Methylation or trimethylsilylation or acetylation of the partially hydrolysed ketolysis product, followed by g.c.-m.s. analysis, afforded information concerning the structure of the monoketal produced.

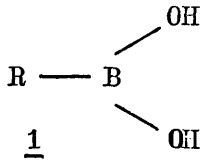
7. The ketolysis reaction was applied to four polybenzeneboronates which contained five- and six-membered benzeneboronate rings. The results showed that a 2-phenyl-1,3,2-dioxaborolane ring can be selectively replaced by a 2,2-dimethyl-1,3-dioxalane ring in good yield.

8. The synthetic use of the selective ketolysis of benzeneboronate esters was demonstrated through the preparation of 1,2-O-isopropylidene-D-mannitol in good yield.

CHAPTER I

GENERAL INTRODUCTION

Boronic acids have the general structure 1. The history of these compounds began in 1859 when FRANKLAND et al[1] obtained diethyl ethylboronate by the controlled oxidation of the spontaneously inflammable triethyl boron.



R = alkyl or aryl .

1

The boronate was subsequently hydrolysed to provide ethylboronic acid.

$$B(C_2H_5)_3 + O_2 \longrightarrow C_2H_5B(OC_2H_5)_2 \xrightarrow{H_2O} C_2H_5B(OH)_2 + C_2H_5OH$$

The synthesis of an arylboronic acid was first accomplished by MICHAELIS et al[2,3], by heating diphenylmercury and boron trichloride in a sealed tube for one hour at 180°, the phenylboron dichloride produced was rapidly hydrolysed to afford benzeneboronic acid.

$$HgPh_2 + 2BCl_3 \longrightarrow 2PhBCl_2 + HgCl_2 \xrightarrow{H_2O} PhB(OH)_2 + 4HCl.$$

Several methods for the preparation of boronic acids have been developed since the original procedures, with the primary objective of attaching one hydrocarbon group to the boron. Two methods have appeared which have received considerable attention and have proved to be useful for the preparation of alkyl- and arylboronic acids. The use of Grignard reagents for the formation of a boron-carbon bond was introduced by KHOTINSKY et al[4], and has been shown to be of general applicability[5,6]. The Grignard reagent is allowed to react with a trialkylborate or a boron trihalide, and the intermediate dialkyl ester or dihalide is usually hydrolysed directly to the acid.

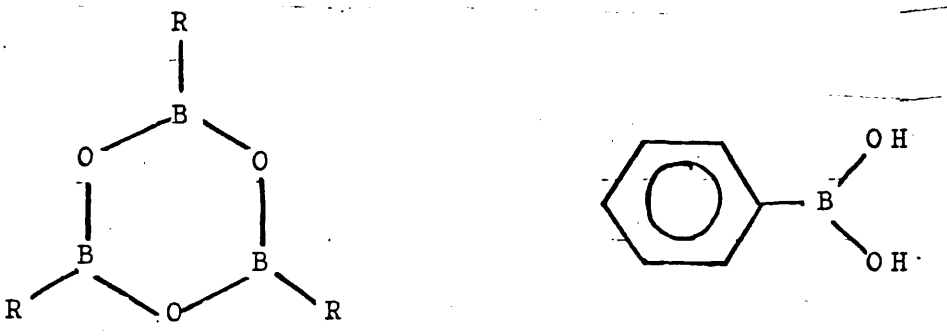
$$B(OR)_3 + R'MgX \longrightarrow MgXOR + R'B(OR)_2 \xrightarrow{H_2O} R'B(OH)_2 + 2ROH$$

The second method involves the reaction of boron trihalides with organometallic compounds, producing alkyl- or arylboron dihalides which readily hydrolyse to the corresponding acids. One particularly useful method for the preparation of phenylboron dichloride, and hence phenylboronic acid, employs the commercially available tetraphenyltin[7].

$$SnPh_4 + 4BCl_3 \longrightarrow 4PhBCl_2 + SnCl_4$$

$$PhBCl_2 + 2H_2O \longrightarrow PhB(OH)_2 + 2HCl$$

Boronic acids readily form anhydrides on standing under vacuum or upon heating. These anhydrides have been shown to have a cyclic structure, (Fig.1(a)), from studies of their molecular weights[8,9], interatomic distances, bond angles and electron diffraction measurements[10], and Raman spectra[11].



(a) (b)

R = alkyl or aryl

Fig.1

Phenylboronic anhydride is a white solid, m.p. 218° [3], and has chemical properties typical of the boronic anhydrides. Various names for this compound have been used, including benzeneboronic anhydride, triphenylboroxine and triphenylboroxole. In this thesis, benzeneboronic anhydride will be used throughout for the purpose of clarity and to emphasise that its direct precursor is benzeneboronic acid, (Fig.1(b)).

Benzeneboronic anhydride undergoes hydrolysis easily to benzeneboronic acid, simply by dissolving the anhydride in hot water. On cooling, the solution deposits benzeneboronic acid . In an analogous manner to boric acid, benzeneboronic acid has a low dissociation constant, $pK_a = 8.86$[12],

behaving as a weak Lewis acid in solution by accepting an hydroxide ion. The planar sp^2 hybridised boron subsequently becomes sp^3 hybridised, and has a tetrahedral arrangement, (Fig.2).

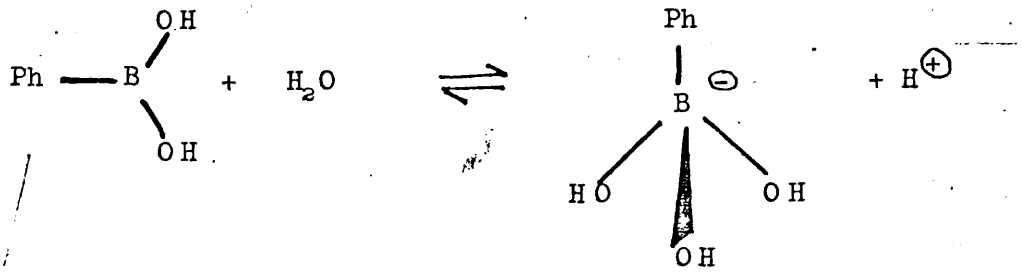


Fig.2

Evidence for the Lewis acid type behaviour of benzeneboronic acid, as opposed to Brønsted acid type behaviour, was first pointed out from results of measurements of dissociation constants of substituted benzeneboronic acids, in an attempt to explain certain 'ortho-effects' [13]. By comparison with the effects of ortho-substituents on the dissociation of benzoic acids, it was anticipated that bulky alkyl groups in the ortho position would increase the acidity of benzeneboronic acids through loss of resonance with the aromatic ring. Thus, a bulky R group in the ortho position was anticipated to significantly reduce the contribution of the structure II, (Fig.3), towards conjugation of the aromatic ring with the boron. The acidic nature of benzeneboronic acid was originally conceived as a proton loss, the conjugate base, III, (Fig.3), would therefore be more easily stabilised if no conjugation between boron and the aromatic ring occurred.

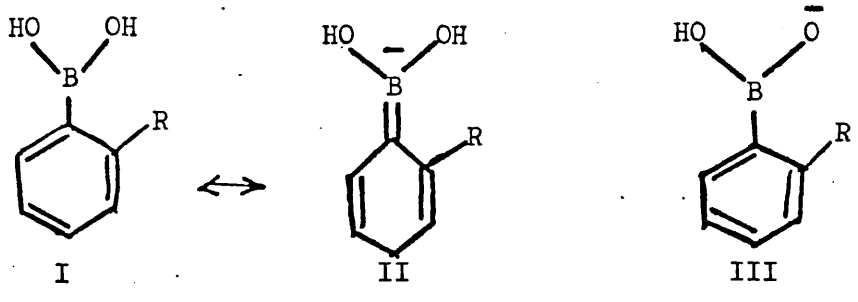


Fig.3

However, it was discovered that a methyl group in the ortho position actually decreased the acidity of benzeneboronic acid. This behaviour could only be accounted for, by assuming that the boron had accepted an hydroxyl ion. The decrease in acidity could then be explained by steric crowding between the methyl substituent and the hydroxyl groups, termed 'F-strain', (Fig.4).

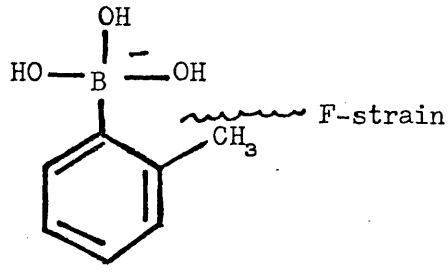


Fig.4

Further evidence for the behaviour of benzeneboronic acid as a Lewis acid was drawn from a comparison of the equilibrium constants for the formation of complexes between benzeneboronate ions and polyhydroxy compounds, and the corresponding constants for borate complexes[14]. It was argued that the constants should be of the same order of magnitude as those of the borate if the complexes are tetrahedral, since it was known that in aqueous solution the borate anion has a tetrahedral structure[15].

Additional evidence for the tetrahedral configuration of the benzeneboronate anion, (Fig.2), was obtained from ^{11}B nuclear magnetic resonance data of benzeneboronic acid in neutral and alkaline solution[16]. Conversion of trivalent planar boron to quadrivalent, tetrahedral boron, was expected to produce a large upfield shift in the ^{11}B resonance, due to increased electron density around the boron atom. A shift of 25.3ppm for benzeneboronic acid on passing from a neutral, to a 10% sodium hydroxide solution in ethanol provided convincing evidence for the tetrahedral arrangement.

When benzeneboronic acid and a polyol are allowed to react in a suitable medium, an equilibrium is established between the free polyol and

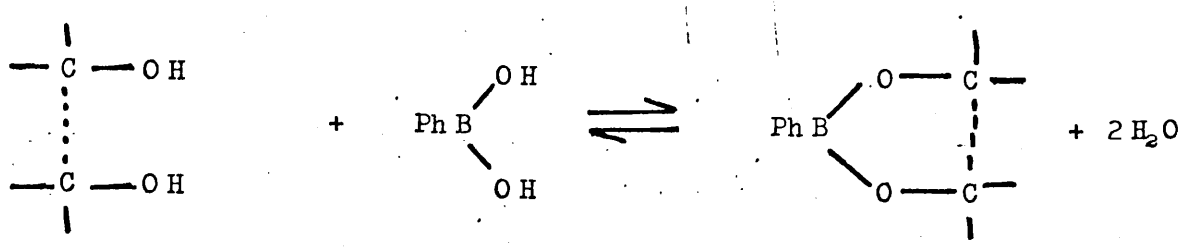
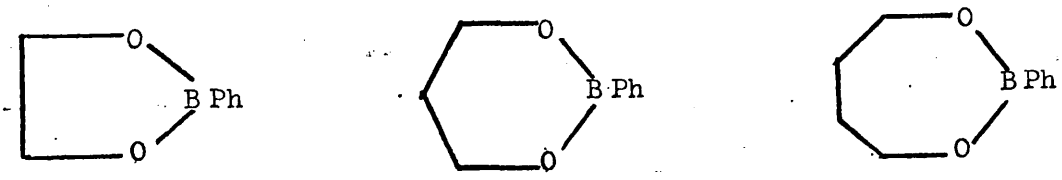


Fig.5

its cyclic ester, (Fig.5). A variety of methods for the preparation of cyclic benzeneboronates have been employed in order to adjust the position of equilibrium in favour of ester formation. Sometimes the product is fortuitously insoluble in water and crystallises from aqueous solution, as for example in the cases of D-glucitol and D-mannitol trisbenzeneboronates[17]. However, the majority of benzeneboronates require the removal of the water produced, for example, by azeotropic distillation from benzene, dioxane[18] or toluene[19].

Simple evaporation of the solvent together with the water liberated, has provided a means of preparation of several boronates. Solvents used include acetone[20], 2-methoxyethanol [21] and pyridine[22]. Several monosaccharide benzeneboronates have been prepared by fusing together the reactants in an evacuated vessel at ~150° [23]. Liquid benzeneboronates formed from several diols have been obtained by simple mechanical removal of the water produced[24].

The susceptibility of benzeneboronates towards hydrolysis may prove to be a problem in certain chemical transformations. Some difficulty has been experienced during methylation of hydroxyl groups using silver oxide and methyl iodide as the methylating reagents[18,25]. However, use of dry, alcohol-free solvents allows many chemical reactions to be performed on the remainder of the molecule. The ease of hydrolysis of benzeneboronates has great value in synthetic chemistry compared to the more traditional blocking groups, as will be shown later.

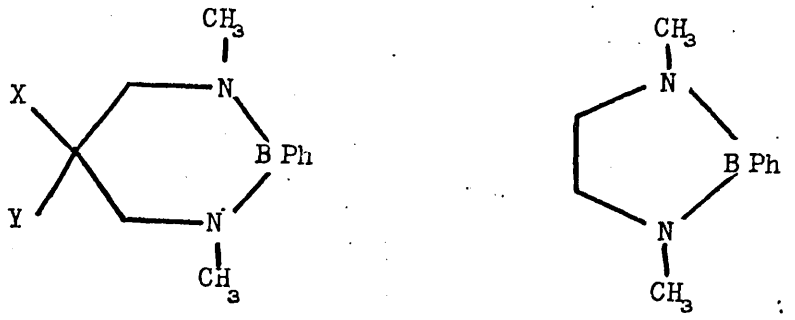


Dioxaborolanes Dioxaborinanes Dioxaborepanes

Fig.6

Reactions of benzeneboronic acid or benzeneboronic anhydride with diols gives rise to cyclic esters, 1,3,2-dioxaborolanes, 1,3,2-dioxaborinanes and 1,3,2-dioxaborepanes, (Fig.6). The 1,3,2-dioxaborocane ring, which might be anticipated from the reaction between benzeneboronic acid and pentane-1,5-diol, could not be isolated as a crystalline compound. The involatile benzeneboronates prepared from pentane-1,5-diol and hexane-1,6-diol were thought to be polymeric[26]. However, the mass spectrum of the reaction mixture of benzeneboronic anhydride and hexane-1,6-diol contained an ion m/e 204, corresponding to a cyclic boronate. Ions corresponding to polymeric forms were not observed[27].

Five-membered ring systems containing trivalent boron are generally considered as having internal strain[28]. The structural requirements of the planar benzeneboronic acid are drastically modified on forming a 1,3,2-dioxaborolane ring; the B O C angle has to be reduced, from the expected 120° required for maximum mesomeric interaction, to nearer the tetrahedral angle of 109°. The oxygen atoms are then considered to be in an sp^3 hybridised state, reducing the effective orbital overlap with the empty p_z orbital on boron. Recent evidence for this was obtained from ^{11}B nuclear magnetic resonance studies on 1,3,2-dioxaborolanes and 1,3,2-dioxaborinanes[29], and by comparison with the chemical shifts of 1,3,2-diazaboracycloalkanes, (Fig.7), in which the nitrogens were deduced to be sp^3 hybridised[30].



1. X = Y = H 2. X = CH_3 Y = H 3. X = Y = CH_3

Fig.7

The internal strain in the five-membered rings of borate esters has also been demonstrated by the stability of complexes formed between 1,2- and 1,3-diols and the borate anion[31]. The 1,2-diols were found to form stronger complexes, contrasting with the reverse behaviour of the corresponding esters. Thus, the borate esters of 1,2-diols frequently hydrolyse on addition of water, liberating the free polyol and boric acid, whereas the 1,3-diol esters can be prepared in aqueous solution, (Fig.8).

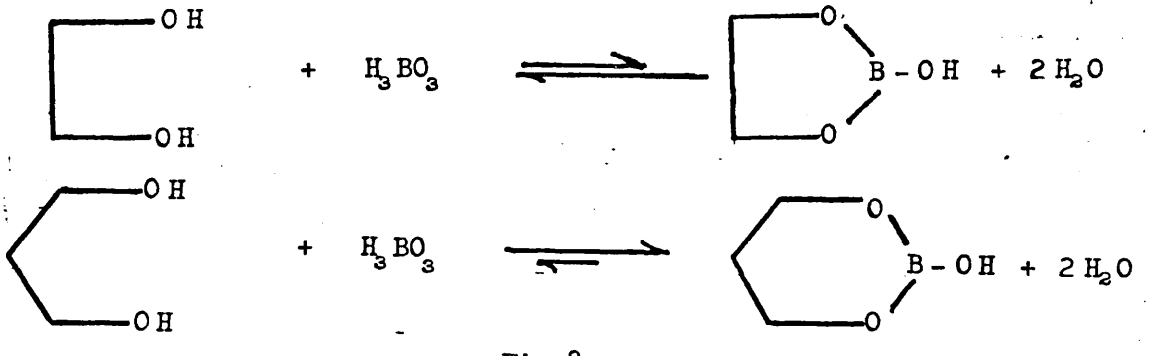


Fig.8

An adequate explanation of these results was provided in terms of a release of internal ring strain in the five-membered rings, on changing the hybridisation of the boron from sp^2 to sp^3. The formation of the tetrahedral boron in a five-membered ring reduces the ring strain without a great loss of mesomeric energy. Whereas the six-membered borate esters do not benefit from such a change in hybridisation since both configurations of boron can be accommodated with relatively little strain.

The strain in five-membered borate esters was also illustrated from investigations of relative complexing tendencies of cyclic borates with

organic bases, such as benzylamine and pyrrolidine[32]. It was shown that a greater exothermal reaction occurred between the 1,2-diols than the 1,3-diols, indicating that the strain in the five-membered ring compounds can be relieved by converting the planar trigonal boron into a tetrahedral arrangement.

Similarly, investigations concerning the stability of benzeneboronate ester complexes with cyclohexylamine, benzylamine and piperidine, showed that the five-membered benzeneboronates formed more stable adducts than the six-membered ring compounds[24]. This was explained in similar terms to those outlined for the borate esters, the greater general stability being attributed to the release of internal strain as a result of change in the hybridisation state of the boron. The six-membered benzeneboronates, considered to exist in a relatively strainless chair conformation, would not benefit greatly from such a change.

Further evidence for the instability of the 1,3,2-dioxaborolane structure relative to the 1,3,2-dioxaborinane ring, came from a study of the solution thermochemistry, in which the benzeneboronates were subjected to oxidative hydrolysis[33]. The results indicated that the 2-phenyl-1,3,2-dioxaborolane ring possesses a strain energy of $\sim 4.18\ \mathrm{KJ\ mol^{-1}}$ greater than the 2-phenyl-1,3,2-dioxaborinane.

However, ring strain in dioxaborolanes can be relieved by intermolecular association[32,34], as was shown from calculations of degree of association[35]. The exact nature of this association is not known. Thermodynamic considerations suggest[36] that no association occurs in the phenyldioxaborolanes or phenyldioxaborinanes, presumably because of steric hindrance

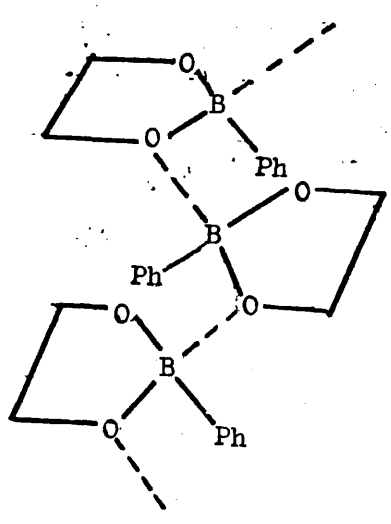


Fig.9

offered by the phenyl group, in the most likely arrangement of 'stacking', (Fig.9).

Formation of the 2-phenyl-1,3,2-dioxaborinane ring in favour of the corresponding dioxaborolane ring was indicated from measurements of $\Delta(\Delta G^{o})$ for the competitive interaction of diols with benzeneboronic anhydride, relative to ethane-1,2-diol[37]. Propane-1,3-diol benzeneboronate was found to be present in 4.2 times the amount of ethane-1,2-diol benzeneboronate.

Diols whose oxygen-oxygen distances are such that formation of a cyclic benzeneboronate is impossible unless large steric interactions result, sometimes form 2,4-diphenyl-1,3,5-trioxa-2,4-diborepane rings. This system is comprised of two boron atoms which enables larger O-O distances to be spanned. This occurs, for example, in methyl α-[18] and β-D-glucopyranoside 2,3-(diphenylpyroboronate) 4,6-phenylboronate[38], (Fig.10).

methyl-α-D-glucopyranoside
2,3-(diphenylpyroboronate)
4,6-phenylboronate

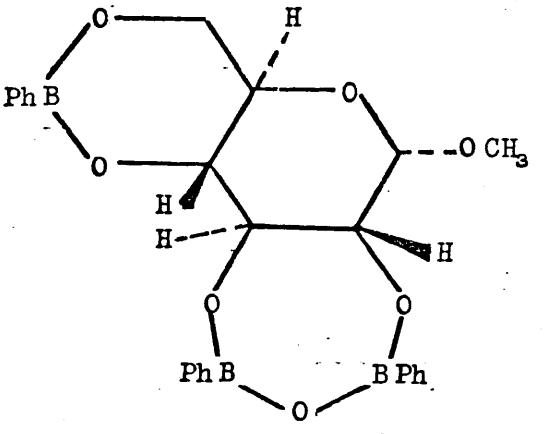


Fig.10

The stereospecific nature of the benzeneboronate grouping has been utilised in the separation of neutral sugars on paper chromatograms[39,40]. When ~0.5% benzeneboronic acid is incorporated into paper chromatographic solvents, compounds containing 1(ax), 3(ax)-diol groupings show increased mobilities. Substitution in the aromatic ring of benzeneboronic acid of a potential ionizing group in aqueous solution, increased the mobility of sugars in paper electrophoresis at pH 7:0[41]. In particular, sulphonated

benzeneboronic acid at 0.05M strength gave a range of mobilities for monosaccharides and alditols greater than borate buffer.

Both these techniques of separation of sugars and their derivatives, involve movement of the ester or complex, and as such require re-chromatography for the removal of the benzeneboronic acid. Similarly, separation of sugars on borate ion exchange columns, eluting with boric acid - borate buffers, requires re-chromatography of the eluted sugars on strongly acidic resin columns for conversion of the borate into boric acid[42]. Recently, a method for the separation of carbohydrates on resin columns, with water as eluent, has been developed by BARKER et al.[43]. A resin matrix was prepared by polymerisation of 4-vinyl-benzeneboronic acid, lightly cross-linked with divinylbenzene, hence immobilising the dihydroxyborono group. Aqueous solutions of sugars were separated by their various tendencies to complex with the resin.

Benzeneboronates of carbohydrates are in general insufficiently volatile for analysis by gas-liquid chromatography, (g.l.c.). However, the analogous butaneboronate esters has been utilised in the study of a variety of bi-functional compounds. Butaneboronates of several diols, hydroxyamines, hydroxyacids and corticosteroids were prepared in micro quantities and analysed by g.l.c. [44-46]. These derivatives are ideally suited to these compounds because of their ease of preparation and regeneration of the parent steroid.

The conventional separation of carbohydrates by g.l.c. as their acetates, methyl ethers or trimethylsilyl ethers, produces multiple peaks representing the various tautomeric and anomeric forms, which usually overlap. Recently, butaneboronates of monosaccharides have been prepared in small quantities[47], followed by silylation[48,49] and analysed by g.l.c. and combined gas-liquid chromatography - mass spectrometry, (g.c.-m.s.).

The chromatograms indicated that the difunctional behaviour of boronic acids and the consequent steric requirements, lead to a preponderance of one particular isomer. In particular[49], quantitative analysis of glucose and fructose mixtures were possible by borosilylation, since these sugars gave single peaks. This is particularly useful in the analysis of glucose and fructose mixtures by g.l.c., since the usual ambiguity resulting from reduction followed by formation of suitably volatile derivatives is eliminated.

In a similar series of experiments, the products of boronation followed by trimethylsilylation of several glycosides and monosaccharides were studied by g.l.c.-m.s.[50]. From an analysis of the mass spectra, the positions of the boronate rings could be assigned. However, not all of the sugars gave a single product.

Formation of benzeneboronates is thought to occur by ruption of the two B-O bonds of benzeneboronic acid, rather than by cleavage of the C-O bonds. Evidence for this was drawn from an analogy with the formation of optically active borates from optically active alcohols and boron trichloride, with retention of configuration[51]. The reaction is thought to proceed via three successive four-centre broadside attacks, (Fig.11).

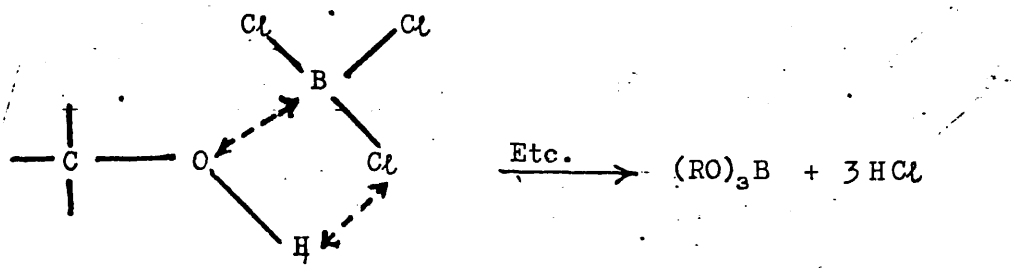


Fig.11

A similar stereochemical test for B-O or C-O ruption was carried out in competitive reactions of erythro- and threo- butane-2,3-diol, with 0.33M benzeneboronic anhydride[37]. The threo-diol ester was initially formed in greater yield, but when equilibrium was attained equal amounts

of the two forms were present, showing that no conversion of threo→erythro had occurred. If an S_N2 reaction had occurred at one diol carbon per diol, then the threo-diol would be converted into the erythro form and vice versa. On the other hand, if an S_N1 reaction had taken place on one of the carbon atoms, equal amounts of both esters would form from both diols.

Furthermore, the hydrolysis of several boronic esters has been shown to occur via B-O fission[52]. Thus, (+)-octan-2-ol was the only isomer produced on hydrolysis of di-(+)-2-methylheptyl phenylboronate. Benzeneboronates of pyranosides gave changes in optical rotation consistant with B-O fission when water was added to their solutions in dioxane[18]. The magnitude of the rotation indicating that the parent glycoside had retained its configuration.

Hydrolysis of benzeneboronates occurs under very mild conditions, usually by addition of water to a solution of the ester in miscible solvents Removal of the benzeneboronic acid produced is effected in several ways. Passage through strongly basic anion exchange resins has been used effectively for the hydrolysis and separation of benzeneboronic acid from carbohydrates[53,54]. Transesterification with propane-1,3-diol is a convenient method for the removal of benzeneboronate groupings, since the propane-1,3-diol benzeneboronate formed can be distilled from the products under reduced pressure[55,56].

Other methods include passage through columns of alumina[54], extraction of the acid with organic solvents[20], paper chromatography[57] and bromine water[58]. The latter method involves conversion of the hydrolysed benzeneboronic acid into bromobenzene[59]. However, the formation of hydrogen bromide may interfere with any acid-labile components present in the compound under investigation.

$$PhB(OH)_2 + Br_2 + H_2O \longrightarrow PhBr + HBr + H_3BO_3$$

Benzeneboronic acid reacts with polyhydroxy compounds to form cyclic boronates in a difunctional manner, so that molecules possessing an odd number of hydroxyl groups form boronates containing one free hydroxyl. This enables specific carbohydrate derivatives to be synthesised by reaction at the unesterified position. For example, D-glucose reacts with benzeneboronic anhydride to form α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate[21,60] (Fig.12).

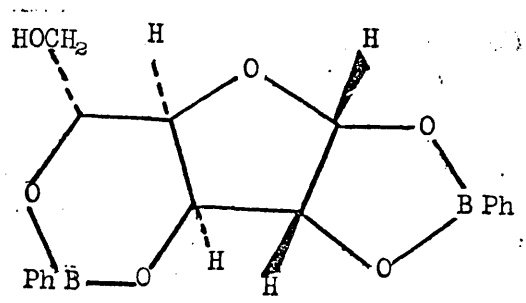


Fig.12

Several C-6 substituted derivatives of D-glucose have been prepared via this compound without rearrangement of the benzeneboronate rings[21,60,61]. Benzeneboronates are generally stable under normal esterifying conditions and the hydroxyl group can be acetylated, tosylated and benzoylated by treatment with acetyl chloride[18], p-toluenesulphonyl chloride[54] and benzoyl chloride[38], respectively. Methyl α- and β-D-xylopyranoside 2,4-benzeneboronates were oxidised with dimethyl sulphoxide-acetic anhydride to give the 3-uloses[62,63] (Fig.13).

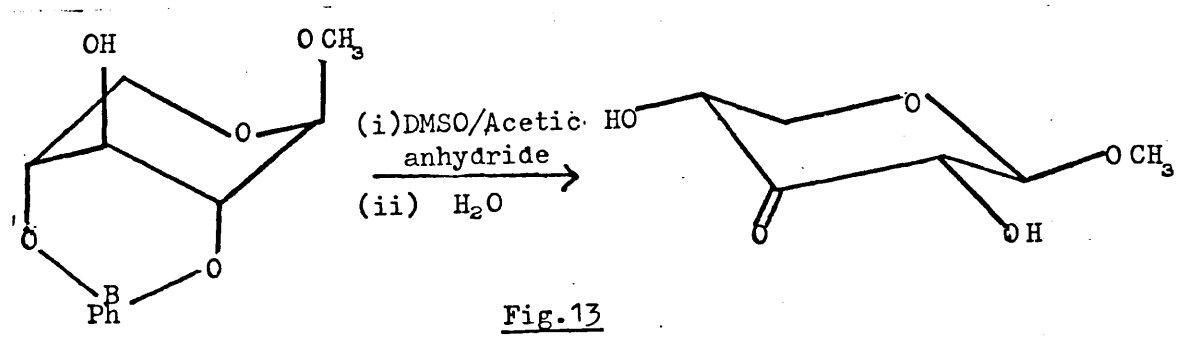


Fig.13

Methylation of carbohydrate benzeneboronates with methyl iodide and silver oxide in dimethylformamide has been attempted, with low yields[18,64]. Cleavage of the boronate ester during methylation may be due to formation of water produced during the reaction, however, addition of desiccants does not improve the yields[18]. Use of diazomethane with

boron trifluoride etherate as a catalyst has proved to be an effective methylating reagent[60].

An interesting synthetic use of benzeneboronates has been demonstrated by MOGEL et al.[61], in which α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate was halogenated at C-6, using triphenylphosphine and carbon tetrachloride, (Fig.14). Hydrolysis of the benzeneboronate rings by transesterification with propane-1,3-diol afforded the 6-halogenated glucopyranose in 79% yield. Similarly, the 6-bromo derivative was prepared in 81% yield. This compares favourably with the preparation of 6-bromo-6-deoxy-D-glucopyranose in 16.5% yield from 1,2; 5,6-di-O-isopropylidene-α-D-glucofuranose with triphenylphosphite and bromine[65].

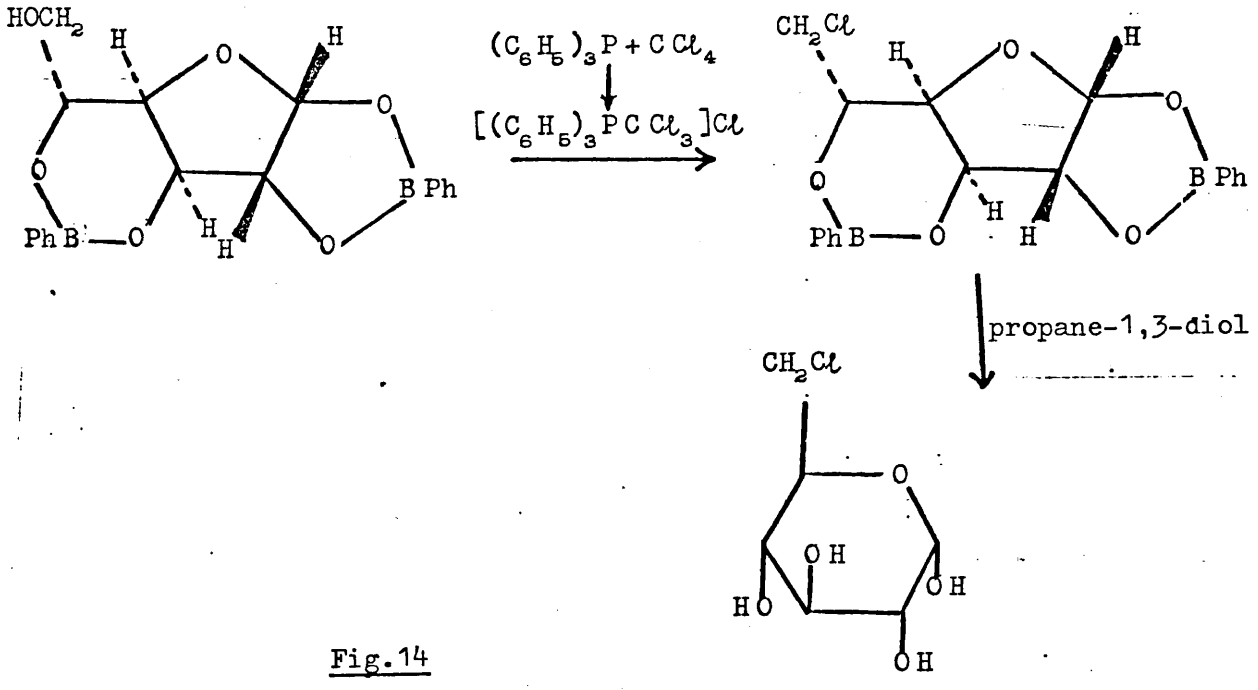


Fig.14

However, halogenation of primary and anomeric carbons has recently been achieved in high yield by reaction of partially protected sugars with triphenylphosphine and the appropriate N-halosuccinimide in N,N-dimethylformamide[66]. The yield of 6-bromo-6-deoxy-1,2; 3,5-di-O-isopropylidene-α-D-glucofuranose, estimated by n.m.r., was 85%.

The presence of structural isomers formed when butaneboronic acid reacts with polyols was apparent from g.l.c. analysis of trimethylsilylated butaneboronates of alditols[49]. Furthermore, structural modifications of benzeneboronates of D-glucose are known to exist in aqueous solution. Proton magnetic resonance spectroscopy was able to detect benzeneboronate complexes of the sugar in furanose and pyranose forms[67]. In addition, an investigation of the composition of benzeneboronates derived from a series of triols revealed the presence of two or more isomeric modifications[58].

As the length of the carbon chain increases with the number of potential hydroxyl groups for esterification, it is expected that more than one cyclic benzeneboronate will form as a result of free rotation of the carbon chain and hence the continuously varying O-O distances. With a view to extending these observations, a study of the isomeric composition of cyclic benzeneboronates, formed when benzeneboronic anhydride reacts with pentitols, was carried out.

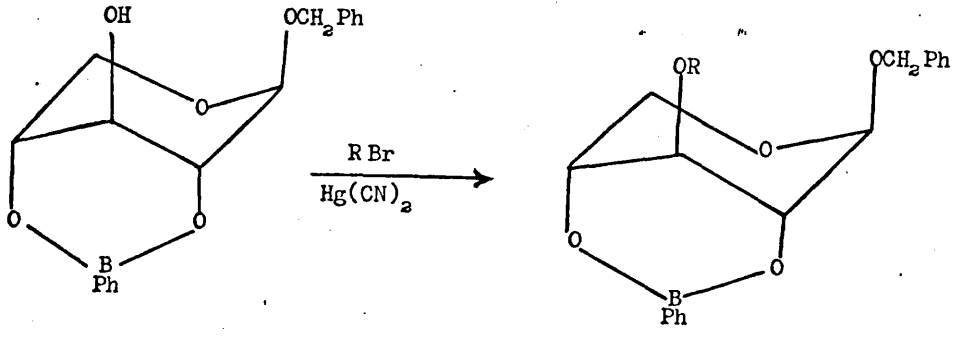
Mass spectrometry has recently provided a means of investigation into the structures of carbohydrate benzeneboronate esters. The high stability of the fused carbohydrate-boronate ring system allows identification of the molecular ion. The use of computer processed high resolution mass spectral data, and measurement of metastable ions have allowed the ring-size of benzeneboronates attached to glycosides to be determined[68]. A study of the modes of fragmentation during mass spectrometry of a series of diol and triol benzeneboronates indicated that this technique could be used to differentiate between five- and six-membered rings[69]. The pentitol benzeneboronates were consequently subjected to mass spectrometry in order to identify ring sizes.

Assignment of ring structure to benzeneboronates of tetritols and hexitols, possessing no free hydroxyl groups, has in the past proved difficult, since attempts to partially hydrolyse one or two rings usually results

in complete hydrolysis of the cyclic esters. However, mass spectrometry of the tris-benzeneboronates of D-glucitol, galactitol[69] and D-mannitol[58] enabled complete structural analysis to be performed. In the light of these investigations, the bisbenzeneboronates of 1-deoxy-pentitols and 1,6-dideoxyhexitols were prepared and subjected to mass spectrometry in an attempt to discover their structures.

The use of benzeneboronates as blocking groups in synthesis has already been mentioned, and some of the work outlined. The main advantage of cyclic benzeneboronates as protecting groups is their ease of preparation as well as ease of hydrolysis, which for other entities such as acetates or ketals, necessitates the use of acid catalysts for removal. This may prove disadvantageous, since disruption of other acid-labile linkages on the sugar may result.

With these aspects in mind, attention was directed to the possible use of benzeneboronic esters as protecting groups in an area of synthetic importance. Of particular interest, the field of disaccharide synthesis shows great promise for the application of benzeneboronates as protective entities. Little attention has been shown hitherto for utilising this grouping in this type of synthesis, with the exception of Ferrier's preparation of 3-O-β-D-glucopyranosyl-D-xylose and 3-O-α- and 3-O-β-D-xylopyranosyl-D-xylose[70] (Fig.15). In an attempt to illustrate their



R = 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl or
2,3,4, -tri - O-acetyl-α- or β-D-xylopyranosyl

Fig.15

potential usefulness, the cyclic benzeneboronate grouping was employed in the synthesis of a specific disaccharide.

The relative stability of five- and six-membered benzeneboronate rings has been mentioned from the aspect of ring strain and their relative ease of formation. Attention was drawn to the fact that selective hydrolysis of a boronate grouping in a polyboronate system was not achieved, although some success may have been anticipated considering the different stabilities of the various ring sizes. Consequently, a different approach was attempted to selectively remove benzeneboronate groupings in compounds containing different sized rings.

CHAPTER II

STRUCTURE OF BENZENEBORONATES OF ACYCLIC POLYHYDROXY COMPOUNDS

1. MASS SPECTROMETRY

II.1.A. GENERAL CONSIDERATIONS OF THE PRINCIPLE AND INSTRUMENTATION

Of all the physical and chemical methods used in the determination of benzeneboronate structures in this investigation, mass spectrometry has been predominant. The extensive use of this technique therefore warrants a discussion to familiarise the reader of its scope and limitations. Although electron impact mass spectrometry has been used throughout this work, a brief outline of various other mass spectrometric techniques will be included to illustrate their application to structural determinations.

A mass spectrometer could be considered as a hypothetical chemical balance capable of weighing individual charged ions. To do this, the sample molecules are ionized in the vapour phase, usually by removal of an electron, separated on passing through a magnetic field according to their mass to charge ratio, and electrically recorded at a collector. The arrangement for a typical single-focussing magnetic deflection mass spectrometer is shown schematically, (Fig.16).

Among the many different methods which have been devised for the production of ions in the mass spectrometer, electron impact production, is the most frequently used mode in organic analysis. Two other methods have more recently received considerable attention, field ionization and chemical ionization, which will be discussed later. Other techniques such as spark discharge, high voltage glow discharge and thermionic emission have specific applications and are not generally used in the field of organic mass spectrometry and will be excluded from the discussion.

Bombardment of a molecule in the ion source with electrons of sufficient energy to remove one electron results in the production of a

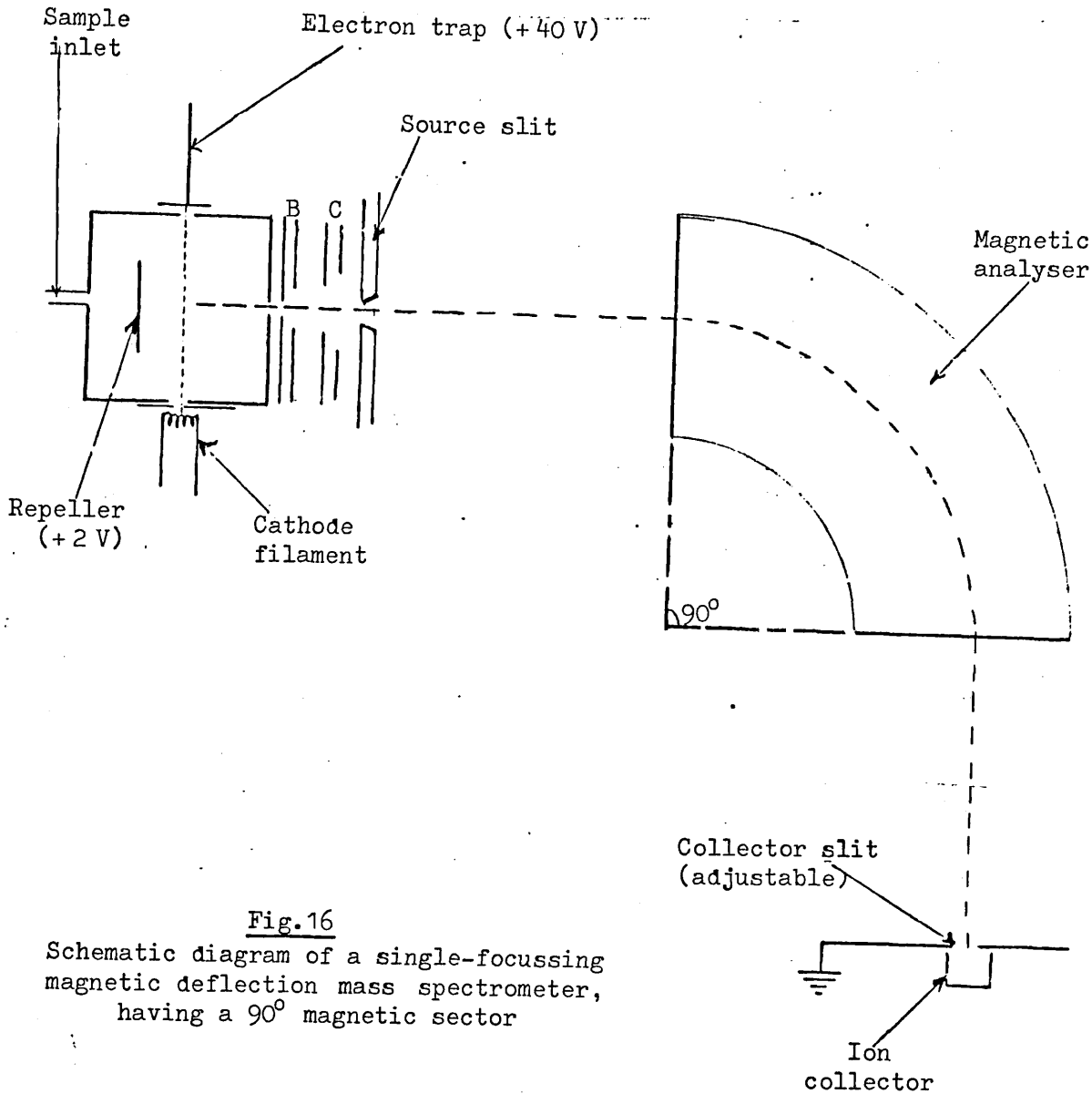


Fig.16
Schematic diagram of a single-focussing magnetic deflection mass spectrometer, having a 90° magnetic sector

positively charged species.

$$M + e \rightarrow M^{+} + 2e .$$

The minimum energy of the electron beam required for this process represents the ionization potential of the compound. Increasing the energy of the electron beam increases the probability of transferring this minimum energy to the molecule during an ion-molecule interaction, thereby increasing the number of ionized molecules.

The relation between electron energy and the abundance of the ions produced is indicated in a graph of ion intensity against electron energy,

(Fig.17). Ideally the plot should be linear with a sharp intersect on the x-axis, indicating the ionization energy of the molecule. However, most commercial ion sources have an inherent spread of about 2 eV in the energy of the electron beam, which results in a curvature at the start of the plot.

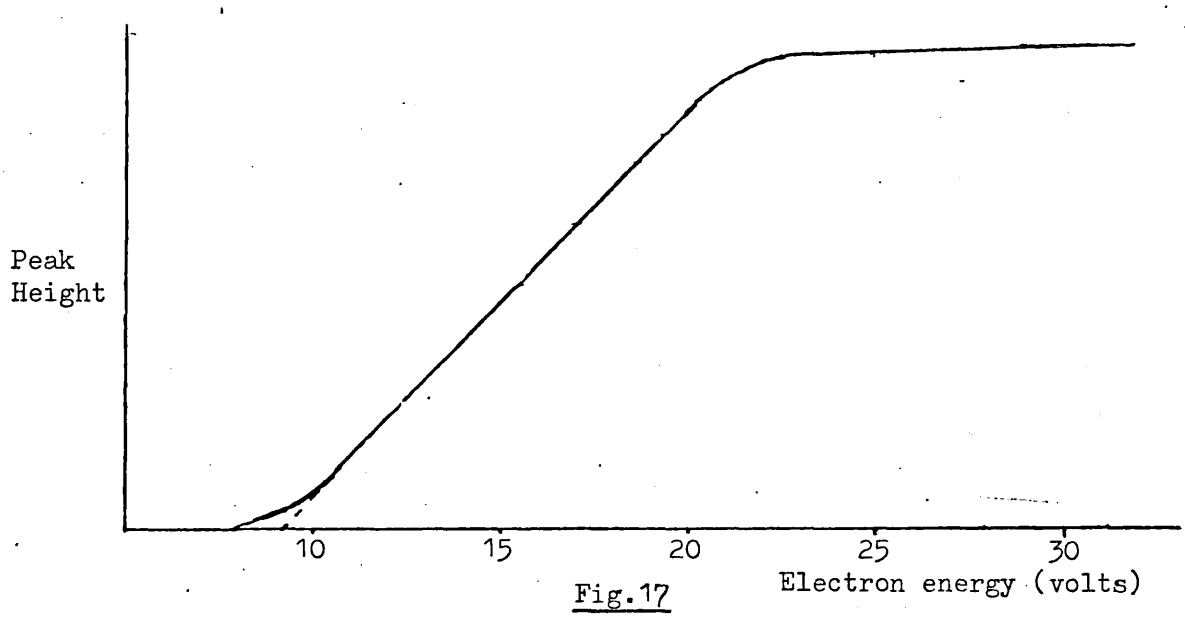


Fig.17

Relationship between ion intensity and electron bombarding energy

When the electron energy exceeds 40-50 eV the abundance of the ion produced does not change appreciably. For this reason most electron impact mass spectra are recorded with an electron beam energy of 60-80 eV. The use of electron beam energies of the order of the ionization potential of organic molecules, i.e. 8 - 15 eV, may not lead to significant fragmentation of the molecular ion. However, the absolute intensity of the ion is considerably decreased and for this reason this method is not generally used to enhance molecular ions in the spectra of organic compounds. Other modes of ionization, such as chemical or field ionization are more applicable for this purpose.

The ions formed in the ion chamber are accelerated through the source slit and the two field plates, B and C, (Fig.16). The plates

are maintained at a high negative potential V , causing the ions to accelerate towards a homogeneous magnetic field with an energy Ve. In the single focussing magnetic deflection type mass spectrometer, used by the author to obtain low resolution mass spectra, the ions enter the magnetic field H and experience a force orthogonal to the direction of the field. The ions are then forced to describe a curved path, the radius of curvature depending upon their mass to charge ratio.

The accelerating force is balanced by the centripetal force, hence

$$Hev = \frac{mv^2}{r} \qquad \ldots (1)$$

where v is the velocity of the ion, and r is the radius of curvature. The kinetic energy relationship can be expressed as

$$Ve = \tfrac{1}{2} mv^2 \qquad \ldots (2)$$

which, on eliminating the velocity term between (1) and (2) gives the equation

$$\frac{m}{e} = \frac{H^2r^2}{2v} .$$

Therefore, for a fixed radius r and an accelerating voltage held constant, by varying the magnetic field H , ions of different mass to charge ratio can be deflected onto the collector.

An instrument of this type can usually effect the separation of ions having nominal mass numbers to about 1000. A mass spectrometer capable of resolving nominal mass numbers in this range has low resolution. The resolution of a mass spectrometer is one of the most important parameters, since a basic requirement for the interpretation of a spectrum is to clearly define peaks differing by one a m u . The most common definition of resolving power presently used, is the numerical value of the highest m/e value for which the height of the valley separating two identically sized peaks is 10% of the height of one of the peaks, i.e. for $\Delta H/H = 0.1$, (Fig.18). The resolution R is then given by $M/\Delta M$.

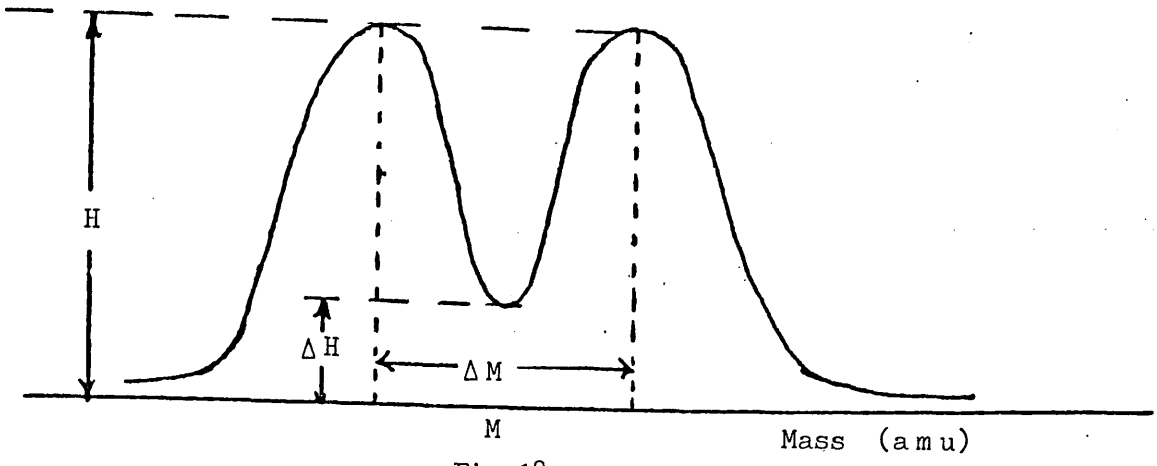


Fig.18

For example, a mass spectrometer capable of separating two ions at m/e 750 and m/e 751, for which $\frac{\Delta H}{H} = 0.1$, has a resolving power of 750.

The ions formed in the ion chamber are accelerated through a few thousand volts, but because they are not formed in the same position in the ion chamber their energy may differ by a few tenths of an electron volt. The velocity dispersion in the ion beam consequently broadens the peaks in the spectrum and therefore limits the attainable resolution. Very much higher resolving power can be obtained by double focussing mass spectrometers, which have in addition to the magnetic analyser, an electrostatic analyser, which essentially produces first order velocity focussing. A schematic diagram of a 90° sector electrostatic analyser is shown, (Fig.19).

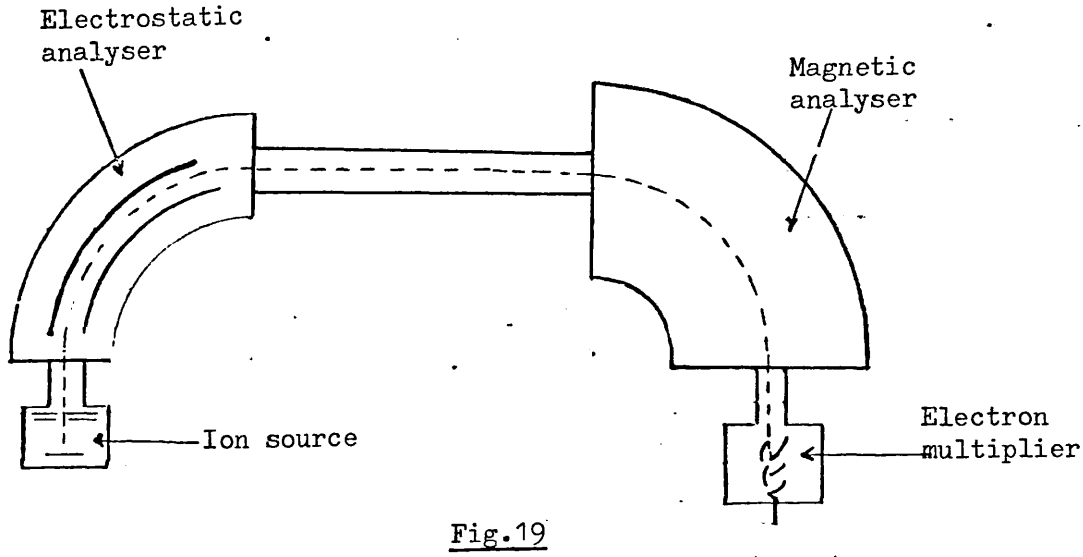


Fig.19

Schematic of double focussing mass spectrometer

Double focussing mass spectrometers have resolving powers in excess of 10,000 and are capable of giving masses to an accuracy of a few parts per million. High resolution mass spectrometry can therefore distinguish between ions of the same nominal mass number but different elemental composition. For example, a single peak occurs at $\frac{m}{e}$ 104 in the mass spectrum of 1-deoxy-$\underline{\underline{D}}$-talitol bisbenzeneboronate which could have the composition $C_6H_5{}^{10}BOH$, since the corresponding ion containing the ^{11}B isotope was present at $\frac{m}{e}$ 105. High resolution mass spectrometry resolved the peak into a doublet, the precise masses being 104.0549 and 104.0433 corresponding to ions of atomic composition $C_6H_5{}^{10}BOH$ and $C_6H_5{}^{11}BO$ in a relative abundance of 1:1.

Ions spend $\sim 10^{-6}$ sec in the ion chamber and take approximately 10^{-5} sec to be accelerated and deflected into the detecting system. Molecular ions formed in the ion chamber will therefore be recorded if their rate constant for decomposition, $k < 10^5$ sec^{-1}. Ions which have a rate constant $> 10^6$ sec^{-1} will fragment, and the daughter ions will be recorded. If the rate constant lies between 10^5 and 10^6 sec^{-1} then decomposition of the parent ion may occur during its passage through the analyser tube. These ions are called metastable ions.

For single and double-focussing mass spectrometers, an ion m_1^+ which fragments to m_2^+ in the field free region before the magnetic analyser, produces a broad peak of low intensity at a position given by the relation

$$m^* = \frac{m_2^2}{m_1}.$$

The detection of metastable ions in the mass spectrum of a compound is important because they provide direct evidence of a particular fragmentation process. For example, a metastable ion at $\frac{m}{e}$ 265.0 was observed in the mass spectrum of $\underline{\underline{D}}$-arabinitol bisbenzeneboronate providing

evidence for the fragmentation of a hydroxymethyl group from the molecular ion.

$$[C_{17}H_{18}B_2O_5]^{+\cdot} \xrightarrow{-\dot{C}H_2OH} [C_{16}H_{15}B_2O_4]^{+}$$

m/e 324 m/e 293

For the process $m_1^+ \rightarrow m_2^+ + (m_1 - m_2)$

$$m^* = \frac{m_2^2}{m_1} .$$

Therefore the calculated position for the metastable ion is

$$\frac{293^2}{324} = 265.0 .$$

The conventional technique of introducing a sample into a mass spectrometer consisted of a reservoir where the sample was vapourised at a pressure $\sim 10^{-2}$ torr. The vapour was then bled into the ion source via a tiny orifice. Quantitative analysis can be carried out with this system, but it has a severe disadvantage that only the more volatile compounds of relatively low molecular weight can be analysed. A modification whereby the sample is inserted directly into the ion source has greatly increased the applicability as well as reducing the amount of material required for a spectrum.

The development of an interface which couples the outlet of a gas-liquid chromatograph to a mass spectrometer has brought together two powerful analytical techniques. The present widespread use of combined gas-liquid chromatography - mass spectrometry, (g.c.-m.s.), is exceedingly obvious from the wealth of literature on the subject. Detailed accounts of the technique and applications of g.l.c.-m.s. are available[71-73], and therefore only a brief discussion concerning the layout of the system will be attempted.

The basic components of a combined g.l.c.-m.s. system are shown schematically, (Fig.20). The gas chromatograph in such a system functions

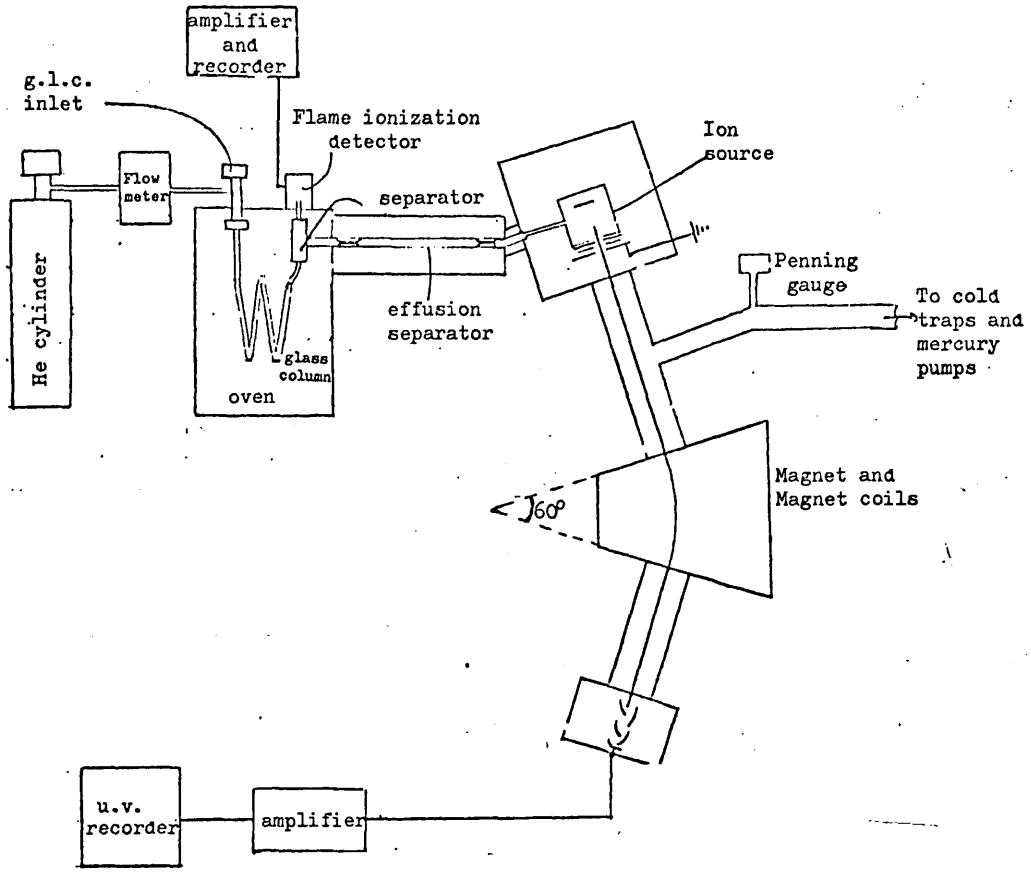


Fig.20
Schematic diagram of gas chromatograph-mass spectrometer combination

in the same way as in the normal mode of gas chromatography. The most significant problem incurred in combining a gas chromatograph with a mass spectrometer is the pressure differential. Under normal operating conditions the chromatograph is operated with an outlet pressure of 760 torr. The mass spectrometer however, requires a pressure at least as low as 10^{-5} torr. The interface system therefore has to reduce the pressure of the carrier gas by eight orders of magnitude while maintaining a usable fraction of the organic sample for the mass spectrometer.

The most widely used interface systems are those which increase the ratio of sample to carrier gas in the chromatograph effluent, and are called molecular separators. Of the many molecular separators in present

use, only the type employed in the present study will be described. This type of effusion separator was designed by WATSON and BIEMANN[74] and has been used in many g.c.-m.s. systems. The separator consists of an ultrafine porosity sintered glass tube enclosed in a vacuum envelope, having fine capillories at either end to provide restrictions from the gas chromatograph, and at the entrance to the mass spectrometer, (Fig.21).

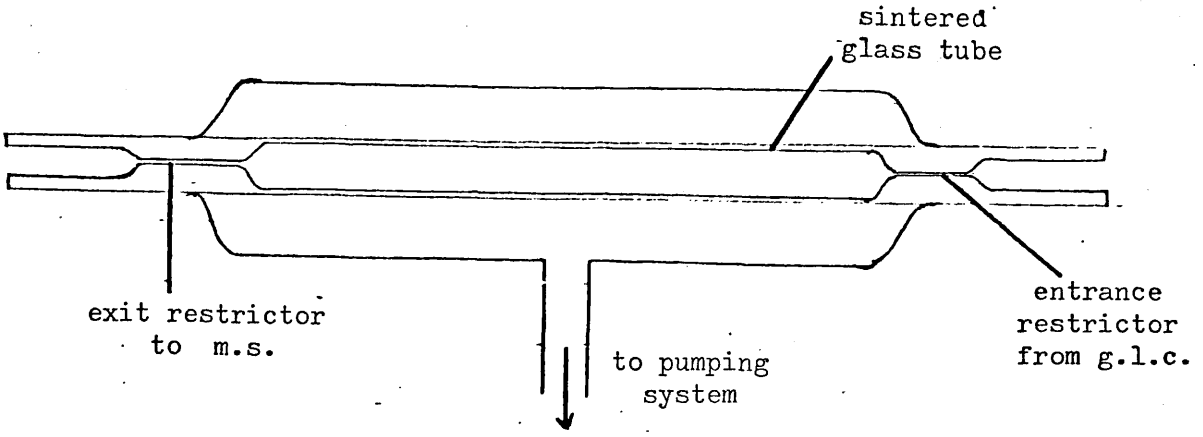


Fig.21
Watson-Biemann effusion separator

The effluent from the gas chromatograph flows through the entrance restrictor, resulting in a drop in pressure to approximately one torr. Fractionation of the sample then occurs by the effusion of the low molecular weight carrier gas, usually helium, through the sintered glass tubing. Using a flow rate from the gas chromatograph of $\sim 17\,cm^3\,min^{-1}$, efficient fractionation was obtained for analysis of compounds in the molecular weight range 300 - 400, whilst maintaining a pressure of 10^{-6} torr in the mass spectrometer.

Recently, the use of alternative ionization techniques, such as chemical ionization and field ionization, in addition to conventional electron impact mass spectrometry, has expanded the field of mass spectrometry in the structure determination of organic compounds. These two methods of producing ionized molecules have the advantage over electron impact mass spectrometry in that the tendency of the molecular ion to fragment is greatly reduced, and as a consequence give abundant ions in the high mass region of the spectrum.

The extensive fragmentation of the molecular ions formed during electron impact mass spectrometry is a result of a large amount of excess energy in the ionized molecule transferred from electrons having considerably greater than the necessary ionization energy. However, in chemical ionization mass spectrometry, ionization of the sample molecules occurs as a result of interactions between the vapourised sample and the primary ions of a high pressure reactant gas, usually methane or isobutane. The reactant gas is introduced into an ionization chamber at a pressure of 0.1 - 3 torr, together with the vapourised sample molecules at approximately 10^{-6} torr. Because of the relatively high pressure of the reactant gas, virtually all of the primary ionization occurs with these molecules. The ionized reactant gas then undergoes ion-molecule reactions with itself to form a steady-state plasma which then reacts chemically with the sample molecules, usually by donating a proton or abstracting a hydride ion. For example, methane predominantly forms the ions CH_5^+ and $C_2H_5^+$ via the following reactions:

$$e + CH_4 \longrightarrow CH_4^{+\cdot} + 2e$$

$$CH_4^{+\cdot} \longrightarrow CH_3^+ + H^\cdot$$

$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH_3^\cdot$$

$$CH_3^+ + CH_4 \longrightarrow C_2H_5^+ + H_2$$

The CH_5^+ ions, which represent ~48% of the total ions formed from methane under these conditions, will then donate a proton to a sample molecule, to an extent depending upon the greater proton affinity of the molecule over methane.

$$CH_5^+ + SH \longrightarrow SH_2^+ + CH_4 \ .$$

However, if the sample is not a good proton acceptor, then hydride abstraction will occur to give an $(M-1)^+$ ion.

$$CH_5^+ + SH \longrightarrow S^+ + CH_4 + H_2 \ .$$

The quasi-molecular ion, i.e. the $(M+1)^+$ or the $(M-1)^+$, is often the most abundant ion in the spectrum. The chemical ionization method is therefore most effective when used in conjunction with electron impact mass spectrometry. For this reason dual purpose sources for both modes of ionization are now commercially available.

Field ionization mass spectrometry is comparable to chemical ionization in that relatively few fragmentation processes occur. Ionization is effected by allowing the sample molecules to pass through a very high positive electric field, ~ 7 - 10 kV, generated at a sharp point or thin blade. An electron is subsequently removed from the molecule and the resulting ion is ejected from the ion chamber. This arrangement provides 10-12 eV for the ionization and excitation of molecules and since most organic compounds have ionization energies in this range, relatively few fragmentation processes occur. As a result, the molecular ion is often observed.

Although field ionization mass spectrometry provides a useful complementary technique to electron impact mass spectrometry, it does suffer from several drawbacks. The most significant being the low sensitivity of the source, the total ion current being 10-100 times less than that produced by an electron impact source. Consequently, even though the molecular ion may have higher relative abundance, the absolute signal may not be greater.

II.1.B MASS SPECTROMETRY OF BENZENEBORONATES

The interpretation of the mass spectra of boron compounds is simplified by the presence of two naturally occurring isotopes of boron, viz. ^{10}B and ^{11}B in a relative abundance of 1:4 approximately. The appearance of boron-containing fragments in this ratio thus facilitates their interpretation and provides a means for the statistical calculation of a number of boron atoms in a fragment.

Cyclic benzeneboronate esters of polyol compounds often give surrisingly few peaks in their spectra. This reflects the tendency of the fused polyol-boronate ring system to remain intact, allowing easy identification of the molecular ion. Also, fission of the boron-phenyl bond occurs infrequently in such compounds owing to its greater strength relative to the C-O and C-C bonds. The strength of the boron-phenyl bond in relation to boron-carbon bonds where the carbon is part of a saturated alkyl group, was demonstrated from a comparison of the mass spectra of tri-n-butyl-, tri-t-butyl-, tricyclohexyl- and triphenylboroxine[75]. The intensities of the molecular ions indicated the relative stability of the boron substituent to fragment, (Fig.22).

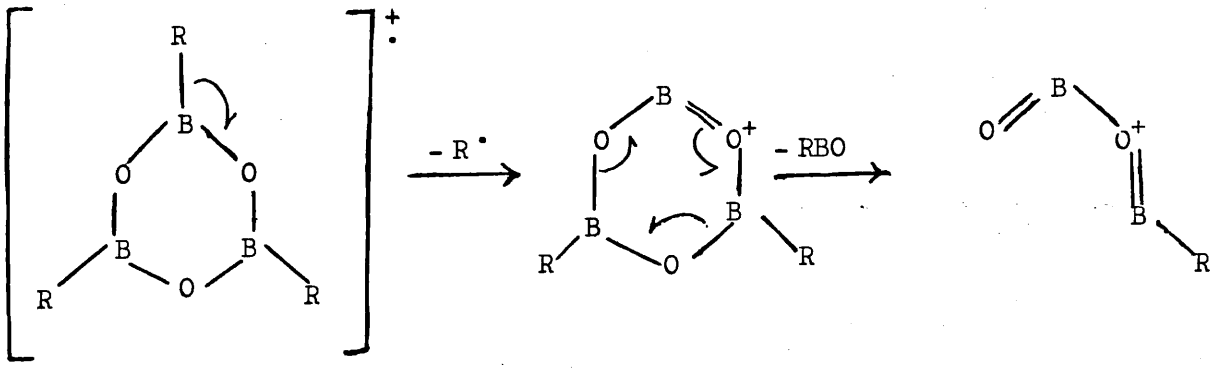


Fig.22

Only one instance of appreciable boron-phenyl fragmentation in cyclic benzeneboronates has been described which results in complete loss of a phenyl radical, that of DL - 4 - (2 - hydroxyethyl) - 2 - phenyl - 1,3,2 - dioxaborinane[27]. Intramolecular coordination of the unsubstituted oxygen

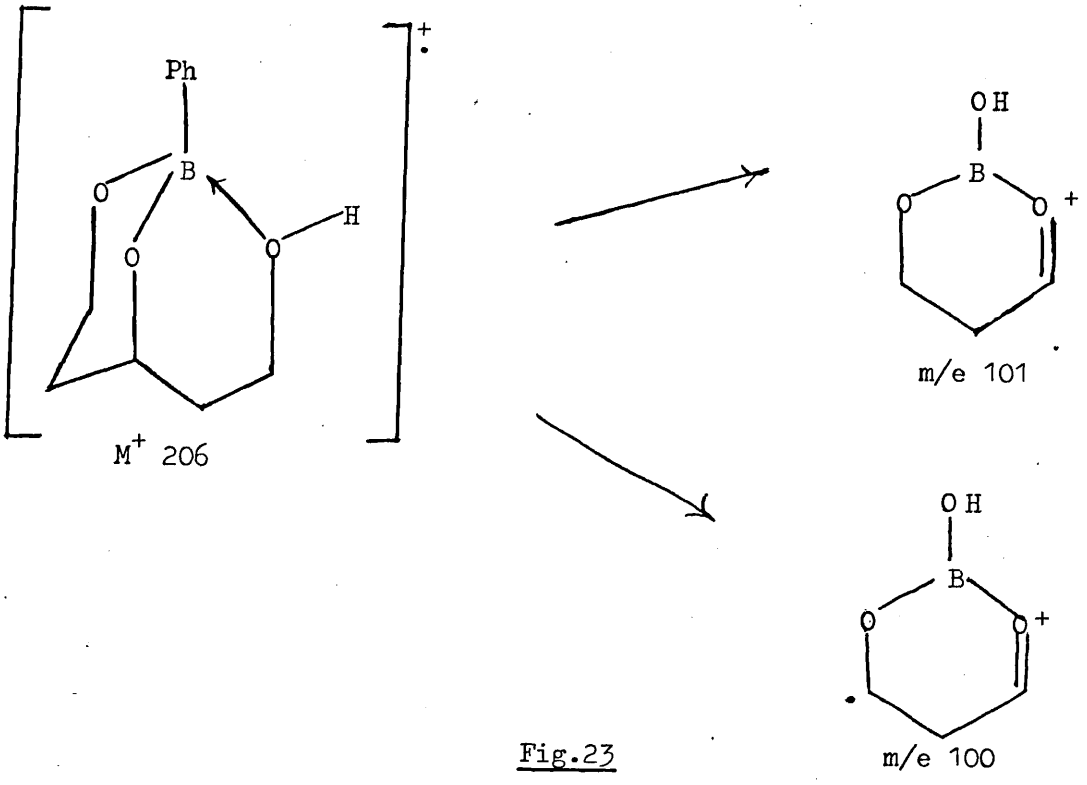


Fig.23

of the hydroxyl group was thought responsible for the weakening of the boron-phenyl bond, (Fig.23). Other substituents attached to boron in the exocyclic position which do not form such strong B-X bonds do, however, undergo bond scission to form cyclic borenium ions[76].

Electron impact mass spectrometry of cyclic benzeneboronates of polyhydroxy compounds has been shown to give rise to four fragmentation modes:

A. Elimination of an oxo-compound.

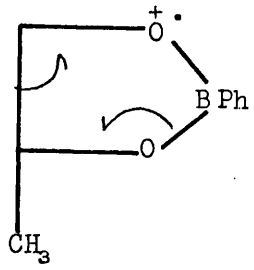
B. Fission of an exocyclic C-C bond.

C. Skeletal rearrangement to hydrocarbon ions.

D. Fragmentation mode exclusive to six-membered ring compounds.

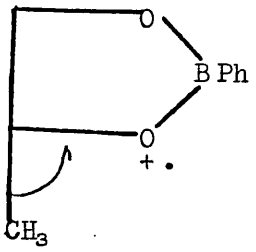
These four modes of fragmentation are shown for 4-methyl-2-phenyl-1,3,2-dioxaborolane and 4-methyl-2-phenyl-1,3,2-dioxaborinane, (Fig.24).

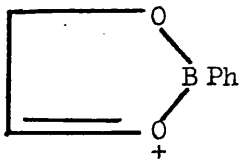
Elimination of an oxo compound. This process of fragmentation of the molecular ions of cyclic benzeneboronates (Fig.24A) has been observed

A. 

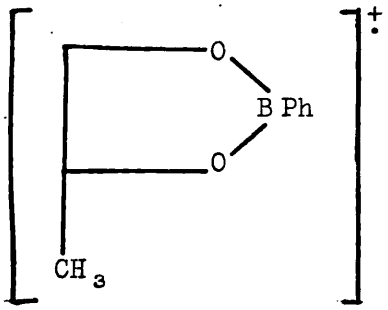
$-C_2H_4O$ → $[PhBOCH_2]^{+\cdot}$

m/e 162 — 1, m/e 118

B. 

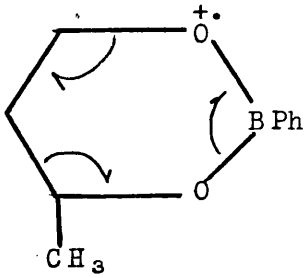
$-CH_3^{\cdot}$ → 

m/e 162 — 2, m/e 147

C. 

$-[C_2H_4BO_2]^{\cdot}$ → $C_7H_7^{+}$

m/e 162 — 3, m/e 91

D. 

$-C_2H_4O$, $-C_2H_4$ → $PHBO^{+\cdot}$

m/e 176 — 4, m/e 104

Fig.24

by a number of workers[69,77,78]. The ion 1, formed by the cleavage of a C–C and a B–O bond, can fragment further to produce the ion $\frac{m}{e}$104 or rearrange to form a hydrocarbon ion $\frac{m}{e}$91, $C_7H_7^+$, (Fig.25).

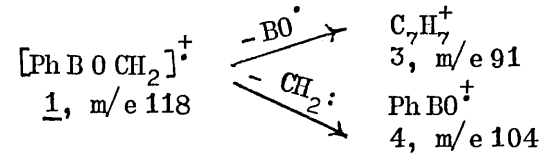


Fig.25

Fission of exocyclic bonds. Exocyclic bond scission in dioxaboron ring systems occurs readily, probably because of the resulting resonance stabilised oxonium ion, 2, (Fig.24). This fragment can undergo rearrangement to form the tropylium ion, $\frac{m}{e}$91, $C_7H_7^+$, (Fig.26).

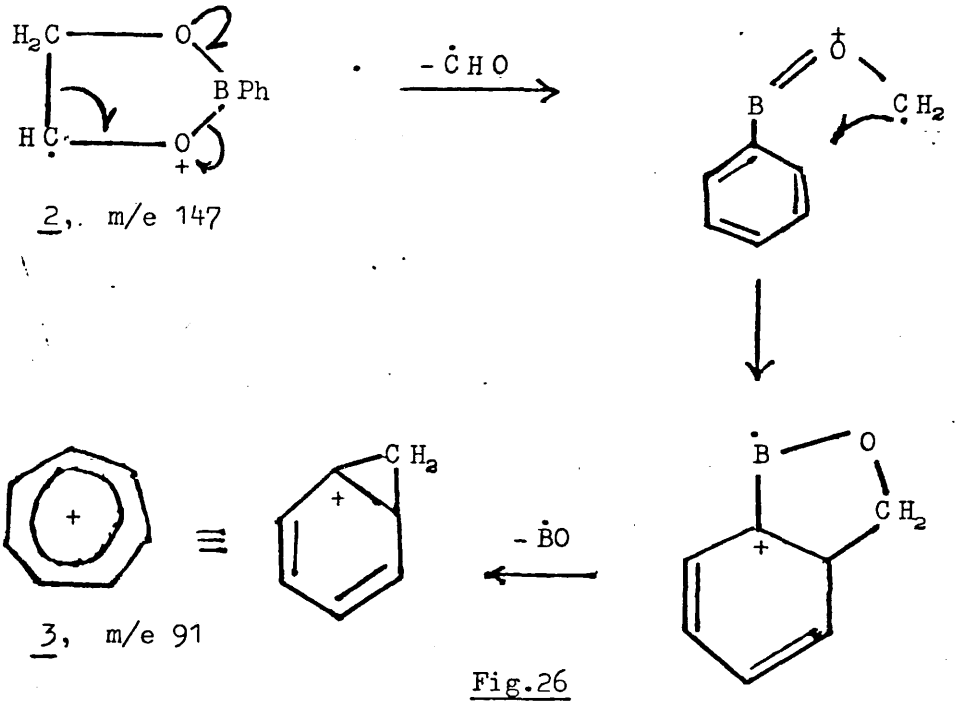


Fig.26

Skeletal rearrangement to hydrocarbon ions. Skeletal rearrangement of the molecular ion to produce the tropylium ion is a well known process common to the fragmentation patterns of aromatic hydrocarbons[79]. Prior to their detection in the spectra of cyclic benzeneboronates, similar rearrangements involving a carbon atom, not directly bonded to the phenyl

group in the parent molecule were rarely observed. Recent interest in fragmentation modes of cyclic boronates has, however, produced a series of publications which have been particularly concerned with the presence of hydrocarbon ions in the mass spectrometer[77,78,80-82]. Besides formation from the previously mentioned fragmentation pathways, hydrocarbon ions are generated directly from the molecular ion. For example, the molecular ion of ethylene glycol benzeneboronate rearranges to give an ion $\frac{m}{e}$ 91, as the base peak in the spectrum[82], (Fig.27).

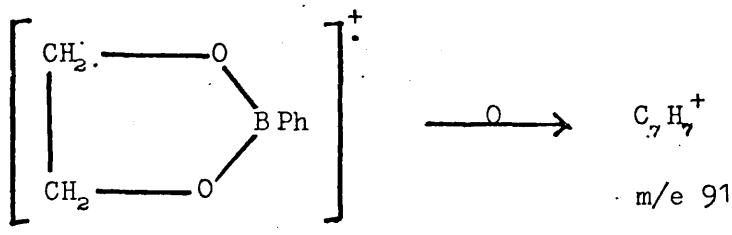


Fig.27

The origin of this ion was indicated from measurements of the metastable ions present, which appear at $\frac{m}{e}$ 55.9 and at $\frac{m}{e}$ 70.2. It was concluded[82] that the ion $\frac{m}{e}$ 55.9 arises from direct rearrangement of the molecular ion. The metastable ion $\frac{m}{e}$ 70.2 was believed to have resulted from fragmentation of the ion $\frac{m}{e}$ 118. This latter ion is formed from the molecular ion by loss of formaldehyde, a process representative of the fragmentation mode A. The origin and constitution of the tropylium ion in the mass spectra of cyclic benzeneboronates was elucidated by deuterium labelling of the parent diols[82]. Thus, the ion $\frac{m}{e}$ 91 in the spectrum of ethylene glycol benzeneboronate shifted to $\frac{m}{e}$ 93 in the spectrum of d_4-ethylene glycol benzeneboronate.

A fragmentation scheme has been proposed which suggests initial α- cleavage to form a stabilised oxycarbonium ion, (Fig.28). Attack at the ortho-position of the aromatic ring by a cationic site mechanism then produces a bicyclic intermediate which can either produce the tropylium

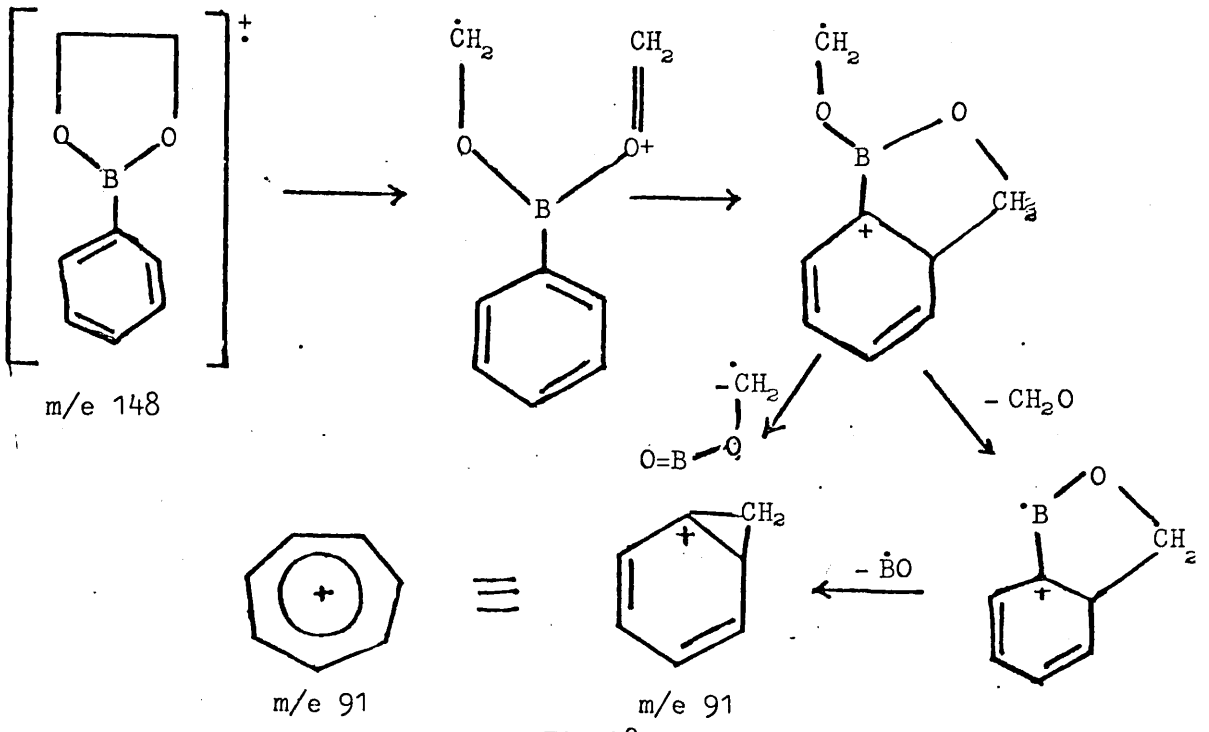


Fig.28

ion directly, or lose formaldehyde and a BO• radical in two steps. This fragmentation pathway was also used to explain the formation of a tropylium ion from propane-1,3-diol benzeneboronate. However, only the metastable ion, $\frac{m}{e}$ 51.5 was observed, corresponding to direct rearrangement of the molecular ion. The two step mechanism would involve loss of C_2H_4O and then the •BO radical.

Fragmentation exclusive to six-membered rings. A mode of fragmentation which occurs during the mass spectrometry of cyclic benzeneboronates, was found to be exclusive to the six-membered ring systems[58,69]. The molecular ions of 2-phenyl-1,3,2-dioxaborinane derivatives fragment to produce the ion $\frac{m}{e}$ 104, C_6H_5BO, or the hydrocarbon ion derived from the boronate ring carbons, directly. For example, metastable ion measurements were able to provide evidence that the molecular ion of 2,2-dimethylpropane-1,3-diol benzeneboronate fragmented directly to give ions at $\frac{m}{e}$ 104, C_6H_5BO, and $\frac{m}{e}$ 56, C_4H_8, (Fig.29).

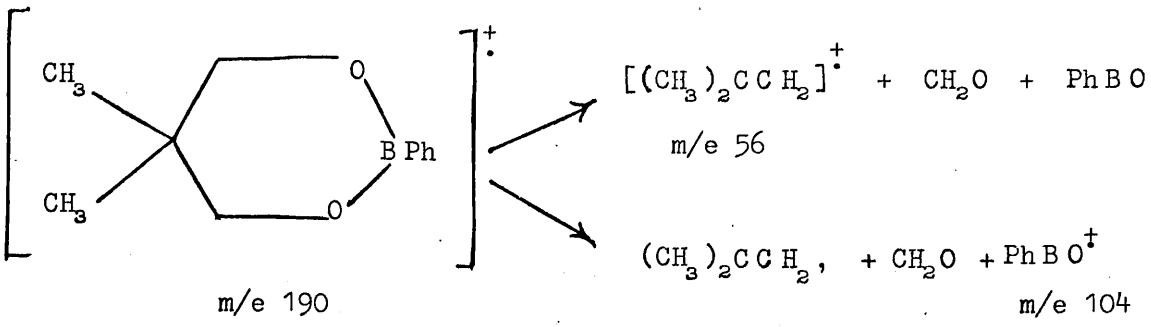


Fig.29

The application of mass spectrometry to the structure elucidation of benzeneboronates containing fused ring systems, which exist for example in erythritol bisbenzeneboronate, has shown that similar considerations to those of the monocyclic boronates can be applied[69]. The fragmentation mode peculiar to six-membered rings, for example, was found to occur in several bis- and trisbenzeneboronates[58]. The presence of certain ions in the mass spectra of bis- and trisbenzeneboronates, together with their atomic composition, as verified by precise mass measurements, provide sufficient evidence for the existence of six-membered rings. However, further proof of a single mode of fragmentation from the molecular ion by

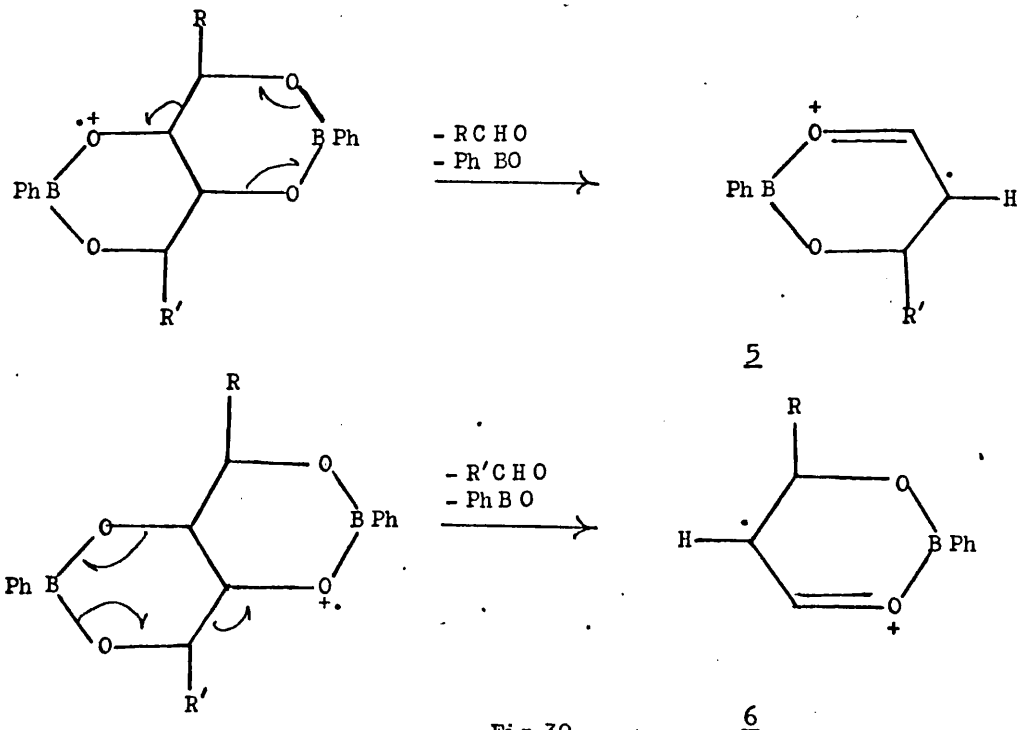


Fig.30

simultaneous elimination of an oxo-molecule and C_6H_5BO, (Fig.30), can be obtained from metastable ion measurements. Production of either 5 or 6 will depend upon the oxygen ionized by electron bombardment in the parent molecule. Generally, both types of ion are observed in the spectrum.

Therefore, mass spectrometry is able to provide a means for the structure elucidation of benzeneboronates of polyols containing even numbers of hydroxyl groups. For example, the complete structure of D-glucitol trisbenzeneboronate was determined solely from a study of its mass spectrum and from the mass spectrum of the 1-d,-D-glucitol trisbenzeneboronate[58].

Using this interpretation of the mass spectra of cyclic boronates, it was possible to assign structures of two 1,6-dideoxy hexitol and three 1-deoxy-pentitol bisbenzeneboronates.

II.2 BENZENEBORONATES OF TETRITOLS

Complete esterification of tetritols with benzeneboronic acid necessitates consideration of three possible structural isomers, (Fig.31).

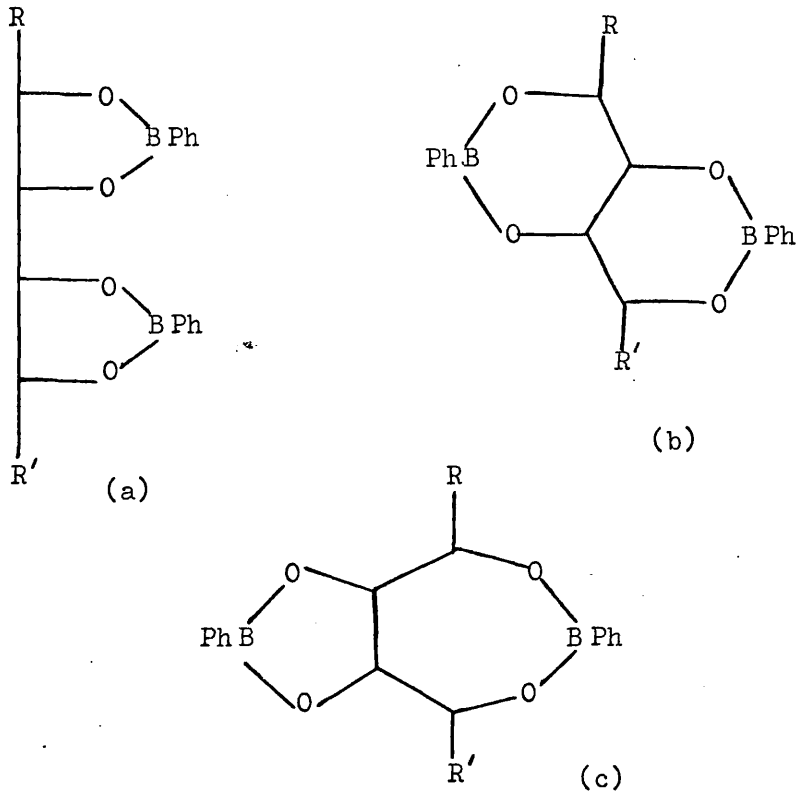


Fig.31

The difunctional behaviour of benzeneboronic acid could theoretically produce three types of ring size, viz. five, six or seven-membered. From a survey of the literature, it appears that formation of a seven-membered benzeneboronate ring is highly unlikely, although a cyclic benzeneboronate has been isolated from butane-1,4-diol[20]. However, in this instance it has to be realised that formation of alternative monomeric benzeneboronates is not possible. Competitive reactions between benzenboronic anhydride, propane-1,3-diol and one of several acyclic diols which had the possibility of forming seven, eight or nine-membered rings, were investigated by g.l.c. and showed that the six-membered ring was always produced as the major component[27].

Formation of two five-membered rings or two fused six-membered rings will intuitively depend upon the orientation and type of substituent on each boronate ring, and also on the type of interaction between the substituent and the remainder of the molecule, i.e. on the nature of R and R^1. For instance, if by forming two trans-fused six-membered rings, (Fig.32), the substituents R and R^1 are in axial orientations on the boronate rings, then steric interaction may be sufficiently large to lead to a preponderance of the alternative two five-membered ring system. Presumably, the interaction energy of the syn-axial methyl group and hydrogen atom will be similar to that found in the methyl cyclohexanes, which amounts to approximately 7.1 KJ $mole^{-1}$ (83).

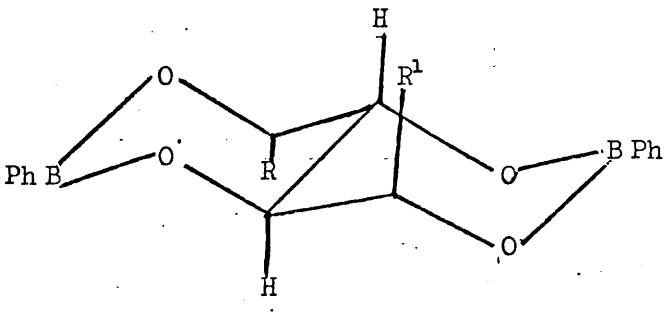


Fig.32

The tetritols investigated were 1-deoxy-pentitols and 1,6-dideoxy-hexitols, i.e. R and R^1 = H or CH_3, hence interaction of this type is expected to influence the structure of the latter compounds to a greater extent. Other types of structure, such as two cis-fused six-membered rings will be discussed in connection with the appropriate tetritol.

Structure determination by chemical methods of benzeneboronates of polyhydroxy compounds containing even numbers of hydroxyl groups is usually not a feasible proposition. Interaction of benzeneboronic acid or benzeneboronic anhydride with such compounds results in complete esterification, providing no free hydroxyl groups for identification by chemical means. Attempts to prepare partially esterified cyclic boronate esters of polyols by addition of less than the stoichiometric amount of benzeneboronic acid, leads to fully substituted polyol, together with residual polyol. However, galactitol 1,3; 4,6-bisbenzeneboronate was prepared by this method(57).

Attempts to selectively remove one boronate ring from a bis- or trisbenzeneboronate ester by mild hydrolysis generally results in complete removal of the rings. Certain compounds which contain a pyroboronate ring have, however, been subjected to partial hydrolysis. For instance the pyroboronate ring in the compounds methyl α-[18] and β-[38] D-glucopyranoside 2,3-(diphenylpyroboronate) 4,6-phenylboronate hydrolyse very easily in water-saturated benzene solutions[38].

From these, and other considerations, it appears that structure elucidation of fully substituted benzeneboronates would be more profitably approached using physical techniques. In the past, infrared spectroscopy has been applied to gain information concerning the conformation of certain boronates. Studies of the infrared spectra of compounds in solutions at concentrations less than 0.005 M yield information concerning the nature of intramolecular hydrogen bonding[84i,ii,iii]. At these concentrations intermolecular hydrogen bonding operates to an insignificant extent, and changes in the O-H stretching frequency (3700-3400 cm^{-1}) can be ascribed to intramolecular hydrogen bonding[84i]. For example, the infrared spectrum of galactitol bisbenzeneboronate[57] showed a single sharp absorption band at 3636 cm^{-1}, indicative of a free hydroxyl group. This afforded the conclusion that the oxygen atoms of the hydroxyl groups are coordinating with the boron atoms in the ester rings, instead of intramolecular hydrogen bonding occurring between the ring oxygens and the free hydroxyl groups.

Correlation of the difference in absorption frequency of non-bonded and intramolecularly bonded hydroxyl groups in diol compounds, to the length of the hydrogen bond has provided useful information regarding conformations of these molecules. Application of this principle to the infrared spectroscopy of benzeneboronates containing a free hydroxyl group must however be regarded carefully since ^{11}B nuclear magnetic resonance (n.m.r.) studies have indicated that back donation of electrons by the oxygen atom in cyclic

boronates occur to a greater degree in six-membered rings than in five-membered rings[29,30]. This reduces the availability of the non-bonding electrons on the ring oxygen for forming hydrogen bonds, and therefore the observed shift in absorption frequency is no longer indicative of the hydrogen bond length.

Several absorption bands in the infrared spectra of acyclic[85] and cyclic[30] boronates have been assigned for example to B-aryl and B-O stretching modes. However, no correlations between stretching frequency and ring size for cyclic boronates have been made.

The advent of sophisticated high resolution mass spectrometers has seen large developments in their use for the assignment of the structures of natural and synthetic compounds. Their application to structure determination of carbohydrate derivatives is no exception. In particular, this technique has been used on several occasions for the study of cyclic benzeneboronate esters of carbohydrates. From considerations of these publications the use of electron impact mass spectrometry of tetritol benzeneboronates was expected to provide information concerning their structures.

Reference has been made earlier, (p.22), to the possibility of the formation of structural isomers when benzeneboronic acid forms cyclic esters with compounds containing three or more hydroxyl groups. From previous experiments concerning the quantitative determination of the products formed from the interaction of benzeneboronic anhydride and several triols[58,86], there appeared a distinct possibility of structural modifications in the products formed from tetritols. Purification by recrystallisation of the benzeneboronates of some triols has been shown[86] to result in a partial fractionation of the isomers. Consequently, the structural assignment of the purified product is not necessarily the structure of the major product. For this reason the bisbenzeneboronates derived from the 1-deoxy-pentitols,

the 1,6-dideoxy-hexitols and the pentitols, to be discussed presently, were not purified. Mass spectrometry carried out on the crude products was expected to indicate the predominant, if not exclusive, structure of the bisbenzeneboronates.

The importance of determining the structure of the major product, rather than the structure of the purified product, enabled correlations between the predominant structure in the product and the expected structure to be made. The expected structure of the bisbenzeneboronate being based on the most favoured ring size and on the tendency to form rings which involve the minimum of steric interaction between the substituents on the ring.

II.2.A 1-Deoxy-Pentitols

The 1-deoxy-pentitols, from which the 1-deoxy-pentitol bisbenzeneboronates were derived, were obtained by reductive desulphurisation of the appropriate diethyldithioacetal, (Fig.33). Hydrogenolytic desulphurisation

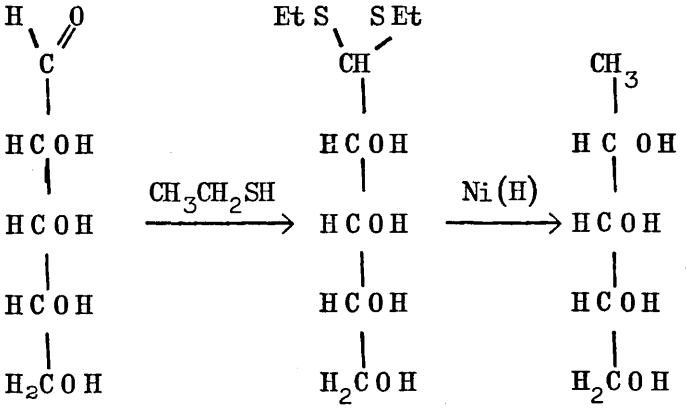


Fig.33

of dithioacetals occurs under mild conditions, with few side reactions. Unexpected products usually result only if less active or deactivated Raney nickel preparations are employed[87]. The mechanism of desulphurisation is still not certain, but at present it is generally believed to proceed via. a free radical mechanism, after chemisorption of the sulphur atom on the surface of the catalyst[88-91].

The radicals produced after fission of the carbon-sulphur bond, may then either recombine or become hydrogenated by the presence of the absorbed hydrogen, (Fig.34). The dimeric products become significant

$$R - S - R^1 \xrightarrow{Ni(H)} R^{\cdot} + R^{\cdot 1} \rightarrow RH + R^1H$$
$$\searrow RR + RR^1 + R^1R^1$$

Fig.34

only if deactivated catalyst is present[91]. Products other than those expected, have been described during the desuplhurisation of diethyl-dithioacetals. For example, ω-deoxy-ω-S-ethyl-polyols were isolated when the mercaptal was treated with a catalyst of low hydrogen content[92], (Fig.35). 1-Deoxy-alditols were easily obtained from the 1-deoxy-1-S-ethyl alditols by further reaction with Raney nickel. Variable yields of the 1-deoxy alditols have resulted in the past probably because of adsorption of the product on the nickel, especially if large quantities of catalyst are used[93]. The present 1-deoxy-pentitols were obtained in 40-50% yields by using a nickel to mercaptal ratio of 4:1 W/W, with reaction times of typically one hour.

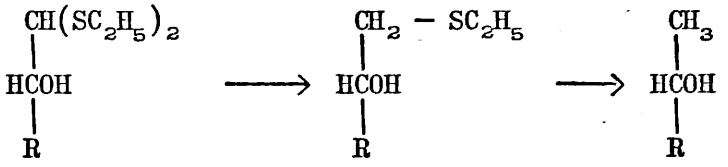


Fig.35

The fully substituted 1-deoxy-pentitol benzeneboronate esters were prepared simply by mixing the alditol together with slightly greater than the stoichiometric amount of phenylboronic anhydride in 2-methoxyethanol. Reaction times were usually one hour over steam, and subsequent removal of the solvent generally afforded a white solid. The crude products were then subjected to mass spectrometry for structural analysis.

II.2.A

(a) 1-Deoxy-D-arabinitol

The low resolution mass spectra of all the benzeneboronates can be found in the appendix. The abundances of the ions have been expressed as a percentage of the total ion current, Σ_x, excluding ions below an m/e

value of x. All the benzeneboronates studied gave molecular ions having the expected atomic composition as verified by precise mass measurements. No ions of higher m/e value were found, thus precluding the possibility of dimeric, trimeric, etc. forms.

The most abundant ion, the base peak, in the spectrum of 1-deoxy-D-arabinitol bisbenzeneboronate occurred at $\frac{m}{e}$160, indicative of the ion 7, resulting from a double elimination process of the six-membered boronate ring carrying the methyl substituent, (Fig.36). The corresponding ion 8, $\frac{m}{e}$174, $C_{10}H_{11}BO_2$ was also present as a result of the alternative ring undergoing the double elimination process. The abundance of this ion is much lower however, probably because the methyl substituent is lost, as a radical to give 9, $\frac{m}{e}$159, $C_8H_8BO_2$, or the methyl substituent is lost initially to give 11, $\frac{m}{e}$293, which then undergoes double elimination to provide the ion 9, (Fig.36). Previous metastable ion measurements[58] have shown the ion 9, $\frac{m}{e}$159, to be produced directly from the molecular ion in other bisbenzeneboronates having the fused six-membered ring structure.

An ion of significant abundance occurred at $\frac{m}{e}$173, having the composition $C_{10}H_{10}BO_2$, indicating the ion 10, (Fig.36). This ion could conceivably have arisen from 8, $\frac{m}{e}$174 through loss of a hydrogen radical, in an analogous fashion to loss of a methyl radical to give 9, $\frac{m}{e}$159.

An abundant ion, $\frac{m}{e}$147, could be readily explained on the basis of an alternative two five-membered ring structure, through bond fission between the rings, (Fig.37). In addition, the ion 12, $\frac{m}{e}$161 could be shown to support this assignment. However, the formation of these ions could be explained equally well by a process of fragmentation which occurs in fused six-membered rings, called 'half-rupture' [94,95] (Fig.38). Fragmentation of the molecular ion via. pathway A would provide the ion 14, $\frac{m}{e}$161, $C_9H_{10}BO_2$, if the residual charge was retained by the oxygen attached

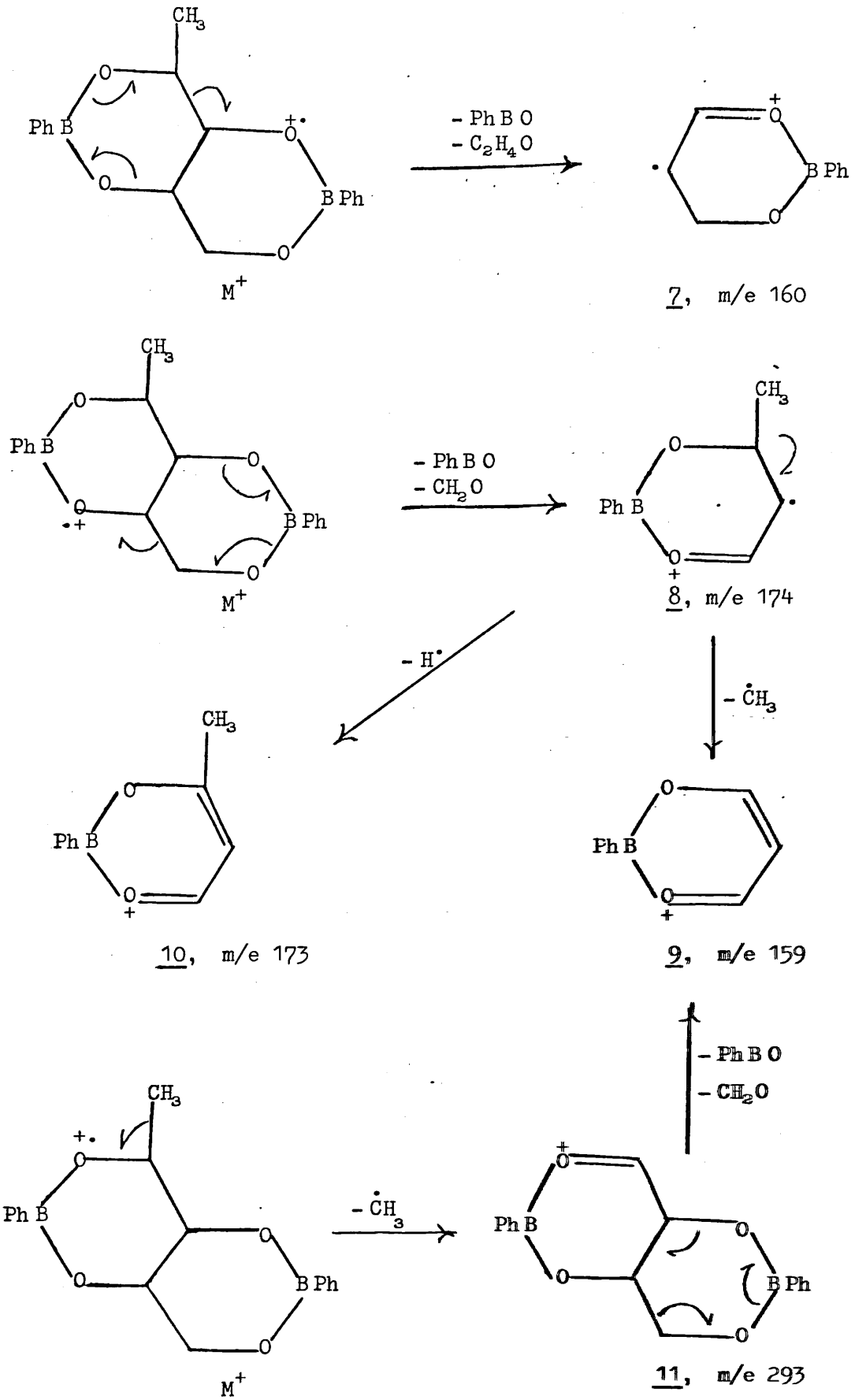
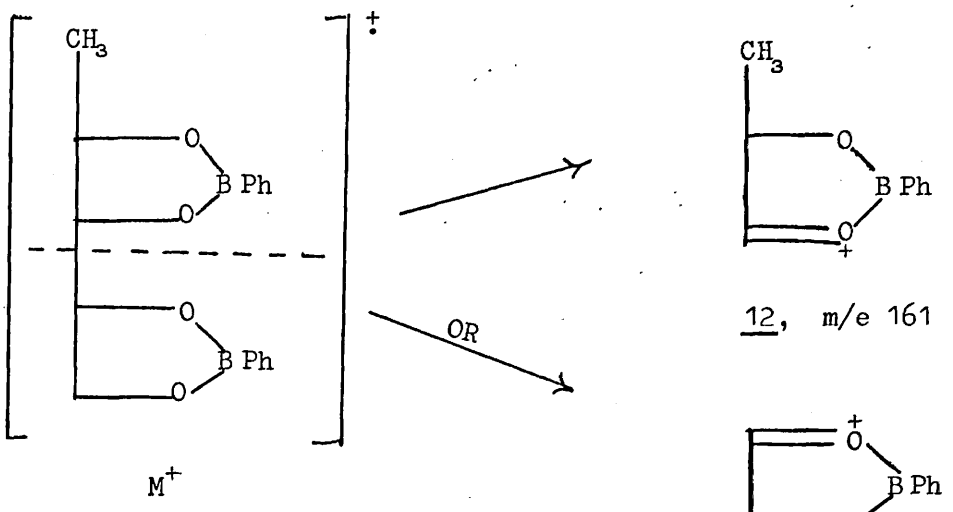

CH3
Ph B
O
B Ph
M+
- Ph B O
- C2H4O
7, m/e 160
- Ph B O
- CH2O
8, m/e 174
- H·
- ĊH3
10, m/e 173
9, m/e 159
- ĊH3
- Ph B O
- CH2O
11, m/e 293

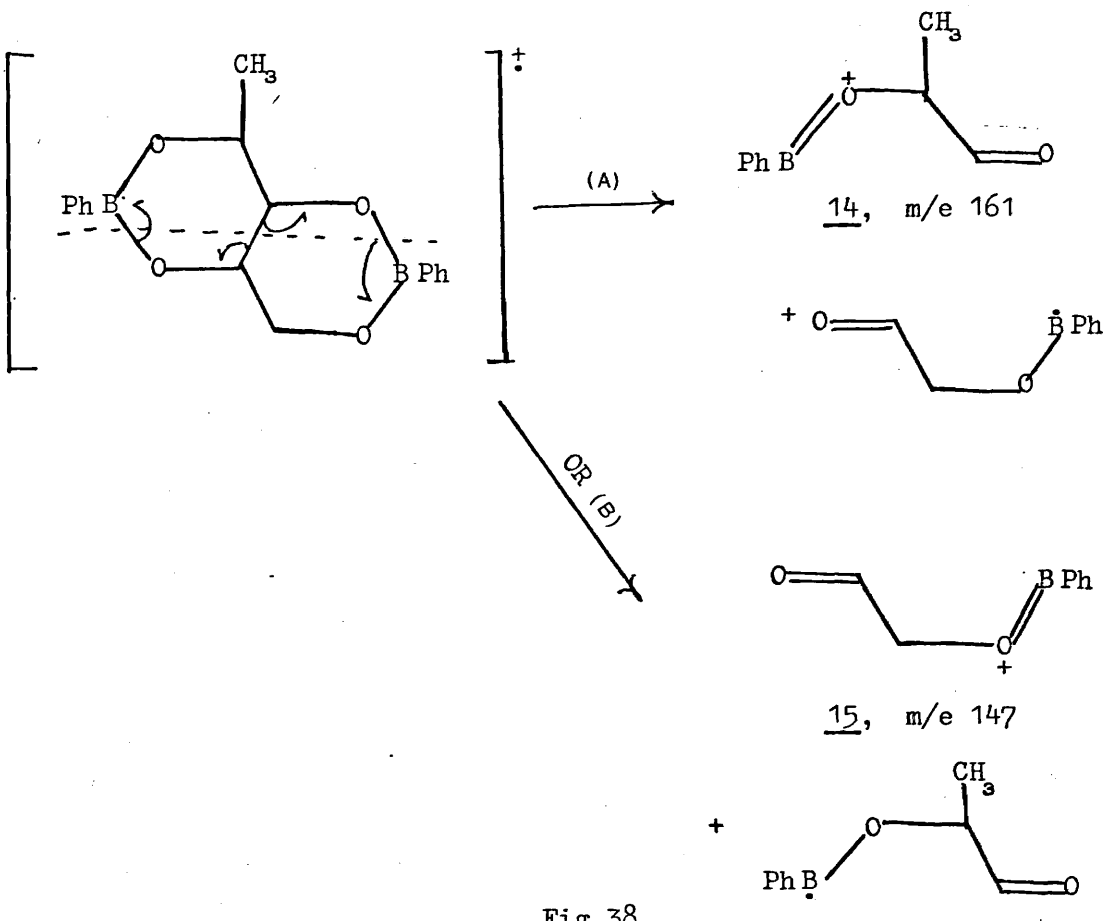
Fig.36



12, m/e 161

13, m/e 147

Fig.37



14, m/e 161

15, m/e 147

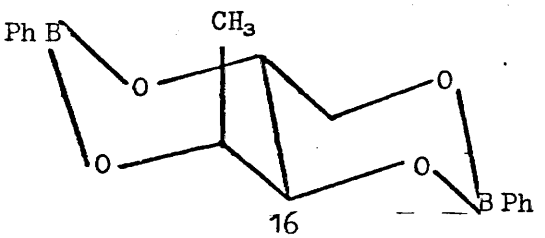
Fig.38

to C-2. Otherwise, retention of the charge at the C-5 oxygen would lead to the ion 15, $\frac{m}{e}$147, $C_8H_8BO_2$. Unfortunately, metastable ion measurements would not differentiate between a 'half-rupture' mechanism or a fission between two five-membered boronate rings, since the position of the metastables are identical. On a study of six bisbenzeneboronates of alditols, the mass spectra of five of these contained a base peak at $\frac{m}{e}$147 (58). It was concluded that the ion $\frac{m}{e}$147 was not suitable for structural identification.

The remainder of the spectrum is typical of cyclic benzeneboronate esters. The ions at $\frac{m}{e}$104 and $\frac{m}{e}$105 were both found to be doublets by high resolution mass spectrometry. The ion $\frac{m}{e}$105, C_8H_9 probably results from the rearrangement of the molecular ion, or the oxonium ions 8, or 10, in an analogous manner to the monobenzeneboronates. The other ion in the $\frac{m}{e}$105 doublet, of atomic composition C_6H_6BO, was found in the spectra of all the benzeneboronates and generally constitutes the greater part of this ion. This ion is also produced from the oxonium ions, such as 8, by loss of an oxo-compound, acetaldehyde and acetylene.

The ion $\frac{m}{e}$104 consisted of two ions of atomic composition $C_6H_6{}^{10}BO$ and C_6H_5BO in a ratio 2:3. The latter ion is produced by double elimination of six-membered boronate rings, but also appears in the spectra of five-membered rings by a two-step fragmentation from the molecular ion, (Figs.24 and 25).

The predominant structure of the bisbenzeneboronate formed when 1-deoxy-D-arabinitol interacts with benzeneboronic anhydride would therefore appear to be comprised of two fused six-membered rings, 16, 1-deoxy-D-arabinitol 2,4; 3,5-bisbenzeneboronate. The configurational arrangement of the hydroxyl groups on the carbon chain of



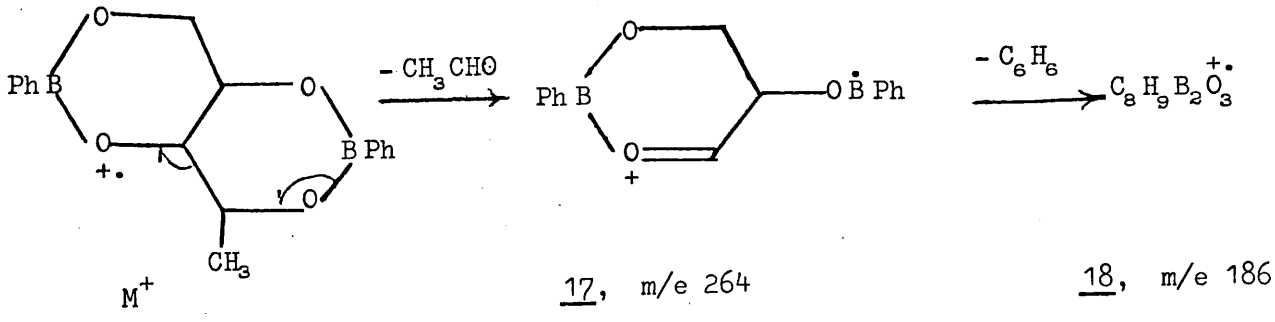
16

1-deoxy-D-arabinitol necessarily requires that two fused six-membered boronate rings will be trans-fused. The methyl substituent is axially orientated, indicating that the steric interaction involved is not sufficient to secure predominant or exclusive formation of two five-membered rings. By comparison, the monobenzeneboronates of a series of triols were found to form a five-membered rings structure exclusively if the alternative six-membered ring had a substituent in an axial orientation[86].

II.2.A

(b) 1-Deoxy-D-ribitol

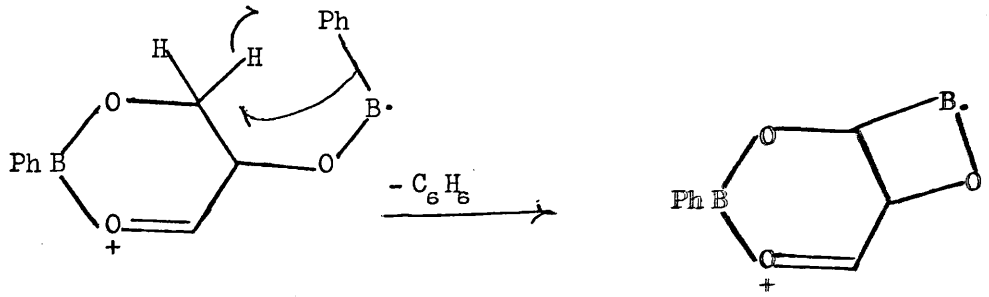
The mass spectrum of the crude product from the reaction of 1-deoxy-D-ribitol and benzeneboronic anhydride was similar to that obtained for the 1-deoxy-D-arabinitol product. The ion $\frac{m}{e}$ 160 again formed the base peak, indicative of the two fused six-membered ring structure. An ion at $\frac{m}{e}$ 186 is rather curious, since its presence is not readily explained by the normal fragmentation patterns of benzeneboronates. A similar peak occurred in the mass spectrum of 1-deoxy-D-xylitol bisbenzeneboronate [58], and its precursor was shown, by measurements of metastable ions, to be the ion 17, $\frac{m}{e}$ 264, $C_{15}H_{14}B_2O_3$, formed by loss of acetaldehyde from the molecular ion, (Fig.39). The atomic composition of the ion 18 by precise mass measurements was $C_9H_8B_2O_3$, corresponding to loss of C_6H_6 from the ion 17, $\frac{m}{e}$ 264. It is



17, m/e 264 18, m/e 186

Fig.39

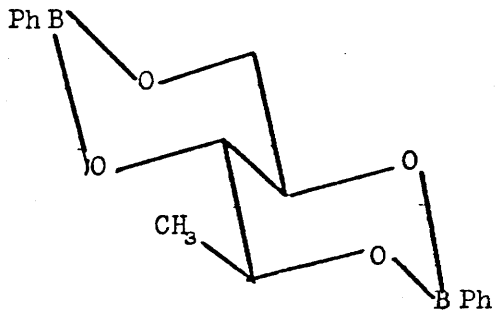
suggested therefore that fission of the B-phenyl bond occurs with either the elimination of a molecule of benzene, or the simultaneous loss of the phenyl radical and a hydrogen atom. The most likely structure for the ion 18, would then involve a four-membered ring, formed after the abstraction of a hydrogen from C 5, (Fig.40).



17, m/e 264 18, m/e 186

Fig.40

The remainder of the spectrum of 1-deoxy-D-ribitol bisbenzeneboronate indicated that the predominant structure is comprised of two fused six-membered rings, using a similar argument for the interpretation of the mass spectrum as that for 1-deoxy-D-arabinitol bisbenzeneboronate. The relationship of the rings will again be trans-fused, with the methyl substituent occupying an equatorial position, 19. This structure is therefore expected to be favoured over the two five-membered ring structure.

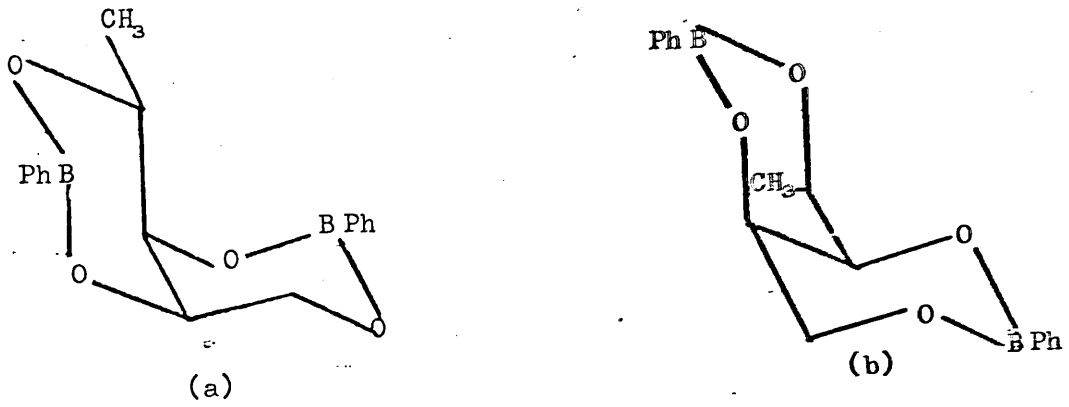


19, 1-deoxy-D-ribitol 2,4; 3,5-bisbenzeneboronate.

II.2.A
(c) 1-Deoxy-D-lyxitol

The mass spectrum of the product obtained from 1-deoxy-D-lyxitol and benzeneboronic anhydride had the ion 7, $\frac{m}{e}160$, $C_9H_9BO_2$ as the base peak, constituting 14.7% of the total ion current. The ion 18, $\frac{m}{e}186$,

$C_9H_8B_2O_3$ was also present, together with its precursor ion 17, $\frac{m}{e}$264, $C_{15}H_{14}B_2O_3$. The ion $\frac{m}{e}$147, $C_8H_8BO_2$ was not so abundant as in the previous two tetritol boronates, which may indicate that 'half-rupture' for this molecule is not so prominent and/or that the structure consists entirely of two six-membered rings.



20, 1-deoxy-D-lyxitol 2,4; 3,5-bisbenzeneboronate

Fig.41

The mass spectrum therefore supplies convincing evidence for the product to consist predominantly of the structure 20, 1-deoxy-D-lyxitol 2,4; 3,5-bisbenzeneboronate, (Fig.41). The configuration of the hydroxyl groups on 1-deoxy-D-lyxitol require the fused six-membered ring structure to adopt a cis-relationship between the rings, (Fig.41). The methyl substituent has the ability to occupy an equatorial position, (Fig.41(a)), or an axial position, (Fig.41(b)), due to the interconvertability of cis-fused six-membered boronate rings. The structure (a) would, however, be the most likely from considerations of steric interactions.

The major fragments observed during the electron impact mass spectrometry of these 1-deoxy-pentitol bisbenzeneboronates and the 1,6-dideoxy hexitol bisbenzeneboronates, to be discussed shortly, are given in Table I, together with their abundances expressed as a percentage of the total ion current, $\%\Sigma_{40}$.

TABLE I

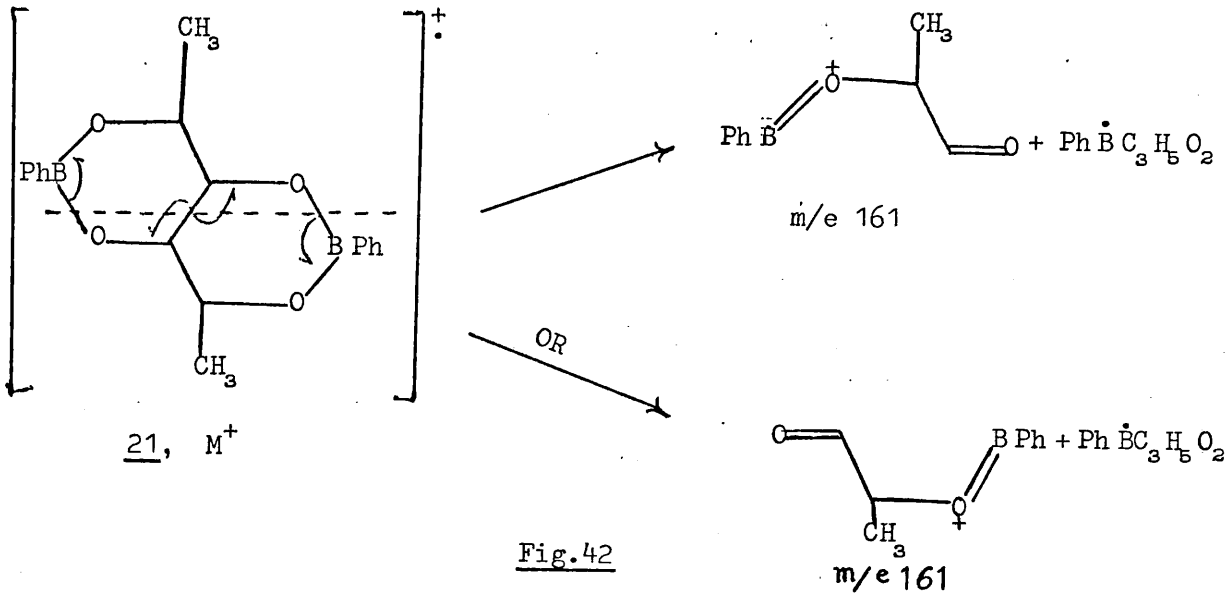
ABUNDANCES OF THE MAJOR FRAGMENTS IN THE MASS SPECTRA OF TETRITOL BISBENZENEBORONATES

Parent Tetritol of Bisbenzeneboronate	Abundances of Major Fragments, % Σ_{40}							Predominant Ring Size
	147	159	160	161	173	174	M^+	
1-deoxy-D-arabinitol	7.2	6.6	8.1	8.0	3.1	0.8	7.1	6
1-deoxy-D-ribitol	3.8	7.6	8.0	7.3	1.3	0.5	7.2	6
1-deoxy-D-lyxitol	1.2	14.9	15.6	4.5	0.8	0.5	12.8	6
1,6-dideoxy-galactitol	0.6	4.0	8.4	17.5	0	0.8	2.1	5
1,6-dideoxy-L-mannitol	0	13.2	2.1	1.4	0	5.2	2.4	6

II.2.B 1,6-Dideoxy-hexitols

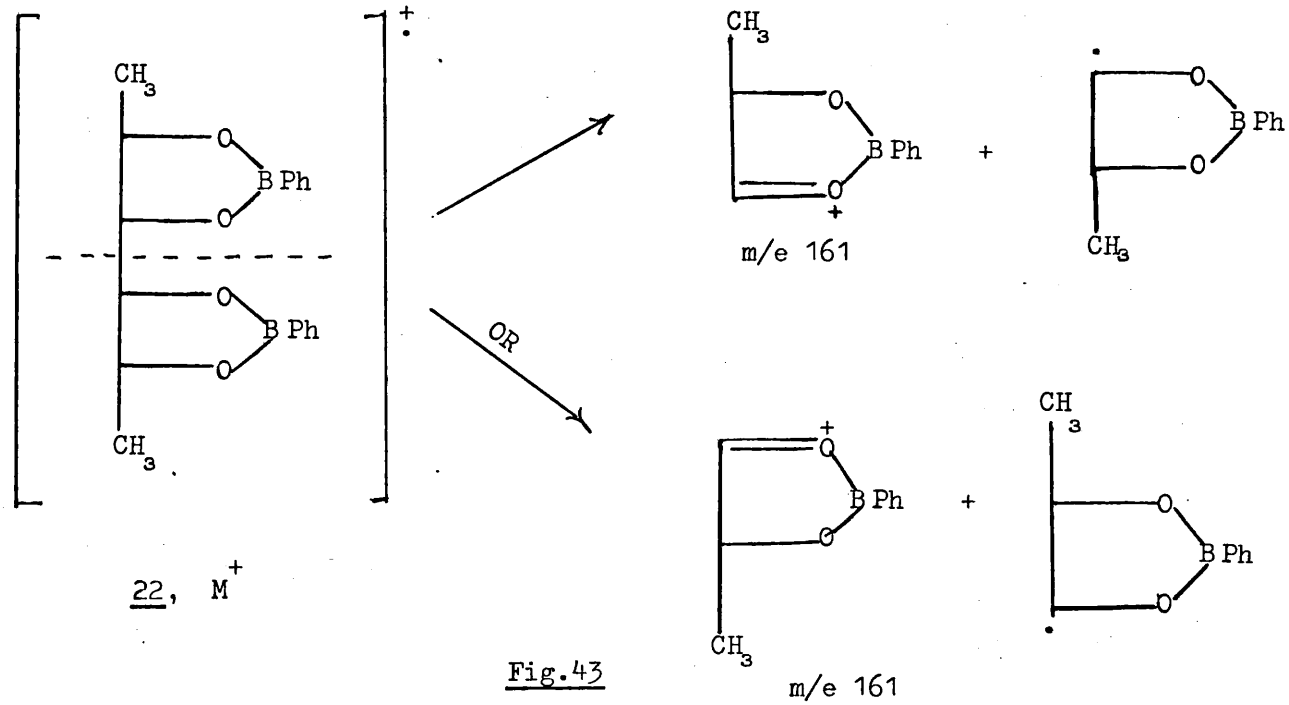
The boronate esters of 1,6-dideoxy-galactitol and 1,6-dideoxy-L-mannitol were prepared by heating the tetritol with benzeneboronic anhydride in a carefully dried solvent, as described for the 1-deoxy-pentitols. The residue in both cases consisted of a white crystalline mass, eminently suitable for direct insertion via. a probe into the mass spectrometer.

The mass spectra of the 1,6-dideoxy-hexitol boronates was anticipated to resemble those of the 1-deoxy-pentitols, with the following exceptions. The molecular ion will necessarily appear 14 m.u. higher. The ion $\frac{m}{e}147$ will also be expected to shift 14 m.u. to $\frac{m}{e}161$. This ion could arise from the 'half-rupture' process in fused six-membered rings, (Fig.42), or from fission of the C-3-C-4 bond in a two five-membered structure, (Fig. 43). Consequently the ion $\frac{m}{e}161$ is of no value in distinguishing between structures 21 and 22.



21, M^+

Fig.42



22, M^+

Fig.43

1,6-Dideoxy-L-mannitol

The abundances of the major fragments occurring in the mass spectra of the 1,6-dideoxy-hexitol bisbenzeneboronates which indicate ring size, are shown in Table I. The spectrum of the product obtained from the interaction of 1,6-dideoxy-L-mannitol and benzeneboronic anhydride was decidedly simpler than the mass spectra of the 1-deoxy-pentitol boronates. A particularly abundant base peak at $\frac{m}{e}159$, $C_9H_8BO_2$, accounted for 13.2% of the total ion current, Σ_{40}, indicating that the structure of the bisbenzeneboronate is predominantly comprised of two six-membered rings. The ion 8, $\frac{m}{e}174$, $C_{10}H_{11}BO_2$, was also present in significant abundance, (Table I), supporting the fused six-membered ring assignment.

Some of the metastable ions that appeared in the spectrum of 1,6-dideoxy-L-mannitol bisbenzeneboronate are given in Table II. The measured values were obtained by a technique developed by DALY et al.[96], in which the normal ions in the ion beam are suppressed. A scintillating device rejects a large proportion of the ions which are produced in the ion source, but enhances those which fragment during their flight between the electrostatic and magnetic analysers.

The metastable ions confirm the occurrence of the double elimination process that occurs during mass spectrometry of six-membered benzeneboronate rings. The ion 8, $\frac{m}{e}174$, $C_{10}H_{10}BO_2$, is produced directly from the molecular ion, as indicated by the presence of a metastable ion at $\frac{m}{e}94$. This fragments further to provide the ion 9, $\frac{m}{e}159$, $C_9H_8BO_2$, evidenced by the strong metastable at $\frac{m}{e}145.3$. The ion $\frac{m}{e}174$ also provides the ion 10, $\frac{m}{e}173$, $C_{10}H_9BO_2$, as suggested in the case of the 1-deoxy-pentitol boronates, as shown by the presence of the metastable at $\frac{m}{e}172.0$.

TABLE II

METASTABLE IONS OCCURRING IN THE MASS SPECTRUM OF 1,6-dideoxy-L-mannitol bisbenzeneboronate

METASTABLE IONS (m/e)		ASSIGNMENT	
Measured	Calculated	Parent	Daughter
240.0	240.0	322	278
124.2	124.2	322	200
94.0	94.0	322	174
34.0	34.2	322	105
248.7	248.8	278	263
143.6	143.9	278	200
108.6	108.9	278	174
172.0	172.0	174	173
145.3	145.3	174	159
63.5	63.4	174	105
146.2	146.1	173	159
144.3	144.3	173	158
69.2	69.3	159	105

The ion 18, $\frac{m}{e}$ 186, $C_8H_9B_2O_3$, which occurred in the mass spectra of the 1-deoxy-pentitols, has an analogous ion in the spectrum of 1,6-dideoxy-L-mannitol bisbenzeneboronate, at $\frac{m}{e}$ 200. Metastable ion measurements suggest that this ion is produced directly from the molecular ion in addition to its formation from the ion 23, $\frac{m}{e}$ 278, $C_{16}H_{16}B_2O_3$, (Fig.44).

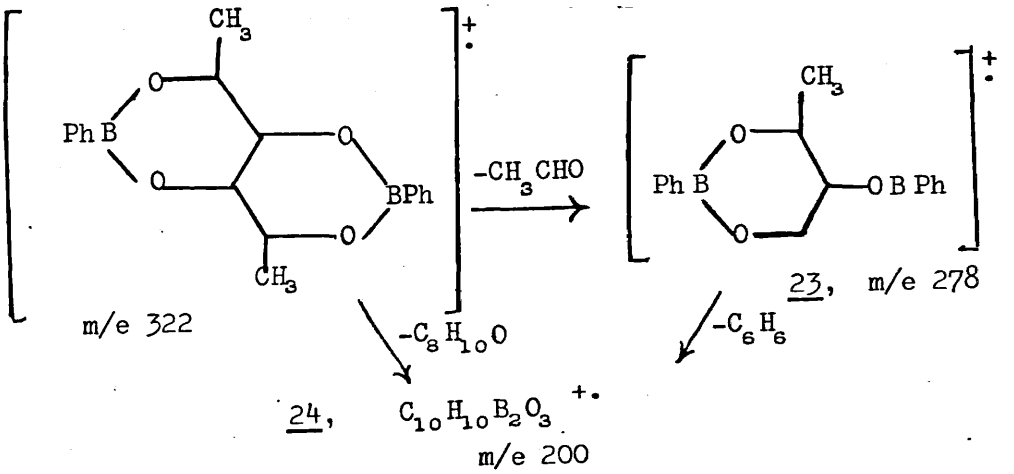
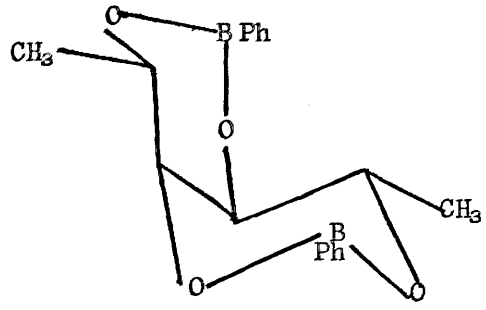


Fig.44

The low abundance of the ion $\frac{m}{e}$161, together with the complete absence of an ion at $\frac{m}{e}$146, indicates that the fused six-membered ring structure is the predominant, if not the only structure present. A molecular model of 1,6-dideoxy-L-mannitol 2,4; 3,5-bisbenzeneboronate shows that two six-membered boronate rings fused onto the 1,6-dideoxy-L-mannitol chain would necessarily adopt a cis-fused configuration with both methyl substituents occupying equatorial positions, 25.



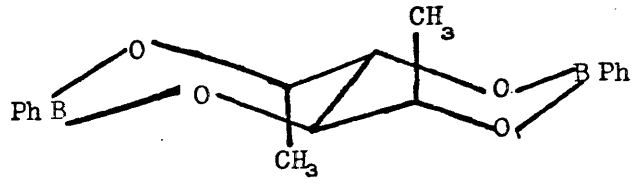
25

1,6-dideoxy-L-mannitol 2,4; 3,5-bisbenzeneboronate

1,6-dideoxy-galacititol

The mass spectrum of this tetritol bisbenzeneboronate differs significantly from the previous dideoxy tetritol boronate indicating a predominance of two five-membered rings. This is concluded from the base peaks at $\frac{m}{e}$161, $C_9H_{10}BO_2$, accounting for 17.5% of the total ion current, and also from the presence of the ion $\frac{m}{e}$146, $C_8H_7BO_2$. However, there is some evidence for the presence of six-membered rings, from the ion $\frac{m}{e}$174, $C_{10}H_{11}BO_2$, and the ion $\frac{m}{e}$159, $C_9H_8BO_2$. A metastable ion $\frac{m}{e}$145.3, indicates that the ion $\frac{m}{e}$174, $C_{10}H_{10}BO_2$, fragments to give the ion $\frac{m}{e}$159, $C_8H_9BO_2$ directly; the only plausible explanation being the existence of two six-membered rings.

From a consideration of the spatial disposition of the hydroxyl groups on 1,6-dideoxy-galactitol, the formation of two fused-six-membered rings would



26

1,6-dideoxy-galactitol 2,4; 3,5-bisbenzeneboronate

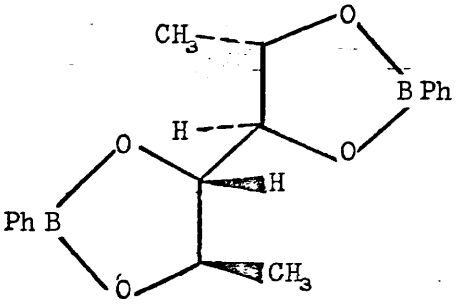
TABLE III

METASTABLE IONS OCCURRING IN THE MASS SPECTRUM OF

1,6-dideoxy-galactitol bisbenzeneboronate

METASTABLE IONS (m/e)		ASSIGNMENT	
MEASURED	CALCULATED	PARENT	DAUGHTER
240.0	240.0	322	278
84.3	84.4	307	161
248.2	248.8	278	263
145.3	145.2	174	159
144.1	144.3	173	158
85.0	85.0	161	117
68.6	68.5	161	105

provide a trans-junction, with both methyl substituents in axial configurations, 26. The preference to form five-membered rings could therefore be explained by unfavourable steric interaction between the axially-orientated methyl groups and axial hydrogen atoms. It is concluded that 1,6-dideoxy-galactitol forms the 2,3; 4,5-bisbenzeneboronate structure, 27, to a greater extent than the 2,4; 3,5-bisbenzeneboronate, 26.



27

1,6-dideoxy-galactitol
2,3; 4,5-bisbenzeneboronate

II.3 BENZENEBORONATES OF PENTITOLS

The presence of five hydroxyl groups for potential esterification provides an opportunity for the formation of a greater number of structural isomers than are possible for the tetritols. However, structure elucidation may be aided by the possibility of forming derivatives from the unesterified hydroxyl group, which results from the bifunctional behaviour of benzeneboronic acid. The problem of structure elucidation of the pentitol bisbenzeneboronates can be divided into two sections. The first section will deal with the problem of determining the position of the unesterified hydroxyl group in the bisboronate. The second will be concerned with the type of ring structure involved.

It was realised at the beginning that there was a distinct possibility for any one pentitol to form more than one bisbenzeneboronate structure on reacting with benzeneboronic anhydride. The structural analysis of the bisbenzeneboronates of the pentitols was therefore carried out on the crude products, for the same reasons that were outlined for the tetritols, (see p.46). With these considerations in mind, a method had to be devised which could distinguish between the various positions of the unesterified hydroxyl groups, and possibly determine the relative amounts of each isomer present, if a mixture of structural isomers existed in the bisboronate product. Mass spectrometry alone would be insufficient to allow identification of all the possible isomers present in a mixture and would not provide a reliable estimate of the relative amounts of each. A chemical method appeared to be more appropriate which would locate the position of the unprotected hydroxyl group.

II.3.A Positions of Substitution

(a) The chemical method

A derivative that has stability at elevated temperatures and confers some volatility on the compound to be applicable for g.l.c. analysis was envisaged for the separation of the isomers of the bisbenzeneboronates of the pentitols. The boronate esters themselves generally lack the volatility to enable g.l.c. to be performed, their butaneboronate analogues have found wider acceptance for this technique[45-50]. Also, the usual derivatives employed for masking polar groups, such as trimethylsilyl or methyl ethers and acetates, are still unable to render the bisbenzeneboronates suitable for separation on g.l.c. columns. However, some success has been achieved with certain benzeneboronates of glycosides followed by trimethylsilylation[50].

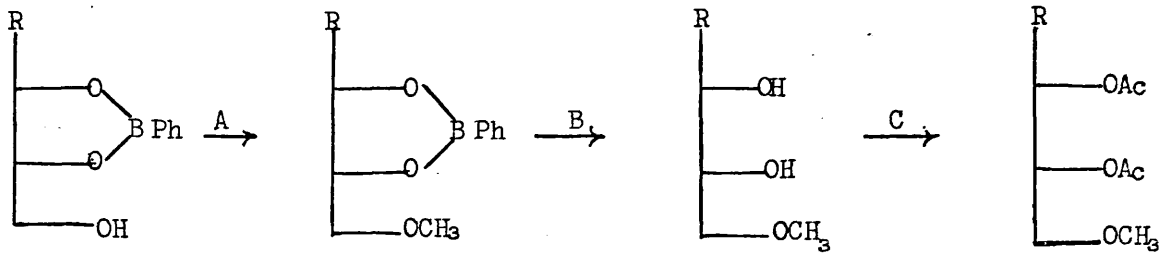


Fig.45

The strategy envisaged to overcome this difficulty was to initially form a derivative from the single unprotected hydroxyl group of the bisbenzeneboronate mixture, remove the boronate ester groups by mild hydrolysis and convert the polar hydroxyl groups to acetate esters, thus rendering the molecule sufficiently volatile for g.l.c. analysis. The scheme for this procedure is outlined for part of a typical pentitol bisbenzeneboronate, (Fig.45). Methylation was chosen as the method for identifying the unprotected hydroxyl group, since a method for the preparation of methyl ethers from hydroxyl groups, in the presence of benzeneboronate esters, has been reported[60]. This particular method is eminently suitable for small scale

work since it proceeds in virtually quantitative fashion, with no migration of the boronate groupings.

The traditional PURDIE and IRVINE technique of methylation[97] of hydroxyl groups in the presence of benzeneboronate esters has been hindered by the concurrent formation of water which tends to hydrolyse the boronate ester, leading to a variety of products[98]. A method of methylating partially protected carbohydrate residues has been developed by GROSS et al.[99,100], who succeeded in methylating tetra-O-acetyl derivatives of D-glucopyranose and D-galactopyranose in high yield.

The technique involves addition of a dry solution of diazomethane in dichloromethane to a solution of the boronate in 1,2-dimethoxyethane containing 0.1% of boron trifluoride diethyl etherate, to act as a catalyst. The mechanism of methylation of alcohols with diazomethane in the presence of catalytic amounts of boron trifluoride has been suggested to occur by nucleophilic attack by the alcoholic oxygen on the zwitterion formed between borontrifluoride and diazomethane[101,102]. This reaction is thought to take place in competition with the formation of polymethylene by two possible routes, involving either a cationic chain mechanism (1), or a continuously rearranging mechanism (2), (Fig.46). The formation of the methylated alcohol occurs until all the hydroxyl groups have been converted to methyl ethers, when the excess diazomethane is polymerised.

In a series of reactions involving the methylation of alcohols with diazomethane using borontrifluoride etherate, or aluminium trichloride as catalysts, it was suggested that the mechanism might involve formation of a zwitterion between the trihalide catalyst and the alcohol[103], (Fig.47). Electrophilic attack of a proton on diazomethane with simultaneous loss of nitrogen gives an ion pair intermediate, which regenerates the catalyst with subsequent formation of the methyl ether. In either event, methy-

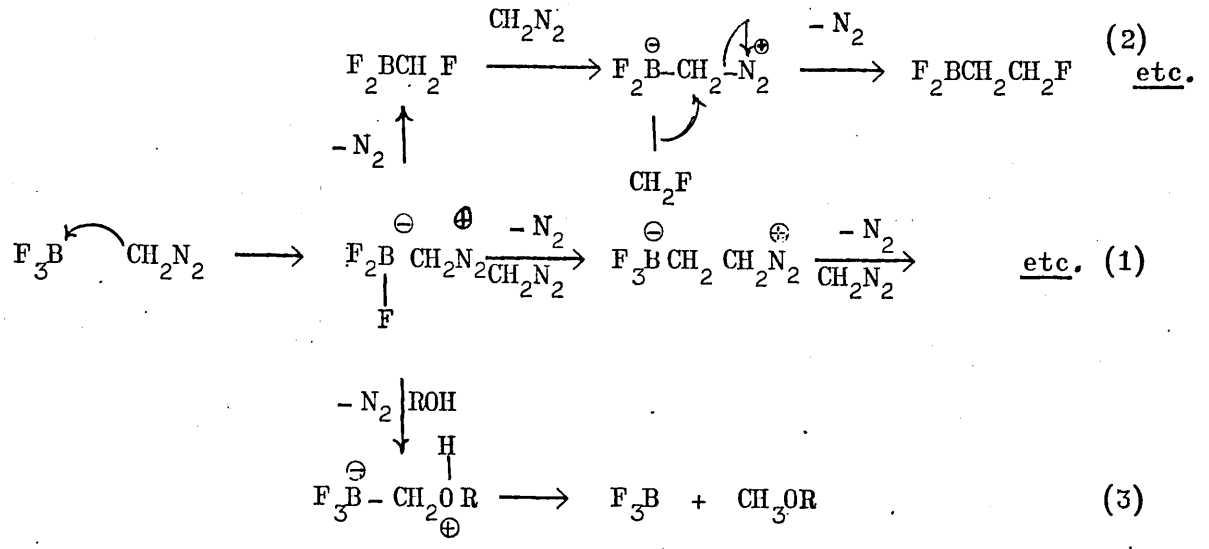


Fig.46

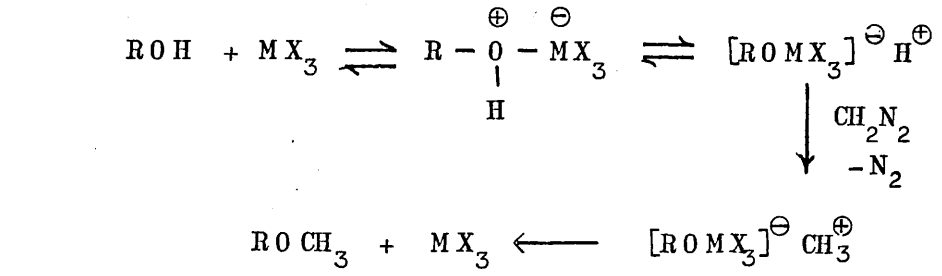


Fig.47

lation of pentitol bisbenzeneboronates proceeded in excellent yield, to give the monomethyl derivatives as detected by paper chromatography.

The hydrolysis of benzeneboronates occurs easily during chromatography on cellulose paper using water-containing organic solvents[39], a property which was of great use in the second stage of structure elucidation, B, (Fig.45). Working on a few milligrams scale enabled the residue from methylation to be chromatographed on preparative paper chromatograms, using a neutral solvent of butanol, ethanol and water. This afforded simultaneous hydrolysis and separation of the ester and the resultant products.

The third step in the analysis, C, proceeds in high yield, to provide the monomethyl-tetra-O-acetyl-pentitol. Separation of these structural isomers was easily accomplished by injecting an aliquot of a chloroform solution of the product onto a g.l.c. column. The majority of separations were achieved using a medium polar stationary phase of cyanopropylmethyl-phenylmethylsilicone, (OV-225), in the temperature range 150-190°. The proportions of each component in the mixtures are given in Table IV, (see p.76). They were obtained by incorporating an electronic integrator between the output of the g.l.c. amplifier and the recorder.

Identification of each isomer was achieved by connecting the gas-liquid chromatograph directly to a mass spectrometer. The mass spectrum of the methylated pentitol acetate could then be obtained directly, and from a knowledge of the fragmentation patters of similar methylated alditol acetates, the position of methylation was determined.

II.3.A
(b) Mass spectrometry of O-acetyl-O-methyl-pentitols

A major reason for an understanding of the fragmentation patterns of partially methylated alditol acetates originated from a desire to recognise the products from methylation studies of polysaccharides. These methylated alditol acetates are the products of the final stage in the now well-defined method of methylation analysis of polysaccharides, using the combined application of gas-liquid chromatography and mass spectrometry[104]. The fragmentation patterns of these compounds have been the subject of intense investigation[105-110] and only a brief discussion of the results and applications will be given to allow an understanding of their importance in connection with this work.

The mass spectra of partially methylated alditol acetates generally do not give an observable molecular ion. The ion of highest m/e value

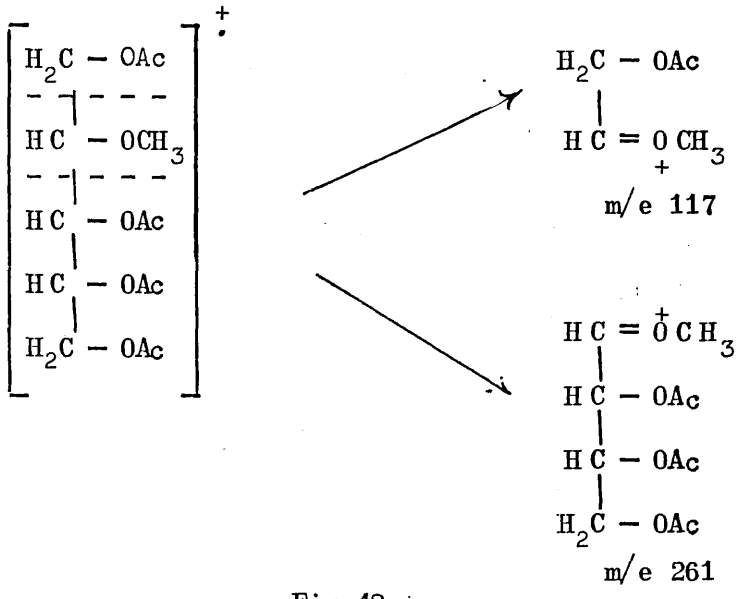


Fig.48

is almost certainly a primary ion, formed by fission between carbon atoms in the chain. The position of cleavage is usually determined by the position of the methylated hydroxyl group. Thus, fission is usually preferred between a methoxylated and an acetoxylated carbon, rather than between two acetoxylated carbons, (Fig.48), with the methoxyl group carrying the formal positive charge.

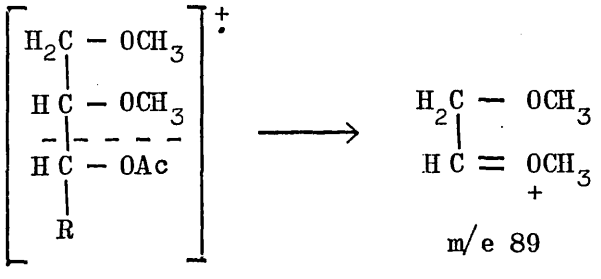


Fig.49

If two methoxylated carbon atoms are adjacent in the carbon chain, then fission between these two occurs more readily than between methoxylated and acetoxylated carbons. One exception to this rule occurs if the adjacent methoxylated carbon atoms are on the end, of the chain, when fission will occur to furnish the ion $\frac{m}{e}89$, (Fig.49).

The presence of a methylene group in the alditol chain inhibits cleavage of the adjacent bond, a phenomena also noted in the mass spectrometry of the fully acetylated alditols[111]. Thus, the mass spectrum of a component produced on methylation and subsequent hydrolysis and acetylation of 1-deoxy-D-talitol bisbenzeneboronate, gave an abundant ion at $\frac{m}{e}$ 59, indicating a methylated hydroxyl group at C-2, however, the ion (M-15), which might be produced by cleavage of the C-1 - C-2 bond, could not be detected, (Fig.50). Similar observations were made on other hexitols with terminal methyl groups.

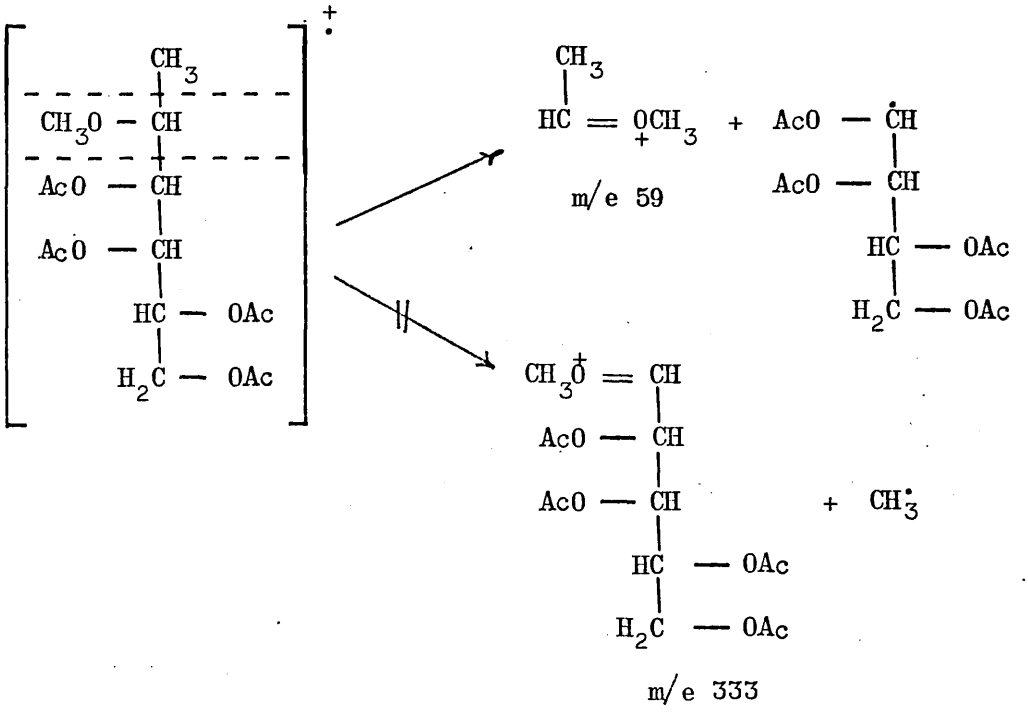


Fig.50

The secondary fragments in the mass spectra of partially methylated alditol acetates are formed from the primary fragments by loss of acetic acid (60 m.u.), ketene (42 m.u.), methanol (32 m.u.), formaldehyde (30 m.u.), methyl acetate (74 m.u.), methoxymethyl acetate (104 m.u.) or acetoxymethyl acetate (132 m.u.). Acetic anhydride, acetic acid and ketene are thought to be lost from the primary ions either via cyclic or acyclic intermediates[109],

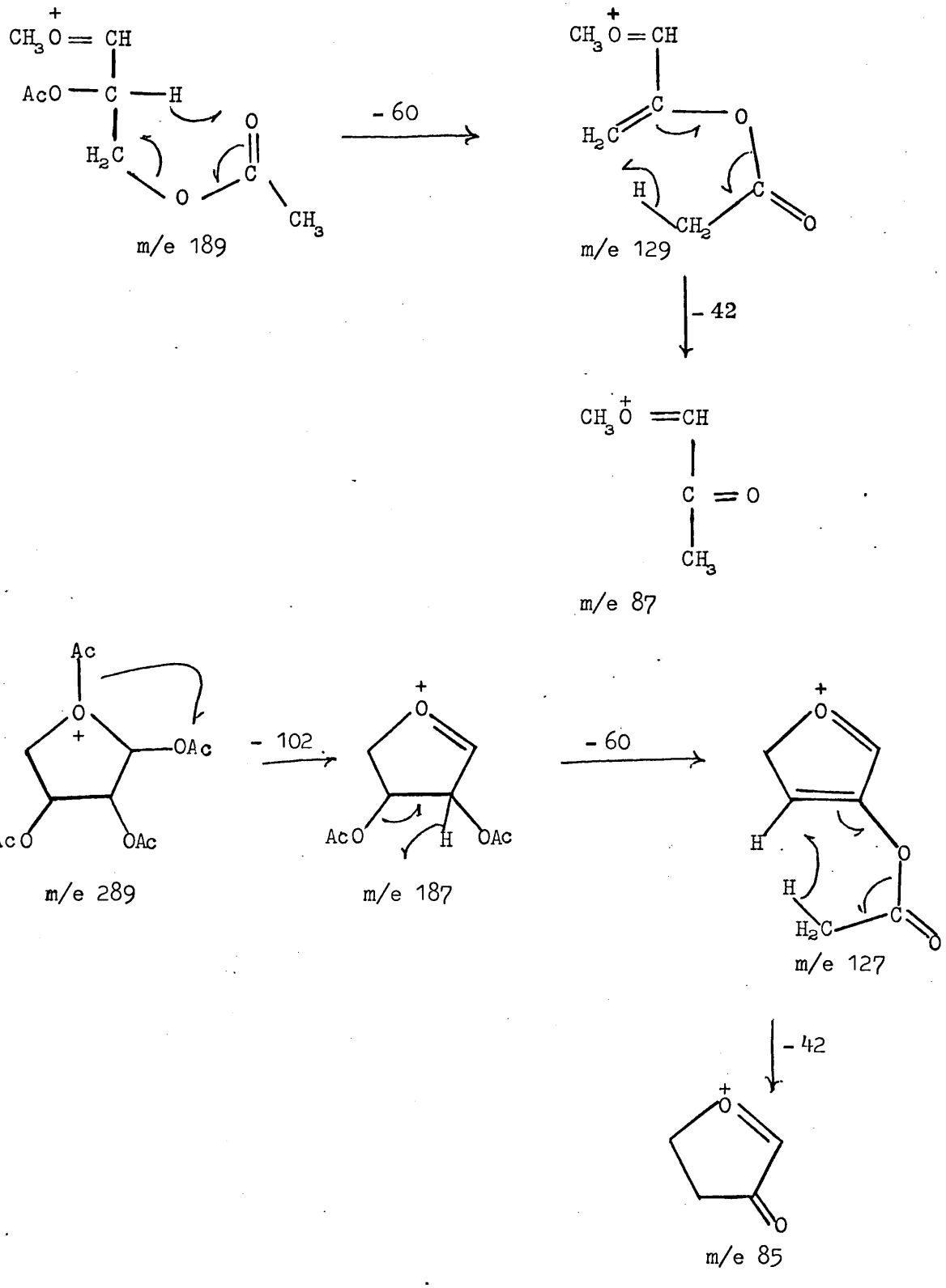
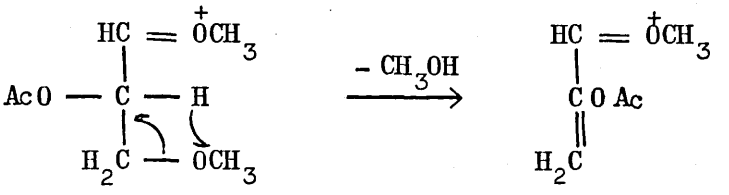

m/e 189
- 60
m/e 129
- 42
m/e 87
Ac
OAc
AcO
OAc
m/e 289
- 102
AcO
H
OAc
m/e 187
- 60
m/e 127
- 42
m/e 85

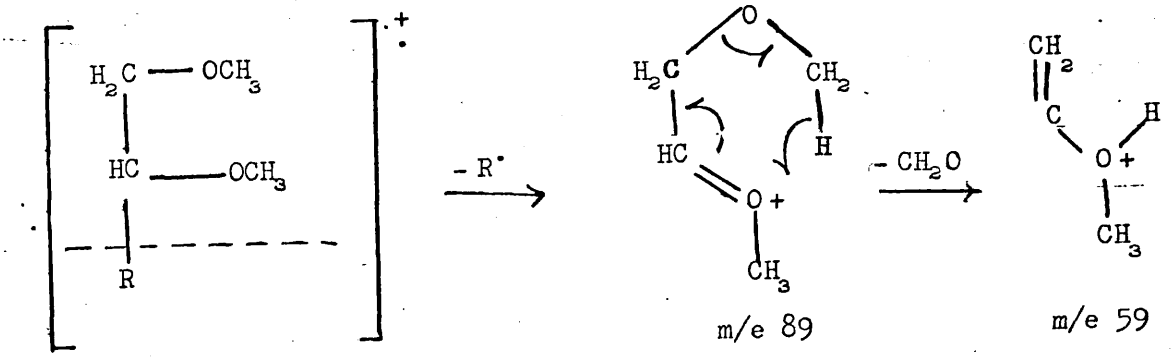
Fig.51

(Fig.51). Methanol is usually lost when the methyl ether grouping is situated at the β-position to the carbon atom considered to carry the formal charge, (Fig.52). Formaldehyde is lost to a significant extent only from the primary fragment $\frac{m}{e}89$, which arises when two adjacent methoxylated groups are in the terminal position, (Fig.53). This latter mode of fragmentation was originally noted in the mass spectra of fully methylated polyols[112].



m/e 161 m/e 129

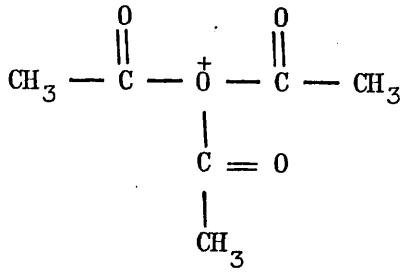
Fig.52



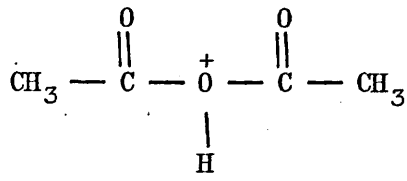
m/e 89 m/e 59

Fig.53

The base peak in the majority of the spectra of O-acetyl-O-methyl alditols is due to the acetylium ion, $\frac{m}{e}43$, $(CH_3 - C^{---}\overset{+}{=} O)$, formed by α-cleavage. Two other ions which are generally present in the mass spectra of compounds containing acetate esters appear at $\frac{m}{e}103$ and $\frac{m}{e}145$, which, for the monosaccharides, have been assigned the structures 28 and 29. However, mass spectral studies on hexa-O-trideuteroacetates of hexitols[111] showed that these ions shifted by mass units of three for the ion $\frac{m}{e}103$ and six for the ion $\frac{m}{e}145$. This latter ion was concluded to compose of two



29, m/e 145



28, m/e 103

isobaric ions resulting from cleavage of the C-2-C-3 bond, or from breakdown of the primary ion 30, $\frac{m}{e}$ 289, (Fig.54). Whereas the ion $\frac{m}{e}$ 103 was depicted as comprising of one acetyl group, not two, as was concluded for the monosaccharide acetates. The origin of this ion was attributed to the primary ion $\frac{m}{e}$ 145, from which it is produced by loss of ketene.

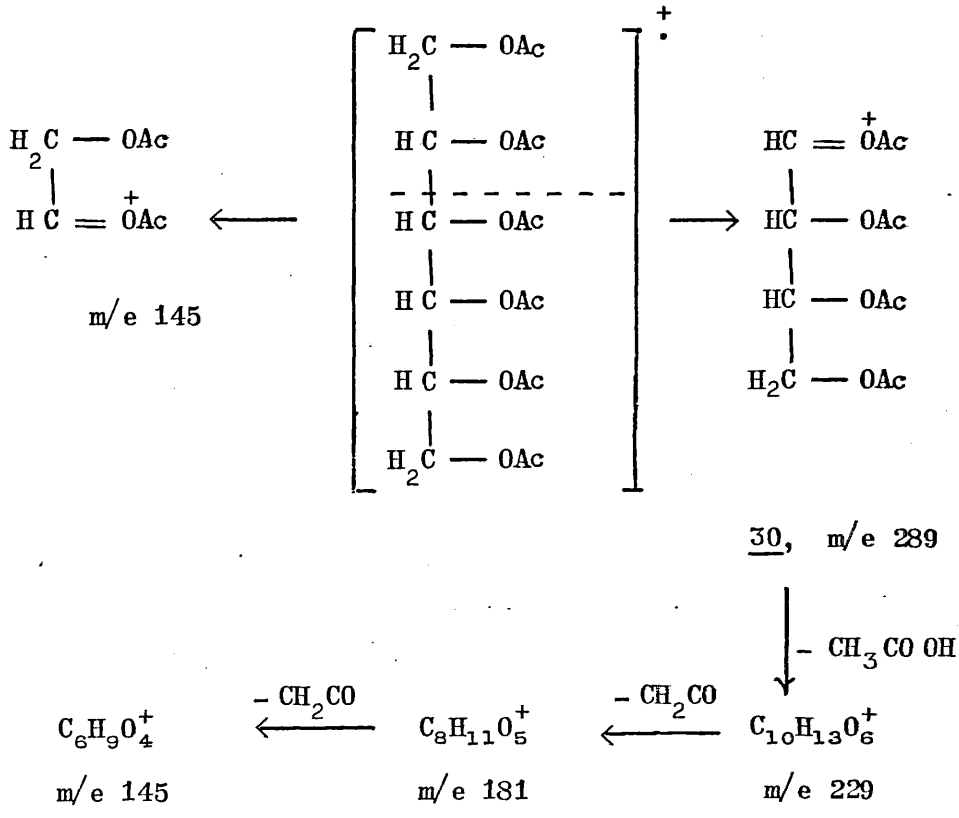
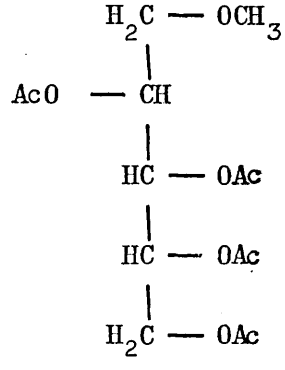


Fig.54

From the above discussion it will be realised that mass spectrometry of O-acetyl-O-methyl pentitols will not distinguish between stereoisomers For example, D-arabinitol bisbenzeneboronate produced a tetra-O-acetyl-mono-O-methyl-D-arabinitol, which was shown by mass spectrometry to

have the methoxy group attached to a terminal carbon. Mass spectrometry could not distinguish between 31, 2,3,4,5-tetra-O-acetyl-1-O-methyl-D-arabinitol and 32, 1,2,3,4-tetra-O-acetyl-5-O-methyl-D-arabinitol. The position of methylation was subsequently determined by labelling C1 with a deuterium atom.



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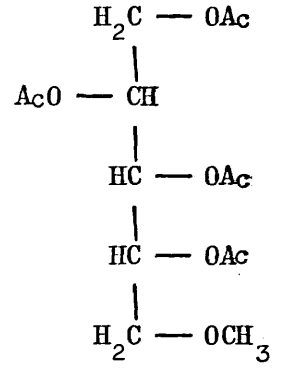


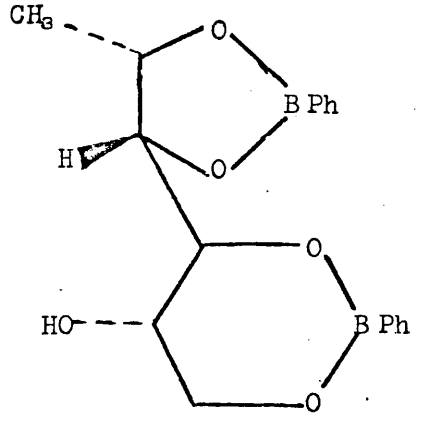
32

The primary ions observed in electron impact mass spectrometry of the mono-O-methyl-tetra-O-acetyl-pentitols, derived from methylation analysis of the parent bisbenzeneboronates, are given in Table IV (p. 76). The ratio of the components found by integration of the peak areas are also given, expressed as mole fractions. The significance of these figures will be discussed presently in conjunction with the appropriate pentitol. Before complete assignment of structure to each bisbenzeneboronate can be made, a knowledge of the boronate ring size must be obtained. With this goal in mind, a study of the electron impact mass spectra of the pentitol bisbenzeneboronate products was undertaken.

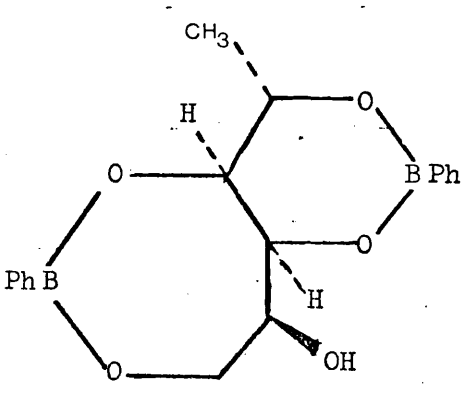
II.3.B Ring Size by Mass Spectrometry

A knowledge of the position of the methoxylated carbon in the pentitol chain from the previously described method, sometimes allows assignment of the size of the boronate ring, in the original bisboronate, directly. For instance, 1-deoxy-L-mannitol bisbenzeneboronate afforded two products on methylation, which by subsequent g.c.-m.s. analysis proved to be the 6-O-methyl and the 5-O-methyl derivatives in a ratio of 3.8:1.

The most likely structure of the bisbenzeneboronate which gave rise to the product 2,3,4,6-tetra-O-acetyl-5-O-methyl-1-deoxy-L-mannitol is 33, 1-deoxy-L-mannitol 2,3; 4,6-bisbenzeneboronate. The alternative structure comprising a six- and a seven-membered ring, 34, is unlikely because the five- and six-membered boronate rings are produced in preference to the seven-membered ring[27,33,36,37]. The 2,3,4,5-tetra-O-acetyl-6-O-methyl-1-deoxy-L-mannitol product, however, could have been produced from a bisboronate comprised of two five-membered rings 35, or two fused six-membered rings, 36.



33



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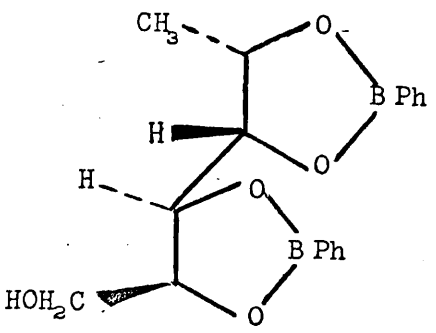
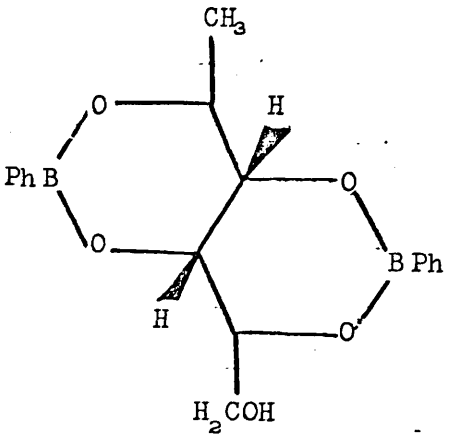


35



36

Mass spectrometry of the original bisbenzeneboronate mixture affords a solution to this problem, since the presence of certain characteristic ions in the spectrum indicate the predominance of either six- or five-membered rings. The fragmentation pathways of the pentitol bisbenzeneboronates were expected to be similar to those of the tetritol boronates, with a few exceptions. For instance, a primary hydroxyl group exocyclic to a boronate ring will produce an abundant ion 37, $\frac{m}{e}$ 31, and the ion (M-31), depending upon which oxygen retains the charge, (Fig.55).

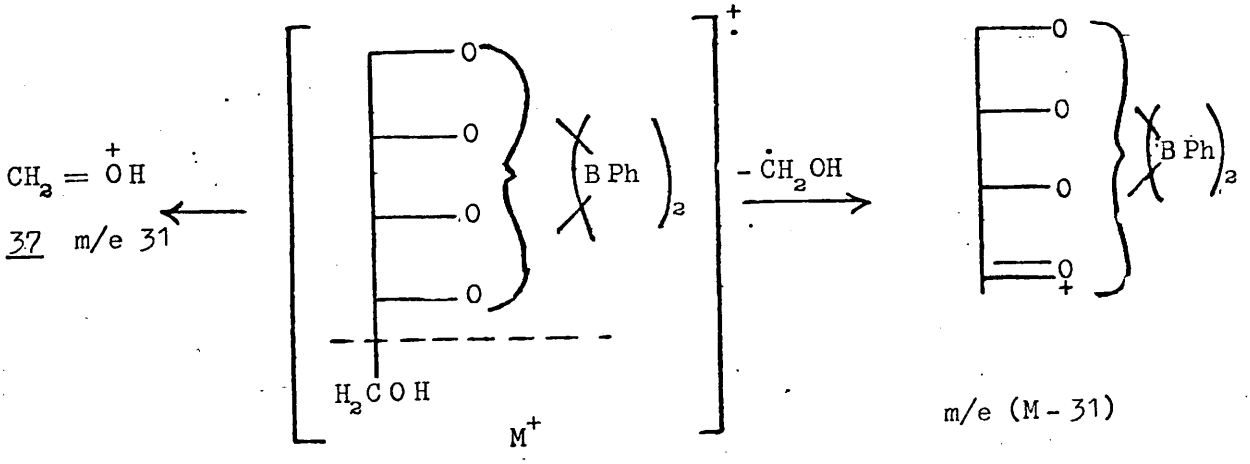


Fig.55

All of the pentitol bisbenzeneboronates gave abundant molecular ions, together with significant abundancies of (M+1) ions, which arise from the addition of a proton to the hydroxyl oxygen, a process characteristic

of compounds containing such groups[113]. Unfortunately the ion $\frac{m}{e}$147, $C_8H_8BO_2$, is again inapplicable for structure identification of five-membered rings since the 'half-rupture' process occurs in the fused six-membered boronate esters to yield the same ion. Also the ion $\frac{m}{e}$177 would not be useful for structure identification for the same reason, (Fig.56).

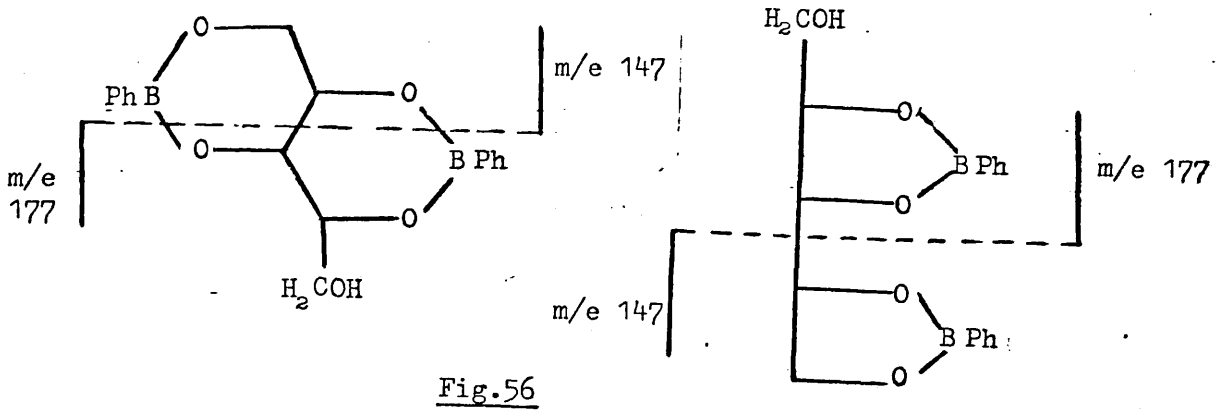


Fig.56

II.3.C The Pentitols Investigated

Xylitol, D-arabinitol and 1-deoxy-L-mannitol were obtained from the corresponding aldoses by reduction with borohydride in water. 1-d_1-D-arabinitol was prepared in a similar manner from D-arabinose using sodium borodeuteride. The bisbenzeneboronates were subsequently prepared from the pentitols by heating a solution of the polyol in 2-methoxyethanol with slightly greater than the stoichiometric amount of benzeneboronic anhydride. The solvent was removed and remaining traces of water were co-distilled with toluene, leaving white crystalline residues.

These crude products were subjected to methylation analysis and mass spectrometry. The results of the methylation studies are shown in Table IV. The relative amounts of each mono-O-methylpentitol, as their acetates, were found by integration of peak areas on the g.l.c. chromatograms and are given as mole fractions.

TABLE IV

ANALYSIS OF BENZENEBORONATES OF PENTITOLS

Parent Pentitol	G.l.c.-m.s. of Products from Methylation, Hydrolysis and Acetylation									Structure of Boronate
	$T^{(a)}$	Mole Fraction	Abundance of primary ions (b) m/e						(c) Identity	
			45	59	117	131	261	275		
Ribitol	0.92	0.86	8.1						5	55
	1.23	0.14			31.1		2.9		2	56
Xylitol	1.25	0.95	7.3						5	59
	1.58	0.05			21.8		1.8		2	60
D-Arabinitol	1.20	0.52	8.2						5	65
	1.70	0.48			33.0		6.5		4	66
1-Deoxy-L-mannitol	1.19	0.79	4.6						6	75
	1.80	0.21			24.8			1.4	5	76
1-Deoxy-D-glucitol	1.57	0.79	10.1						6	77
	2.08	0.21			29.3			2.6	5	78
1-Deoxy-D-talitol	0.86	0.42	7.9						6	86
	1.06	0.40		48.0					2	87
	1.91	0.18				11.6	3.7		3	88
1-Deoxy-L-gulitol	1.21	0.74	4.8						6	93
	1.48	0.19		44.2					2	94
	1.60	0.02			21.1				5	95
	1.80	0.05				8.3	1.3		3	96

(a) Retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol

(b) Expressed as $\% \Sigma_{40}$

(c) Refers to C atom carrying MeO group

II.3.C

(a) Ribitol

Application of the chemical analysis outlined above, (p.63), to the bisbenzeneboronate derivative obtained from ribitol afforded two components as detected by g.l.c. in the ratio of 5.9:1. Combined g.c.-m.s. analysis permitted on-line mass spectrometric analysis of the mono-O-methyl-tetra-O-acetyl-ribitol isomers. The mass spectrum of the major component, which had the shorter retention time, (T, 0.92), had a base peak at $\frac{m}{e}$ 43, indicating O-acetyl groups in the molecule. The second most abundant ion at $\frac{m}{e}$ 45 was assigned to the primary ion 38, arising from a primary methoxylated carbon atom, (Fig.57).

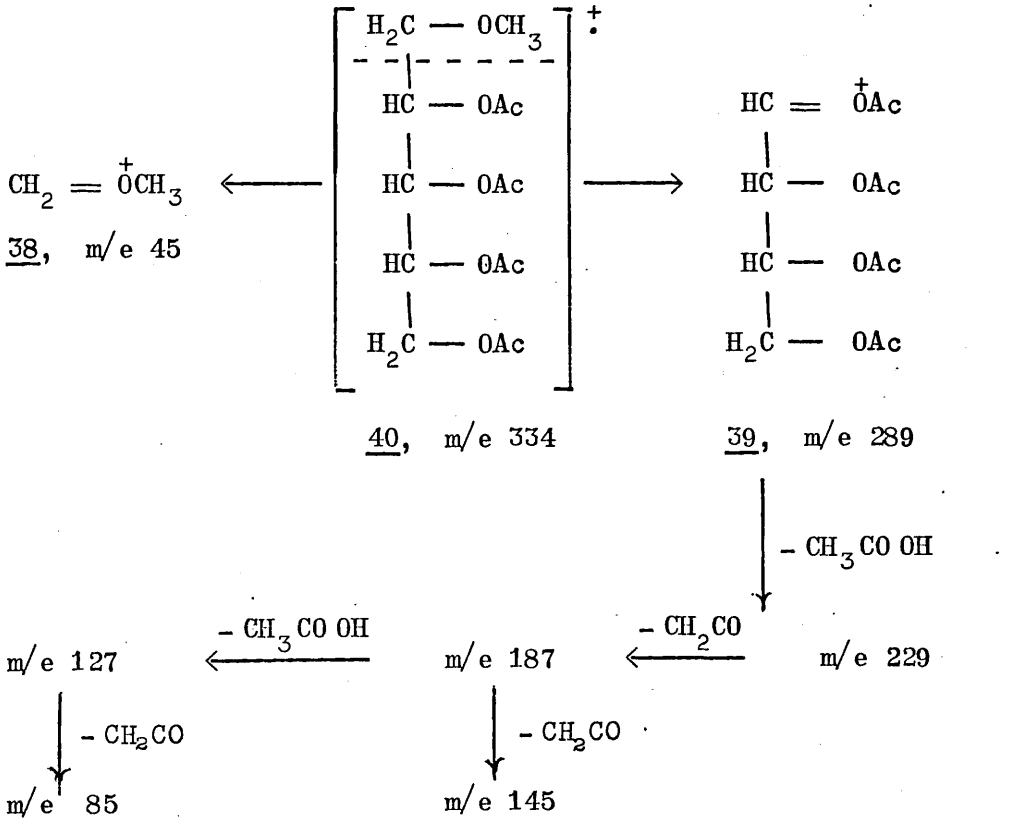
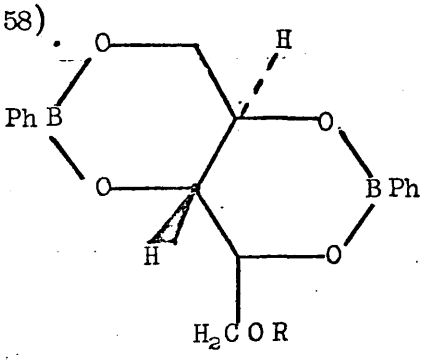


Fig.57

The ion 39, arising from the same bond fission but with the charge residing on the tetra-O-acetyl fragment, (Fig.57), was not observed, although secondary fragments from the breakdown of this primary ion were recorded. The overall spectrum was therefore similar to that of a fully acetylated

pentitol. The acetates of 1-O-methyl-alditols are known to give spectra similar to fully acetylated alditols, with the exception of the primary ion, 38 [105]. As a result, ions such as $\frac{m}{e}$ 187, 127, 145 and 85 appearing in the spectrum indicate the presence of four adjacent acetoxy groups. However, it should be realised that some of these ions may also arise from other fragmentation processes. For example, the ion $\frac{m}{e}$ 145 can also be produced directly from the molecular ion by C-4 - C-5 fission in the carbon chain, (Fig.54).

The major component of the product produced from the methylation studies was consequently assigned the structure as shown for the molecular ion, 40, (Fig.57). The possible structures of the parent bisbenzeneboronate and the intermediate O-methyl derivative that gave rise to this compound are shown, (Fig.58). Of these three structures, 41, DL-ribitol 1,3; 2,4-bisbenzeneboronate, and its 5-O-methyl derivative, and 42, DL-ribitol 1,2; 3,4-bisbenzeneboronate, and its 5-O-methyl derivative are the most likely since the five- and six-membered boronate rings are usually formed in preference to the seven-membered ring in situations where all three may be formed [27,58].



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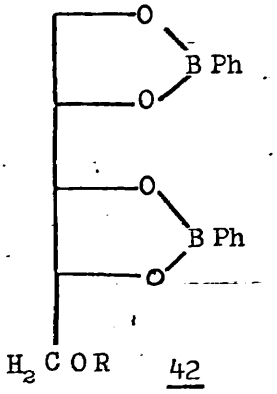

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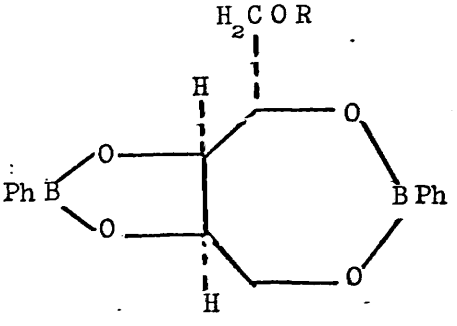


Only one enantiomer shown. 43 R = H or CH_3

Fig.58

The minor component in the product, (T,1.23), analysed by g.c.-m.s. gave a completely different mass spectrum. Besides the base peak at $\frac{m}{e}$ 43, a peak at $\frac{m}{e}$ 117 dominated the spectrum, with very little of any other fragment. This ion was assigned the structure 44, which arises from the fission of the C-2 - C-3 bond in the carbon chain of a pentitol having a methoxy substituent at C-2, (Fig.59). The low abundance of secondary fragments from this ion reflects its tendency to remain intact, a property noted for this ion when it is produced as a primary fragment in such compounds(106).

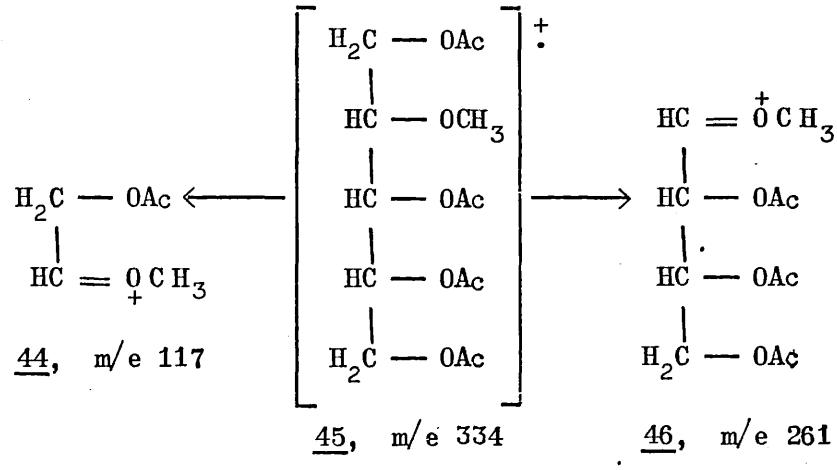
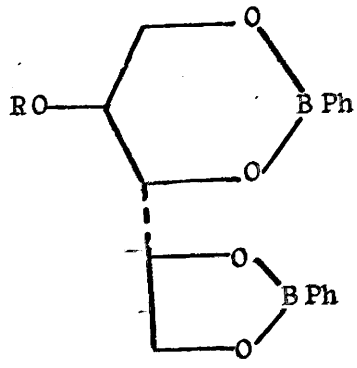


Fig.59

Fission between C-1 and C-2 provided another primary ion of low abundance at $\frac{m}{e}$ 261. Secondary ions from this fragment were observed at $\frac{m}{e}$ 201 , 159 and 99, through loss of acetic acid, ketene and a further molecule of acetic acid, respectively. The minor component was therefore identified as 1,3,4,5-tetra-O-acetyl-2-O-methyl-DL-ribitol. The most likely structure of the bisbenzeneboronate and the intermediate monomethyl bisbenzeneboronate derivative, 47, DL-ribitol 1,3 ; 4,5-bisbenzeneboronate and 2-O-methyl-DL-ribitol 1,3 ; 4,5-bisbenzeneboronate, respectively, contains one five- and one six-membered ring.

From the results of the methylation studies it can be concluded that the product obtained from the interaction of ribitol with benzeneboronic anhydride contains at least two structural isomers, 41, and 47,

with the possibility of a third, 42. Also the isomer having a hydroxyl group at C-2 comprises 14% of the total product.



47

R = H or CH_3

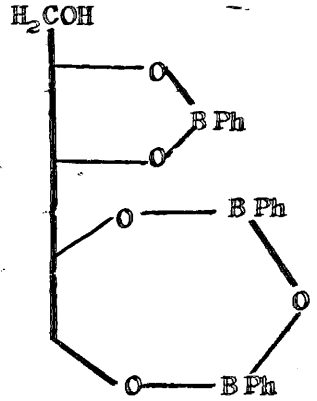
Only one enantiomer shown

The conclusions drawn here, from the methylation analysis of the crude bisbenzeneboronate of ribitol should now be correlated with those drawn from the low and high resolution mass spectrometry data of this material. The major ions in the mass spectra of the pentitol bisboronates are given in Table V, and are expressed as a percentage of the total ion current, $\%\Sigma_x$, inclusive of peaks greater than $\frac{m}{e}$ x.

TABLE V

ABUNDANCES OF MAJOR IONS IN THE MASS SPECTRA OF PENTITOL BISBENZENEBORONATES

Parent Pentitol of Bisbenzeneboronate	Abundance of Major Ions $\%\Sigma_{40}$										
	31	147	159	160	161	172	173	174	177	M-31	M
ribitol	2.0	14.0	8.8	6.0	5.0	1.2	1.0	0.5	0.8	0.5	1.4
D-arabinitol	2.6	8.2	2.2	0.1	0.1	0.2	0	0	0.7	0.2	2.2
Xylitol	2.8	17.0	8.8	5.3	0.7	1.4	0	0	4.9	0.2	3.7
1-deoxy-L-mannitol	0.6	6.1	8.2	1.8	1.4	1.8	1.4	0.7	0.2	0	1.1
6-deoxy-D-glucitol	3.0	1.2	0.8	2.3	6.9	0.2	0.2	0.7	0	0	0.2
1-deoxy-D-glucitol	4.0	3.2	3.2	1.8	2.8	1.2	0.8	0.6	0	0.7	2.0
1-deoxy-D-talitol	2.0	5.5	8.1	4.8	5.5	1.2	1.2	0.6	1.8	0.4	2.0



48

An abundant molecular ion ($\frac{m}{e}$ 324.1354, $C_{17}H_{18}B_2O_5$) in the mass spectrum, of the crude benzeneboronate of ribitol, precluded structures containing a 2,4-diphenyl-1,3,5-trioxa-2,4-diborepane ring, such as 48. The fused six-membered ring structure, 41, was shown to be present from abundant ions at $\frac{m}{e}$160, $C_9H_9BO_2$ and $\frac{m}{e}$ 159,

$C_9H_8BO_2$. The ion 49 fragments directly from the molecular ion via the double elimination process characteristic of six-membered benzeneboronate rings, (Fig.60). The ion 50 can also be produced from the molecular ion directly, as indicated from metastable ion measurements in the mass spectrum of purified ribitol bisbenzeneboronate, suggested to have the structure 41 [58,69], (R = H). Alternatively, 50, could have the precursor 51, but the low abundance of this ion suggests that this is not the main parent ion

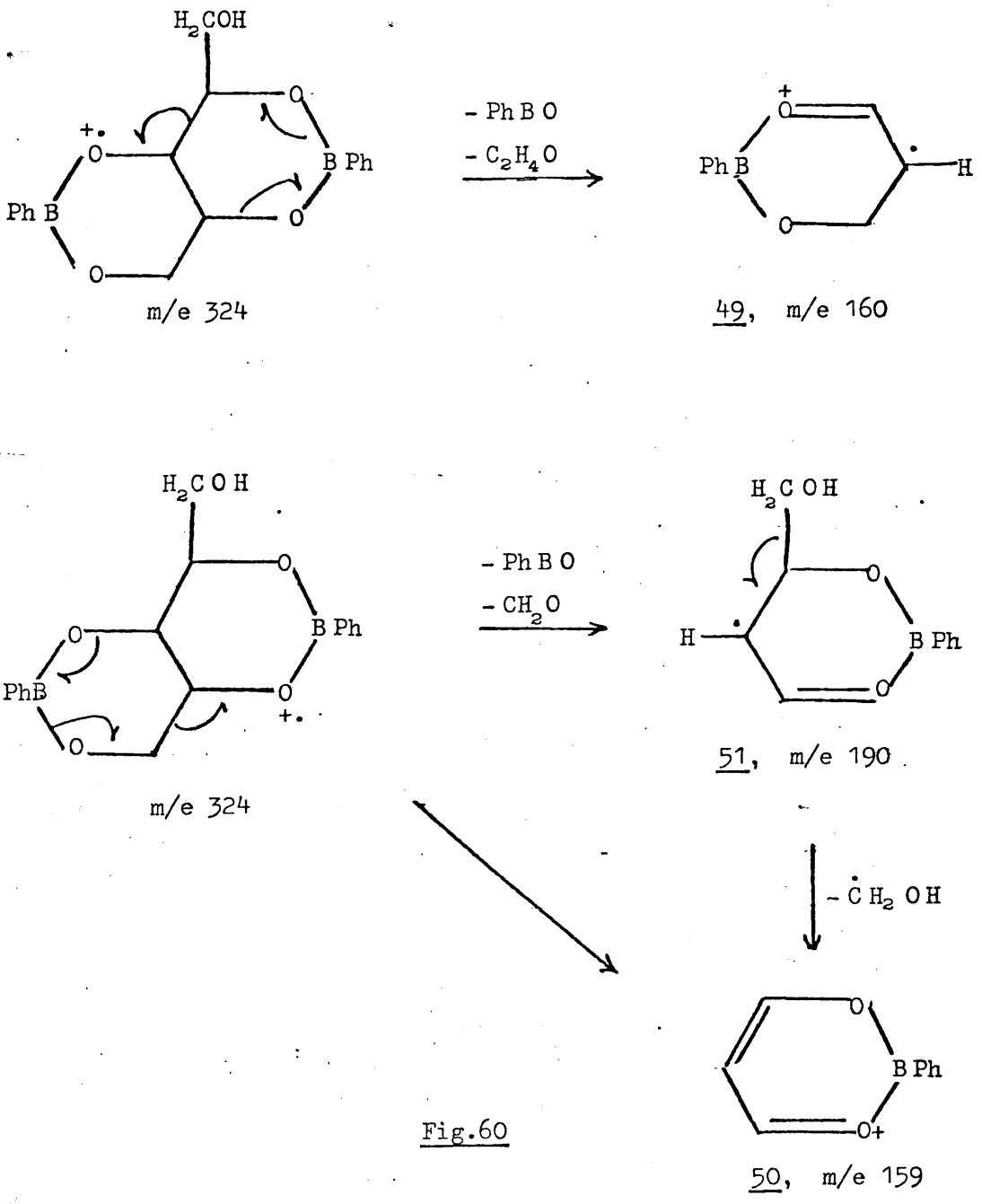
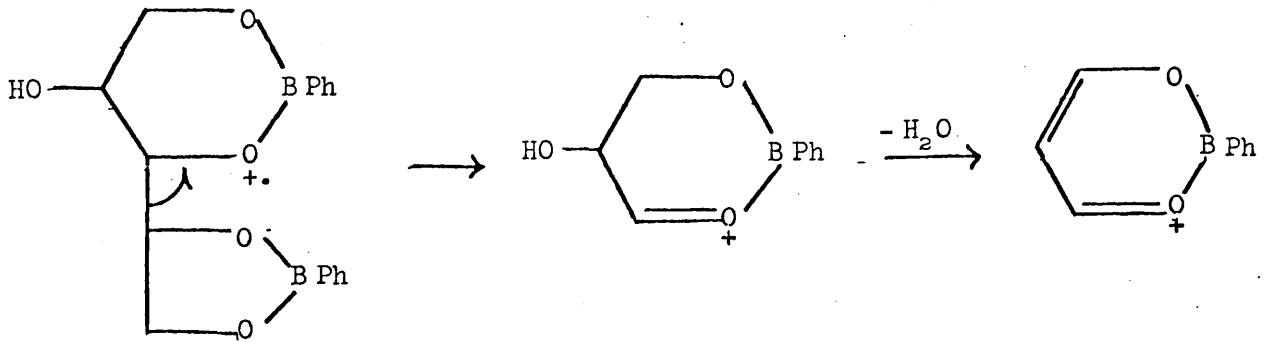


Fig.60



m/e 324 — 52, m/e 177 — 50, m/e 159

Fig.61

of 50. However, the ion 50, $\frac{m}{e}$159, could have arisen from the breakdown of 47. This latter structure could produce the ion 52, $\frac{m}{e}$177, through loss of the five-membered boronate ring, (Fig.61). Subsequent elimination of water would produce 50, $\frac{m}{e}$159. However, it has been shown[114,115] that electron impact induced dehydration usually occurs via. a 1,4-elimination involving a six-membered transition state, (Fig.62), rather than by a 1,2-elimination, which would operate in the elimination of water from 52 to produce the ion 50. Also, it is unlikely that the third most abundance peak in the spectrum, accounting for 8.8% of the total ion current, should be produced by the fragmentation of the component which is present only to the extent of 14% in the mixture.

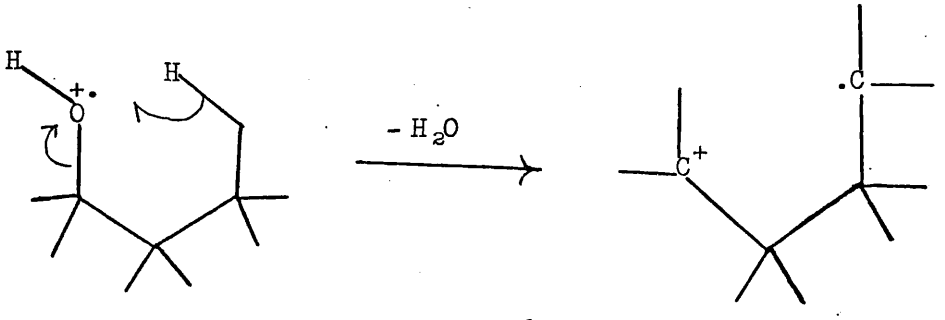
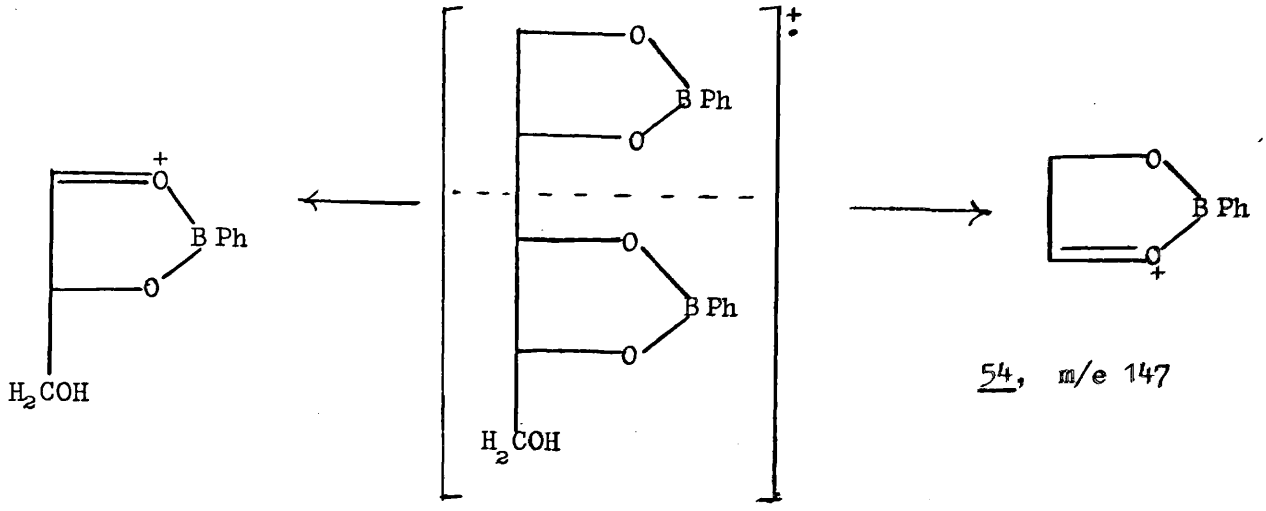


Fig.62

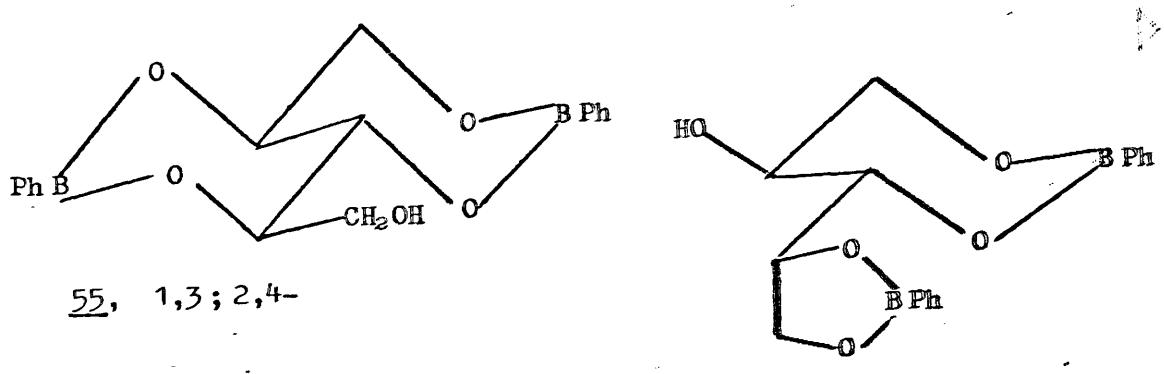
The possibility that the crude benzeneboronate of ribitol also contains a component having the structure 42, i.e. two five-membered rings, cannot be excluded. This structure would produce the ions $\frac{m}{e}$147 and $\frac{m}{e}$177,



53, m/e 177 m/e 324 54, m/e 147

Fig.63

(Fig.63). However, these ions can also be produced from the two fused six-membered ring structure, 41, via. the 'half-rupture' process, (Fig.56). In addition, the low abundance of the ion 53 indicates that 42 is not the predominant structure.



55, 1,3 ; 2,4- 56, 1,3 ; 4,5-

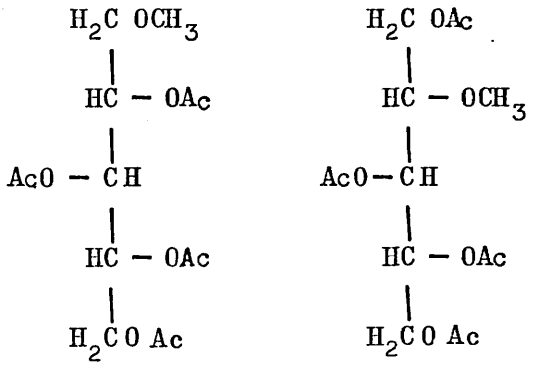
Fig.64

DL-Ribitol bisbenzeneboronates; only one enantiomer shown.

Mass spectrometry of the crude ribitol bisbenzeneboronate mixture has therefore provided evidence to show that the major component has a structure composed of two fused six-membered rings, necessarily trans-fused by virtue of the configuration of the hydroxyl groups on the ribitol chain, 55. The smaller component consists of one six- and one five-membered ring, 56, the two substituents occupying equatorial orientations (Fig.64).

II.3.C
(b) Xylitol

Methylation analysis on the crude product obtained from the interaction of xylitol and benzeneboronic anhydride afforded two products on g.l.c. analysis. The component of shorter retention time, T, 1.25, (Table IV, p.76), constituted the major fraction, being present in 95% of the total product. The mass spectrum of this derivative resembled that of the derivative obtained from ribitol bisbenzeneboronate, assigned the structure 40, 2,3,4,5-tetra-O-acetyl-1-O-methyl-DL-ribitol. Additional evidence for methylation to have occurred at the primary hydroxyl came from the observation of a small peak at $\frac{m}{e}$ 289, resulting from C-1 – C-2 cleavage with localisation of charge on the acetoxy group of C-2, (Fig.57). This component was therefore assigned the structure 55, 2,3,4,5-tetra-O-acetyl, 1-O-methyl-DL-xylitol, (Fig.64).



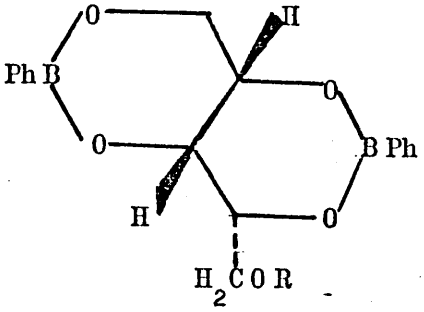
55 56

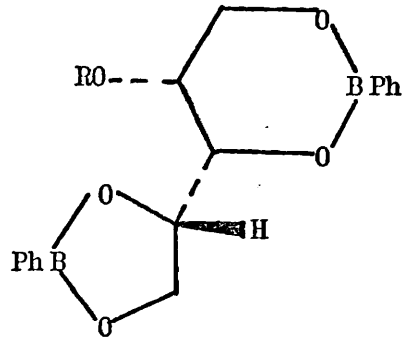
Only one enantiomer shown.

Fig.64

The mass spectrum of the minor component was also similar to the smaller component in the ribitol mixture. The $\frac{m}{e}$ 117 ion was the second most abundant, the base peak being due to the acetylium ion, $\frac{m}{e}$ 43. The other primary fragment, $\frac{m}{e}$ 261, was also observed, together with the expected secondary ions, (Fig.59). This second component was therefore concluded to have the structure 56, 1,3,4,5-tetra-O-acetyl-2-O-methyl-DL-xylitol, (Fig.64).

The proposed structures for the two bisbenzeneboronates and the intermediate mono-O-methyl derivatives that gave rise to the methylation products are shown, (Fig.65). By virtue of the high proportion of the bisbenzeneboronate containing a primary hydroxyl group in the mixture, i.e. 95%, the most abundant ions in the mass spectrum of the crude bisbenzene-





57, DL-xylitol 1,3;2,4-bisbenzenboronates

58, DL-xylitol 1,3;4,5-

Only one enentiomer is shown.

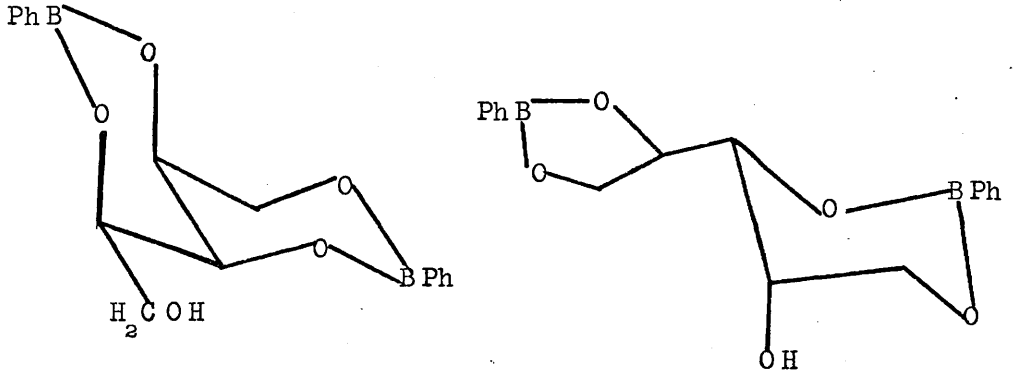
Fig.65

boronate product were expected to reflect this structure accordingly. The structure of the minor component was expected to have a six- and a five-membered ring, 58. However, since the bisbenzeneboronate having an unesterified secondary hydroxyl group was present in only 5%, the ions arising from this structure were anticipated to be small.

The mass spectrum of the crude product did in fact contain a large ion, $\frac{m}{e}$147 of atomic composition $C_8H_8BO_2$, (147.0605), which may in part be due to the five-membered ring grouping, assigned to the minor component, but it most probably arose from the 'half-rupture' process of two fused six-membered rings, (Fig.56). The ion $\frac{m}{e}$177, $C_9H_{10}BO_3$, could also be attributed to a five-membered boronate ring having an exocyclic hydroxymethyl group, (Fig.56). However, the fragmentation process producing this ion would not be distinguishable from the 'half-rupture' process, using the enhanced metastable spectrum, since the $\frac{m}{e}$ values of the daughter and parent ions are the same, (Fig.56). The presence of an abundant ion, $\frac{m}{e}$160,

$C_9H_9BO_2$, does, however, indicate the six-membered boronate structure. This ion can be produced directly from the molecular ion via. the double elimination process, (Fig.60), evidenced by a metastable peak at $\frac{m}{e}$ 79.0.

Similarly, the ion $\frac{m}{e}$159, $C_9H_8BO_2$, was deduced to fragment directly from the molecular ion by observation of a metastable ion $\frac{m}{e}$78.0, (Fig.60). The major component in the crude product was therefore concluded to have the structure 59, DL-xylitol 1,3; 2,4-bisbenzeneboronate, the cis-fused rings presumably adopting the conformations shown, with the hydroxymethyl substituent in an equatorial orientation. The alternative, with cis-fused rings in chair conformations, would have the hydroxymethyl group in an axial orientation, which would involve unfavourable steric interactions with the adjacent boronate ring. The minor component was concluded to have the structure 60, DL-xylitol 1,3; 4,5-bisbenzeneboronate, adopting the conformation shown. The more bulky dioxaborolane substituent occupying the equatorial orientation to minimise unfavourable steric interaction.



59, DL-xylitol 1,3; 2,4- 60, DL-xylitol 1,3; 4,5-bisbenzeneboronates

Only one enantiomer shown

Fig.66

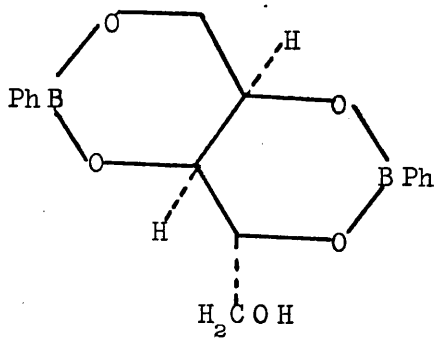
A comparison of the relative amounts, in the crude product, of these isomers with those found for ribitol bisbenzeneboronate is instructive at this point. The boronates of these two pentitols have each been shown to be a mixture of two structural isomers. The isomer having two fused six-

membered boronate rings was found to be in significantly greater proportion. The minor component, which has the proposed structure consisting of one five- and one six-membered ring, was found to be in lower yield in the xylitol product. This suggests that whatever chair conformation is adopted by 60, there is one axial substituent, either the hydroxyl or the 2-phenyl-1,3,2-dioxaborolane group, which is offering substantially more steric interaction compared to 56, which has both substituents in equatorial orientations, (p.83). Consequently, the formation of 60, is less favourable compared to 56.

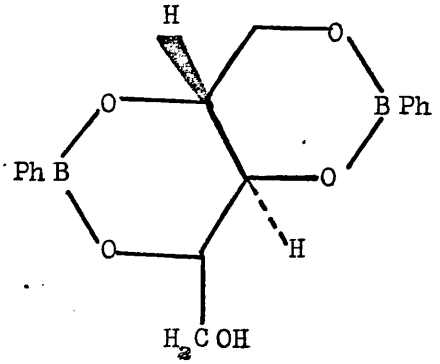
II.3.C
(c) D-Arabinitol

The crude product obtained from the interaction of D-arabinitol and benzeneboronic anhydride afforded two products on methylation with diazomethane, in almost equal amounts. The product of shorter retention time, (T,1.2), was found to have a primary methoxylated carbon, indicated by an abundant ion at $\frac{m}{e}$ 45 in its mass spectrum. The remainder of the spectrum resembled a fully acetylated alditol, as expected, (see p.77). The second component, (T, 1.7), gave a mass spectrum indicative of a methoxylated carbon at position C-2 or C-4, evidenced by an abundant ion at $\frac{m}{e}$ 117, and an accompanying primary ion at $\frac{m}{e}$ 261, (Fig.59).

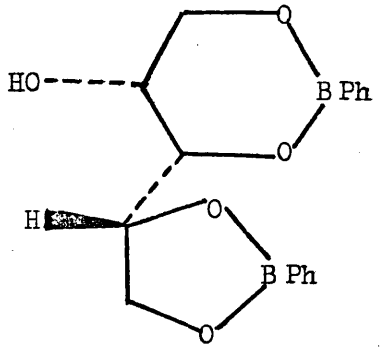
The formation of a terminal methoxylated carbon is direct evidence for the presence of a primary hydroxyl group in the original bisbenzeneboronate. However, the hydroxyl groups on 1-O-methyl-D-arabinitol and 6-O-methyl-D-arabinitol are stereochemically not equivalent. Consequently, there are two possible bisbenzeneboronate structures, having the fused six-membered ring arrangement, with the rings either cis- or trans-fused, (Fig. 67). Similarly, the other product from the methylation analysis could be 1,3, 4,5-tetra-O-acetyl-2-O-methyl-D-arabinitol or 1,2,3,5-tetra-O acetyl-4-O-methyl-D-arabinitol, produced from the structures 63, and 64 respectively, (Fig.67).



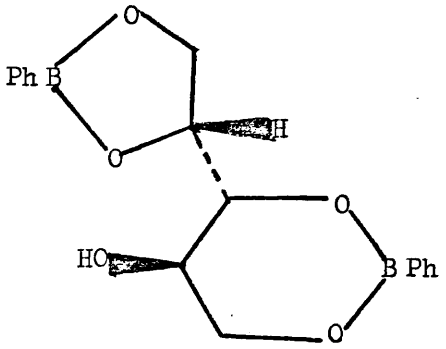
61, 1,3 ; 2,4-



62, 2,4 ; 3,5-



63, 1,3 ; 4,5-



64, 1,2 ; 3,5-

D-Arabinitol bisbenzeneboronates

Fig.67

A possible resolution to this problem involved labelling the C-1 position with a deuterium atom, which would shift the ion $\frac{m}{e}45$ to $\frac{m}{e}46$ if this position was methylated, (Fig.57). In addition, a methoxy group attached to C-2 would shift the ion $\frac{m}{e}117$, in the mass spectrum of the methylated product, to $\frac{m}{e}118$, (Fig.48).

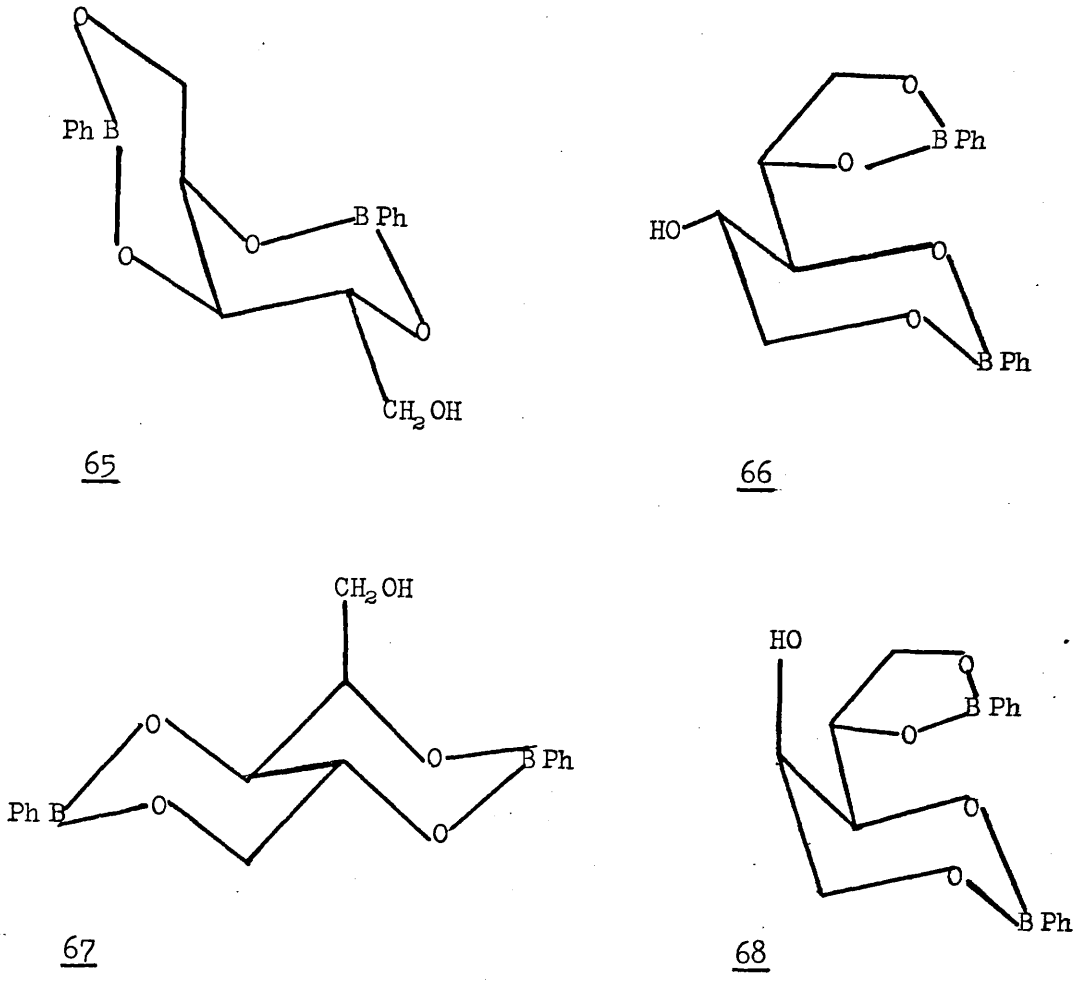
Methylation was therefore carried out on the product obtained from the interaction of 1-d_1-D-arabinitol and benzeneboronic anhydride. Combined g.c.-m.s. analysis showed that the relative intensity of the ion $\frac{m}{e}45$ to the ion $\frac{m}{e}46$, the latter ion probably being due to $H_2{}^{13}C=\overset{+}{O}CH_3$, had not changed for the component T = 1.2. It was concluded therefore, that only the C-5 hydroxyl group had been methylated.

The spectrum obtained from the second component, (T, 1.7), again contained an abundant peak corresponding to the ion $\frac{m}{e}117$. However, the

alternative primary ion, $\frac{m}{e}$ 261, (Fig.48), shifted completely to $\frac{m}{e}$ 262, indicating that methylation had occurred exclusively at the C-4 hydroxyl group. It was concluded from these experiments that the most likely structures for the two bisbenzeneboronates in the crude product are 61, D-arabinitol 1,3; 2,4-bisbenzeneboronate, and 64, D-arabinitol 1,2; 3,5-bisbenzeneboronate.

The mass spectrum of the crude boronate product afforded peaks indicative of a fused six-membered ring structure. Metastable ion measurements supported the double elimination process. Thus, the molecular ion, $\frac{m}{e}$ 324, $C_{17}H_{18}B_2O_5$, was shown to fragment directly to $\frac{m}{e}$ 160, $C_9H_9BO_2$, via. the double elimination process, evidenced by a metastable ion at $\frac{m}{e}$ 79.0. The ion $\frac{m}{e}$ 159, $C_9H_8BO_2$, was also shown to have the molecular ion as a precursor through the presence of a metastable ion $\frac{m}{e}$ 78.0, (Fig.60).

The mass spectrometric and methylation studies therefore, suggest the crude product consists of the 1,3; 2,4- and 1,2; 3,5-bisbenzeneboronates 65 and 66 respectively. It will be noticed that if the hydroxyl group at C-1 had remained unesterified, then the proposed two fused six-membered ring structure would have the hydroxymethyl substituent in an axial orientation, 67, (Fig.68). The complete absence of this structure indicates the significance of steric interactions in the formation of benzeneboronates. Similarly if methylation had occurred on the C-2 hydroxyl, the proposed structure for the bisbenzeneboronate 68, has one equatorial and one axial substituent on the six-membered boronate ring.

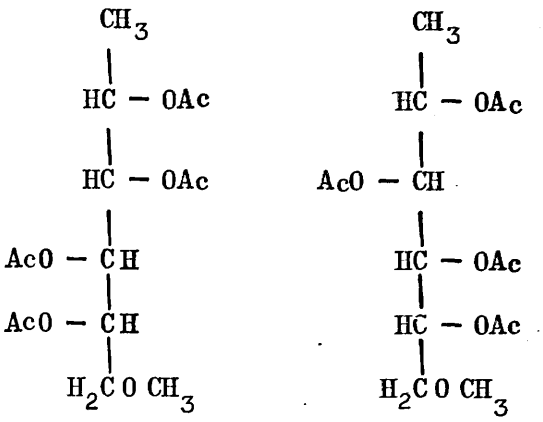


65 66 67 68

Fig.68

II.3.C
(d) 1-deoxy-L-mannitol and 1-deoxy-D-glucitol

The results obtained for the bisbenzeneboronates of 1-deoxy-L-mannitol and 1-deoxy-D-glucitol are similar, and it will be instructive to consider them together. Both pentitol bisbenzeneboronates afforded two products on methylation analysis, as detected by combined g.c.-m.s., in a ratio of 3.8:1. The components of shorter retention time gave similar spectra, characteristic of a terminal methoxylated carbon, and were therefore assigned the structures 2,3,4,5-tetra-O-acetyl-1-deoxy-6-O-methyl-L-mannitol 69, and 2,3,4,5-tetra-O-acetyl-1-deoxy-6-O-methyl-D-glucitol, 70.



69 70

The smaller components of the two pentitols, having longer retention times, T, 1.80 for the mannitol and T, 2.08 for the glucitol derivative, also gave similar spectra. In both cases an abundant peak, $\frac{m}{e}$ 117, was sufficient evidence to show methylation had occurred at the C-5 hydroxyl group. An ion, $\frac{m}{e}$ 275 provided further evidence for a C-5 methoxylated carbon, since this primary ion results from C-5 - C-6 cleavage (Fig.69). Secondary ions produced by successive loss of acetic acid and ketene from this latter ion were also observed.

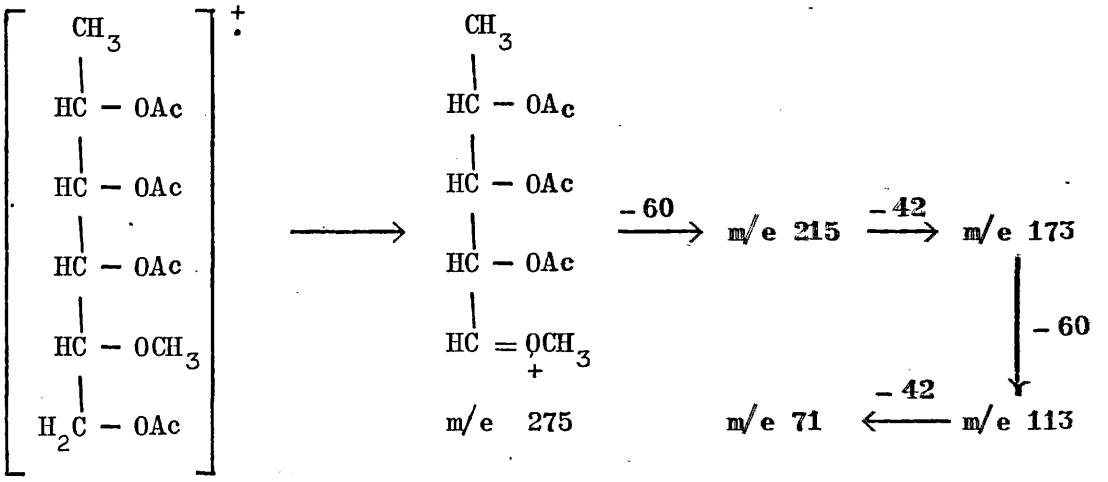
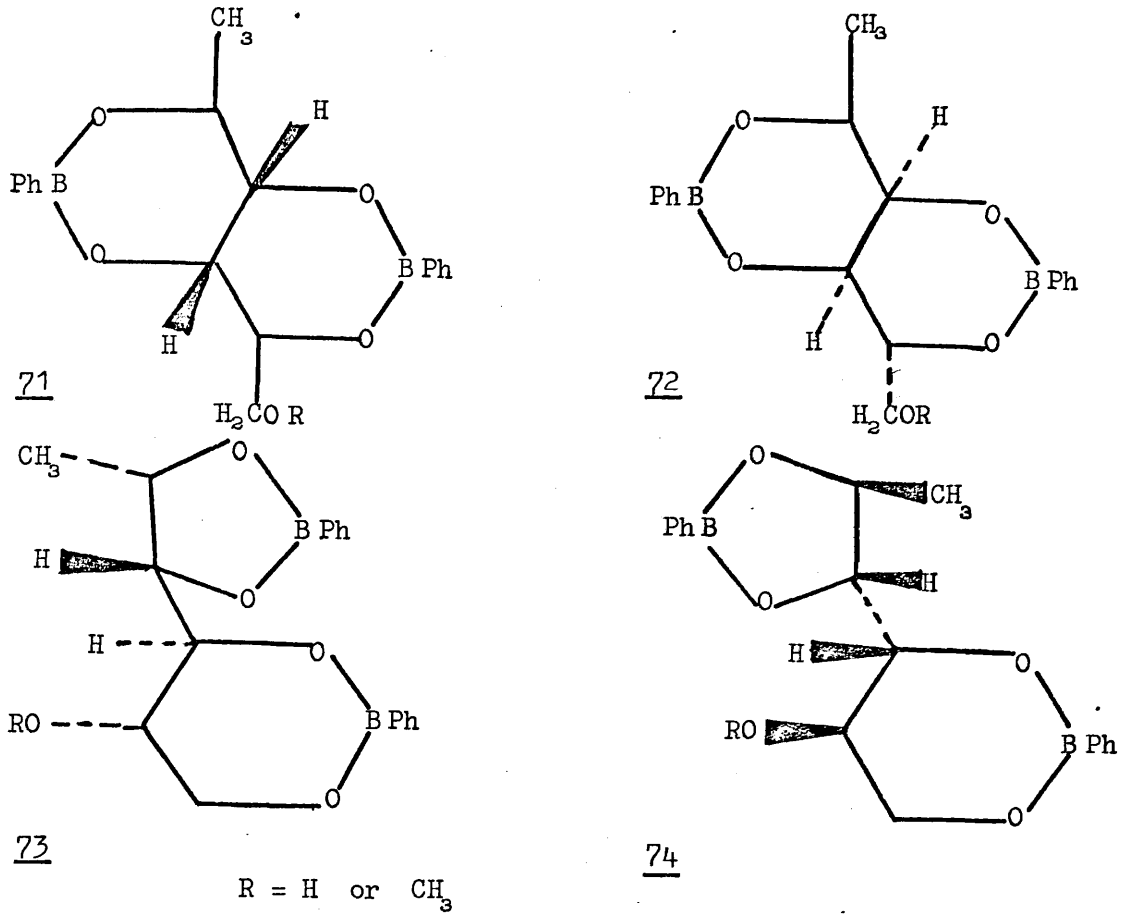


Fig.69

The structures of the bisbenzeneboronates and the intermediate monomethyl bisboronate derivatives for these pentitols having the hydroxyl and methoxy substituents at the terminal position, are expected to be 71, 1-deoxy-L-mannitol 2,4 ; 3,5-bisbenzeneboronate, and 72, 1-deoxy-D-glucitol



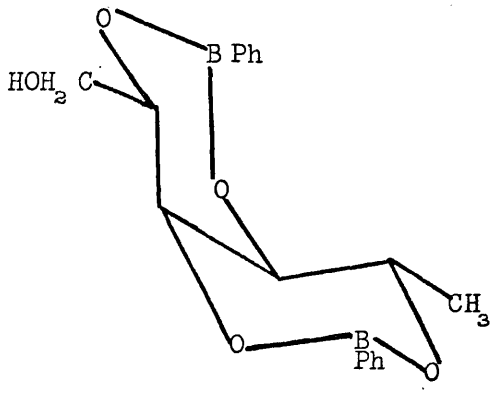
R = H or CH_3

Fig.70

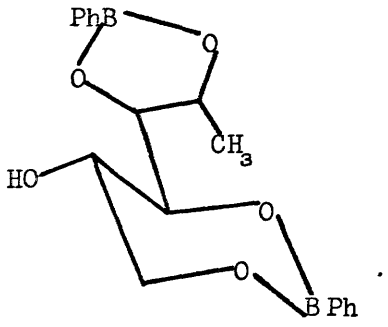
2,4; 3,5-bisbenzeneboronate, (Fig.70). Whereas the most likely structures for the bisboronates having an hydroxyl or methoxy group at C-5 are 73, 1-deoxy-L-mannitol 2,3; 4,6-bisbenzeneboronate and 74, 1-deoxy-D-glucitol 2,3; 4,6-bisbenzeneboronate, (Fig.70).

The mass spectrum of the crude product obtained from the interaction of 1-deoxy-L-mannitol and benzeneboronic anhydride gave clear indication of a predominant six-membered ring structure containing two fused rings. Thus, the second most abundant ion, $\frac{m}{e}$159, $C_9H_8BO_2$, was indicated to fragment directly from the molecular ion $\frac{m}{e}$338, $C_{18}H_{20}B_2O_5$, evidenced by a metastable ion $\frac{m}{e}$74.8, lending support to the two fused six-membered ring structure, 71. Although the ion $\frac{m}{e}$174 was present, indicating the ion 8, (Fig.36, p. 50), arising from double elimination of the six-membered ring having the exocyclic hydroxymethyl substituent, a metastable ion confirming this process was not present in the enhanced metastable spectrum.

The predominant bisbenzeneboronate in the crude product of 1-deoxy L-mannitol was therefore assigned the structure 75, with both methyl and hydroxymethyl substituents in equatorial dispositions, (Fig.71). The minor component, present in 21%, was assigned the structure 76, with both substituents in equatorial orientations.

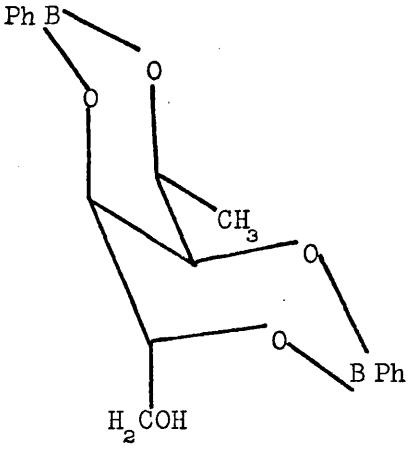


75, 2,4 ; 3,5-

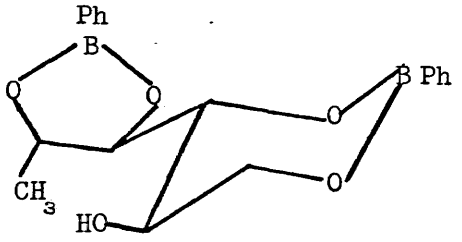


76, 2,3 ; 4,6-

1-deoxy-L-mannitol bisbenzeneboronate



77a, 2,4 ; 3,5-

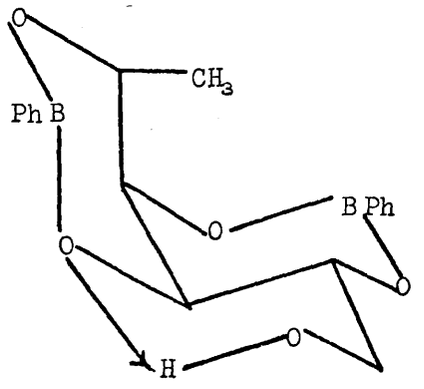


78, 2,3 ; 4,6-

1-deoxy-D-glucitol bisbenzeneboronate

Fig.71

The mass spectrum of the crude product obtained from 1-deoxy-D-glucitol contained a similar set of peaks to the spectrum of the 1-deoxy-L-mannitol product. Using the same arguments for those applied to the latter product, the reaction product obtained from 1-deoxy-D-glucitol was considered to consist of the two bisboronate structures 77a and 78, (Fig. 71). The two cis-fused six-membered boronate rings proposed for the major component of the 1-deoxy-D-glucitol product have one axial and one equatorial



77b, 1-deoxy-D-glucitol 2,4 ; 3,5-bisbenzeneboronate

Fig.72

substituent. The latter structure would be expected to be less stable, with respect to steric interaction of the group occupying the axial orientation, compared to the mannitol bisboronate structure 75. The methylation studies however indicated that these two structures were present to the same extent in both products. However, the glucitol bisbenzeneboronate, 77a, could conceivably be stabilised in the alternative chair conformation, 77b, by intramolecular hydrogen bonding, (Fig.72).

II.3.C
(e) 1-Deoxy-D-talitol

The case of 1-deoxy-D-talitol bisbenzeneboronate was found to be more complex than those discussed so far. Methylation gave three products, which were present in the mole fractions 0.42, 0.40 and 0.18, as measured from the peak areas, having the corresponding retention times T, 0.86, 1.06 and 1.91. The component with T, 0.86 produced the familiar mass spectrum of a terminal methoxylated carbon, and was therefore assigned as 2,3,4,5-tetra-O-acetyl-1-deoxy-6-O-methyl-D-talitol. The component of retention time T, 1.06, gave a mass spectrum containing a base peak at $\frac{m}{e}$59, instead of the familiar acetylium ion, $\frac{m}{e}$43. The ion $\frac{m}{e}$59, was conclusive evidence for methylation to have occured at C-2, (Fig.73). The alternative primary fragment formed by loss of the terminal methyl group was not expected to appear, since deoxy groups in the alditol chain are known to inhibit such cleavage[110]. The remainder of the spectrum consisted of a few peaks of low abundance which supported the assigned structure 3,4,5,6-tetra-O-acetyl-1-deoxy-2-O-methyl-D-talitol.

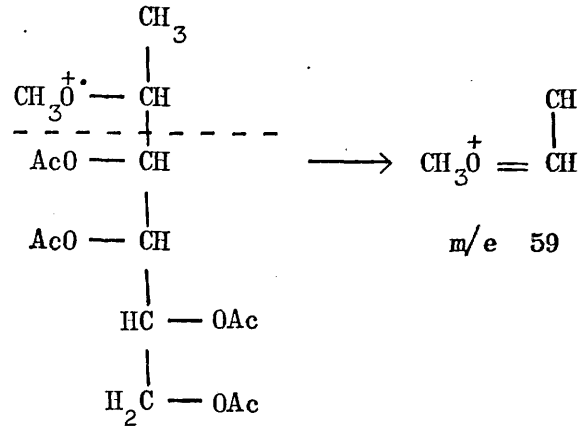


Fig.73

The third component of the methylated mixture was deduced to be 2,4,5,6-tetra-O-acetyl-3-O-methyl-1-deoxy-D-talitol, for the following reasons. The second most abundant ion in the mass spectrum of this component occurred at $\frac{m}{e}$ 131, suggesting a three carbon chain carrying the terminal deoxy, an acetoxy and methoxy groups, 79, (Fig.74). The ion of highest mass number occurring in the spectrum appeared at $\frac{m}{e}$ 261, indicative of the ion 80, produced by C-2-C-3 fissions (Fig.74). The other peaks in the spectrum could quite easily be assigned to secondary fragments resulting from further breakdown of these two ions.

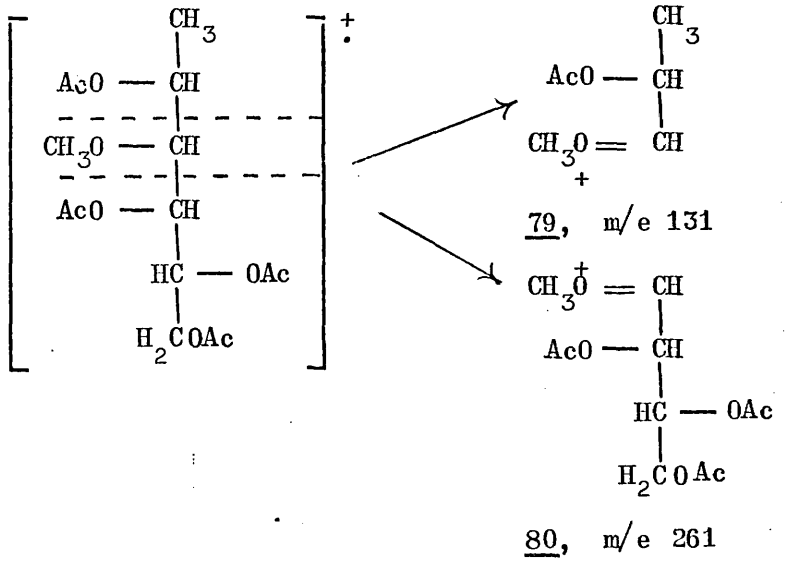
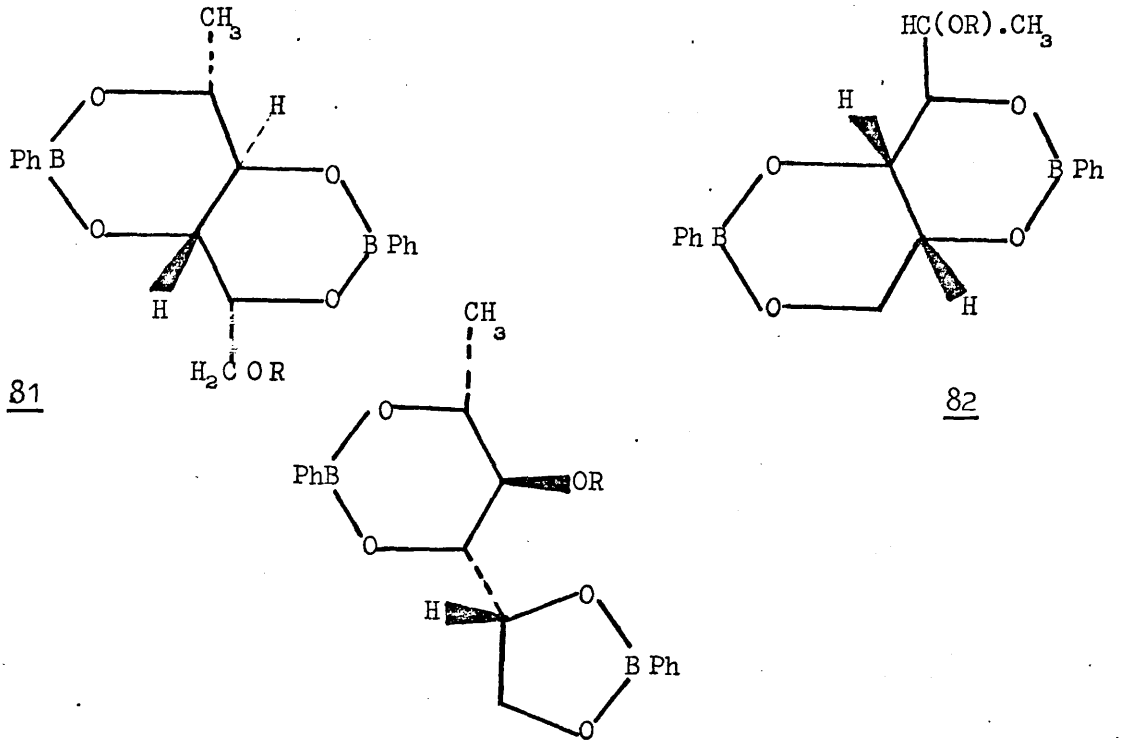


Fig.74

The proposed structures for the three isomeric bisbenzeneboronates and the intermediate mono-O-methyl derivatives which gave rise to the

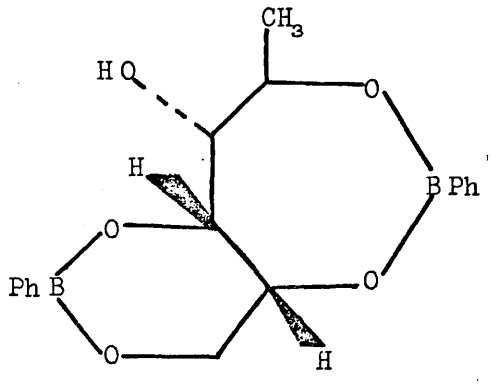


81 82 83

Fig.75

methylation products are shown, (Fig.75). The high and low resolution mass spectra of the crude product from 1-deoxy-D-talitol gave ample evidence of six-membered ring boronate structures, (see Appendix). The proposed trans-fused six-membered boronate ring structure containing an hydroxyl group at C-6, 81, (Fig.75), was supported by a fragment at $\frac{m}{e}$174, $C_{10}H_{11}BO_2$, 8, (Fig.36), produced by a double elimination process in the neighbouring six-membered ring. The structure 81 will also be responsible for the ions $\frac{m}{e}$177, $C_9H_{10}BO_2$, and $\frac{m}{e}$161, $C_9H_{10}BO_2$, produced by the 'half-rupture' fragmentation mode of the molecular ion, (Fig.38).

Although direct evidence from the mass spectral data could not definitively identify the structures 82 and 83, the methylation products suggested that these were the most likely structures. The alternative structures would involve a seven- and a six-membered ring 84 and two five-membered rings 85, (Fig.76), which would give rise to the products 2,4,5,6-tetra-O-acetyl-1-deoxy-3-O-methyl-D-talitol and 3,4,5,6-tetra-O-acetyl-1-deoxy-2-O-methyl-D-talitol, respectively.



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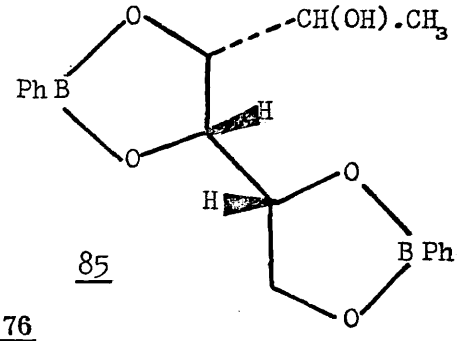
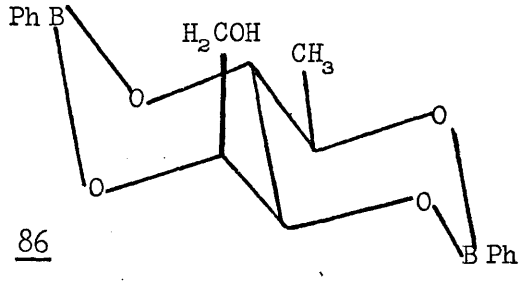


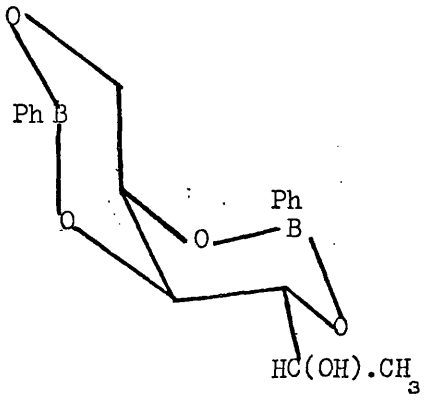
85

Fig.76

The most likely conformations of the three structural isomers of the bisbenzeneboronates formed from 1-deoxy-D-talitol are shown, (Fig.77). It will be noted that the major isomer in the product, 86, has an axially disposed hydroxymethyl group. The alternative two-fused six-membered ring structure will only have one substituent, the 1-hydroxyethyl, in an equatorial position in its most favourable conformation, 87. Since the second isomer is present in almost the same amount in the crude product, the two structures must be of comparable stability.



86



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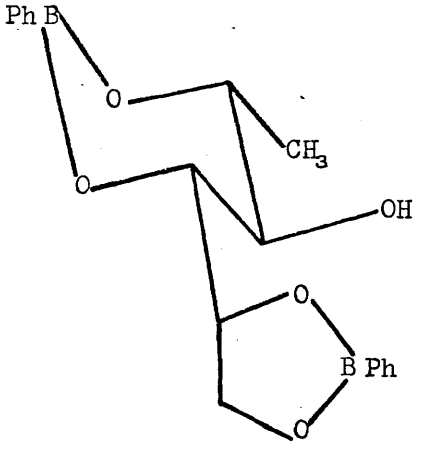


88

Fig.77

II.3.C

(f) 1-Deoxy-L-gulitol

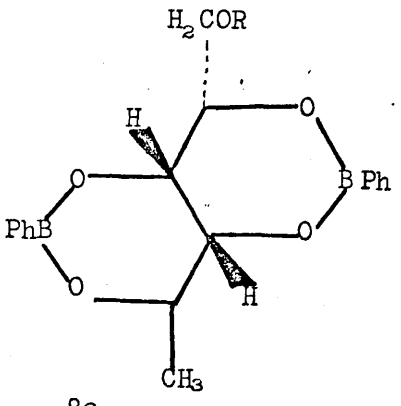
Methylation analysis on the crude product obtained from this pentitol yielded four monomethyl derivatives, Table IV. The first component, (T, 1.21), gave the characteristic spectrum for a methoxylated group at C-1 and was therefore assigned 2,3,4,5-tetra-O-acetyl-1-deoxy-6-O-methyl-L-gulitol.

The component, (T, 1.48), afforded a very simple mass spectrum, having two abundant peaks at $\frac{m}{e}$43 and $\frac{m}{e}$59, the latter being the base peak. By a similar argument to that used for the second component of 1-deoxy-D-talitol, p.94, this product was concluded to be 3,4,5,6-tetra-O-acetyl-1-deoxy-2-O-methyl-L-gulitol.

The third product, (T, 1.60), which constituted only 2% of the total methylation product, afforded an abundant ion at $\frac{m}{e}$117, indicative of the ion 44, (Fig.59), and hence evidence of a C-5 methoxylated carbon. This product was therefore designated 2,3,4,6-tetra-O-acetyl-1-deoxy-5-O-methyl-L-gulitol.

Finally the component, (T, 1.80), was identified as 2,4,5,6-tetra-O-acetyl-1-deoxy-3-O-methyl-L-gulitol from the appearance of ions at $\frac{m}{e}$131 and $\frac{m}{e}$261, (Fig.74). From these results the most likely structures of the bisbenzeneboronates and the intermediate methylated bisboronates which gave rise to the methylation analysis products are shown, (Fig.78).

Mass spectrometry provided evidence for the existence of fused six-membered boronate ring structures in the crude product. Thus, a metastable ion, $\frac{m}{e}$78.4, indicated that the double elimination process, characteristic of the dioxaborinane ring, was occurring to produce the ion 50, $\frac{m}{e}$159, $C_9H_8BO_2$, (Fig.60), directly from the molecular ion. This ion could have been produced from 91, 1-deoxy-L-gulitol 2,3 ; 4,6-bisbenzeneboronate, (Fig.78), via. a fragmentation process analogous to that discussed



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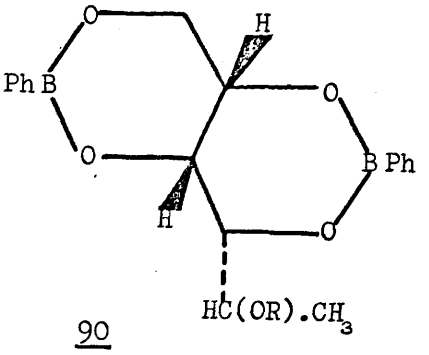

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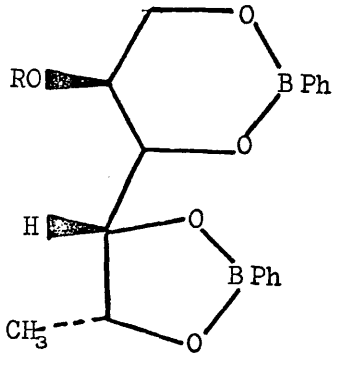
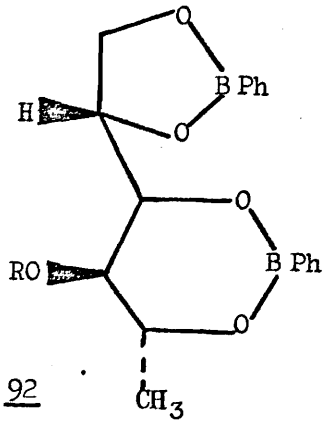


91

R = H or CH_3

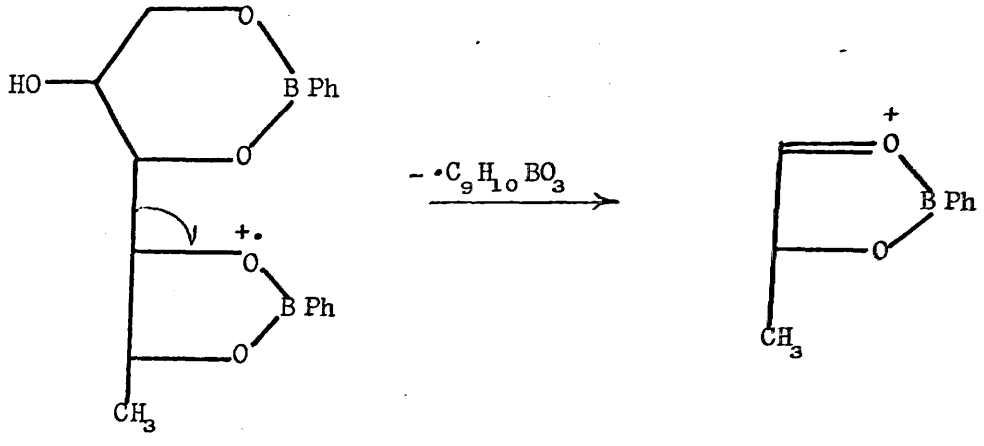


92

Fig.78

for DL-ribitol 1,3 ; 4,5-bisbenzeneboronate, (p.82). However, for the reasons given earlier, this possibility can be discounted.

A metastable ion $\frac{m}{e}$ 76.7, indicated fragmentation of the molecular ion, $\frac{m}{e}$ 338, to the ion $\frac{m}{e}$ 161 directly. This ion could be produced by the fission of the C-3 - C-4 bond in the structure 91, 1-deoxy-L-gulitol 2,3 ; 4,6-bisbenzeneboronate, (Fig.79). Alternatively $\frac{m}{e}$ 161 can also be

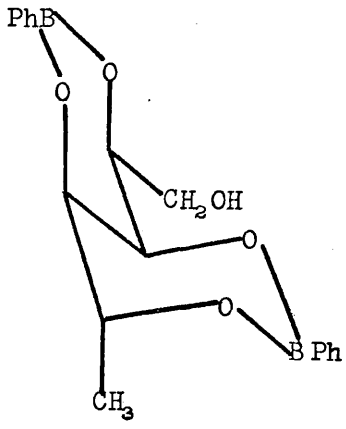


m/e 338

Fig.79

m/e 161

produced from 89, 1-deoxy-L-gulitol 2,4; 3,5-bisbenzeneboronate via the 'half-rupture' mechanism (Fig.38). Consequently, the ion $\frac{m}{e}$ 161 is not a useful aid for structure determination for these compounds.



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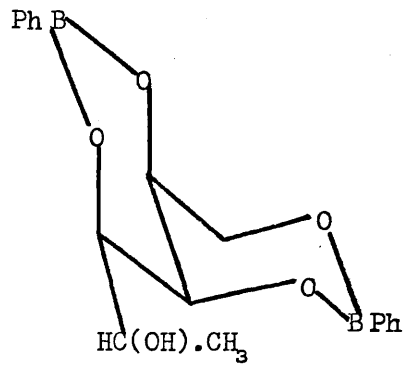

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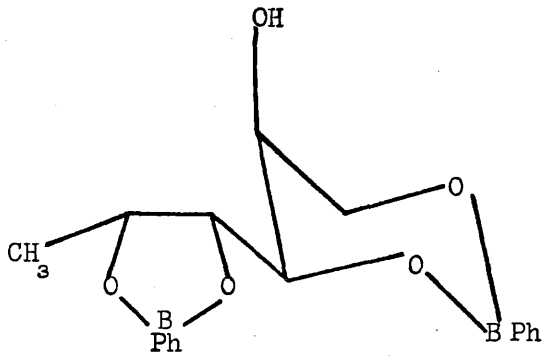

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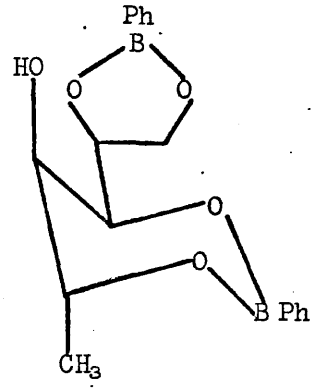


96

Fig.80

The four components of the product obtained from 1-deoxy-L-gulitol have therefore been assigned the structures 93, 94, 95 and 96. It will be noticed that the major component 93, 1-deoxy-L-gulitol 2,4; 3,5-bisbenzeneboronate, has one equatorial and one axial substituent on its two cis-fused, six-membered ring structure. The steric interaction of the axial substituent is probably the main reason for the presence of significant amounts of the other structural isomers, as indicated from the methylation studies, (Table IV).

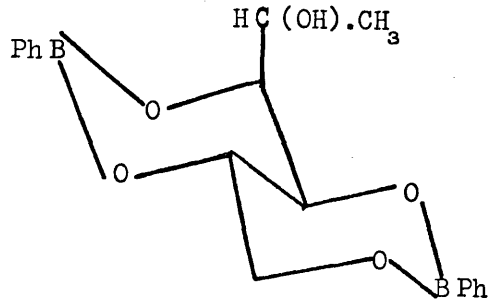
CONCLUSION

The structures of bisbenzeneboronates formed from seven pentitols on interaction with benzeneboronic anhydride have been investigated by chemical and mass spectrometric methods. All of the pentitols have been shown to produce mixtures of structural isomers. From considerations of the relative abundance of each isomer in the crude product and its mass spectrum, several generalisations can be formulated.

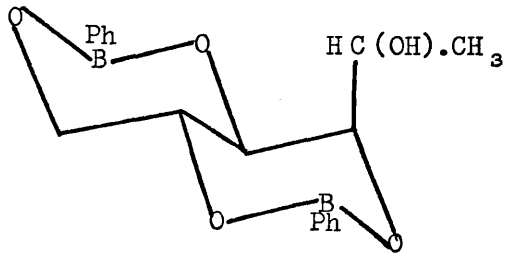
The most abundant isomer in all the bisbenzeneboronate products contained a primary hydroxyl group. All of these boronates have been assigned fused six-membered ring structures. This latter arrangement of 2-phenyl-1,3,2-dioxaborinane rings is probably a consequence of the greater thermodynamic stability of this arrangement over the two five-membered ring system. However, steric interactions of axial substitutents on the boronate rings may be sufficient to result in other structural forms. In an investigation of benzeneboronates of six triols[88], five of which gave rise to mixtures of structural isomers, the six-membered ring structure was found to form the major component if the non-hydrogen substituents were equatorially disposed. Whereas the five-membered ring isomers were found to be exclusive products if the substitutents on the six-membered chair conformation, were axially disposed.

By analogy, the bisbenzeneboronates studied here form trans-fused six-membered rings if possible, in preference to the cis-fused six-membered rings. However, the latter will predominate if axial substitutents are present in the former. For instance, both cis- and trans-fused rings are possible for 1-deoxy-L-mannitol bisbenzeneboronate, but the trans-fused rings do not appear in the product since their formation would involve an axially disposed 1-hydroxyethyl group, (Fig.81). A similar situation arises in the case of 1-deoxy-D-glucitol, where a cis-fused system has been

proposed, 77, (Fig.71), the trans-fused arrangement, which is not present would have the 1-hydroxyethyl substituent in an axial orientation, (Fig.81).



1-deoxy-L-mannitol
3,5 ; 4,6-bisbenzeneboronate



1-deoxy-D-glucitol
3,5 ; 4,6-bisbenzeneboronate

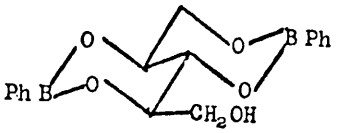
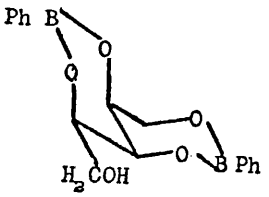
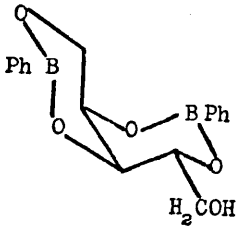
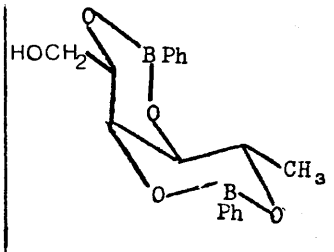
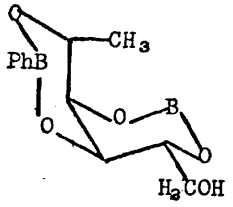
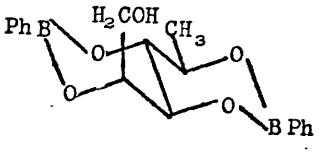
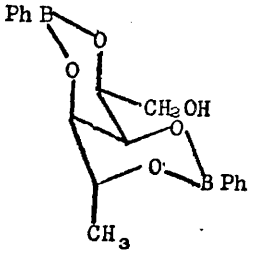
Fig.81

The most favourable conformations of the main boronate produced from the pentitols are given in Table VI. From a comparison of the relative amounts of each isomer present in the mixtures of boronates, it appears that the most favoured structure for a bisboronate will be the one which has equatorially disposed substituents.

The major product in all of the pentitols does, however, have a primary hydroxyl group. The tendency to form this structure in one case, 1-deoxy-L-gulitol, leads to the formation of a structure which appears to be sterically more unfavourable than one of its isomers. Thus, the main product is the one shown, Table VI, having one equatorial and one axial substituent on the two, cis-fused, six-membered rings in chair conformations. A structural isomer of this compound, also present in the product, has the two, cis-fused, six-membered ring structure with only one equatorial substituent in the chair conformation, 94, (see p.100), present in 19% of the total product. Therefore, the most likely structure, on steric grounds, may not be the major product.

TABLE VI

CONFORMATION OF MAJOR ISOMER IN BISBENZENEBORONATE PRODUCTS

Parent Pentitol	Main Product	Mole Fraction	Ring Fusion	Disposition of substituent (s)
ribitol		0.86	T	1 E
xylitol		0.95	C	1 E
D-arabinitol		0.52	C	1 E
1-deoxy-L-mannitol		0.79	C	2 E
1-deoxy-D-glucitol		0.79	C	1 E , 1 A
1-deoxy-D-talitol		0.42	T	1 E , 1 A
1-deoxy-L-gulitol		0.74	C	1 E , 1 A

III. CYCLIC BENZENEBORONATES AS PROTECTIVE GROUPS IN SYNTHESIS

III.1 INTRODUCTION

The continuing demand for synthetically prepared complex organic molecules has led to a fervent interest in the use of protective groups[116,117]. Until recently, benzeneboronate esters had not enjoyed the widespread use as protecting groups for polyhydroxy systems as the more conventional ketal and acetal groupings. This is somewhat surprising considering the ease of preparation and removal of the cyclic boronate entity, and its ability to remain intact under similar conditions to those previously employed with ketal and acetal as protecting groups.

Earlier work on the preparation of monoglycerides[118-120] indicated the utility of boronate esters and their susceptibility to hydrolyse in neutral or weakly acid conditions. Thus, saturated and unsaturated monoglycerides of long chain fatty acids had formerly been prepared by acylation of 1,3-benzylideneglycerol with the acyl chlorides. The protecting acetal group could then be removed by hydrogenolysis. However, preparation of unsaturated 2-monoglycerides precludes the use of this method for removal of the benzylidene acetal. Instead, the protective grouping was cleaved by reacting with boric acid, to yield the intermediate 1,3-borate ester, (Fig.82). The ester was hydrolysed under very mild conditions, simply by mixing with water, to yield the symmetrical monoglyceride.

A similar procedure was carried out for the hydrolysis of 1-acyl-2,3-O-isopropylidene glycerols[119,120]. The usual method of removing the acetal grouping involves use of mineral acids, which unfortunately were found to cause partial isomerisation to the 2-monoglycerides. The reaction of the protected monoglyceride with boric acid afforded the 1-acyl-2,3-borate ester, which was conveniently hydrolysed under neutral conditions to the 1-monoglyceride, (Fig.83).

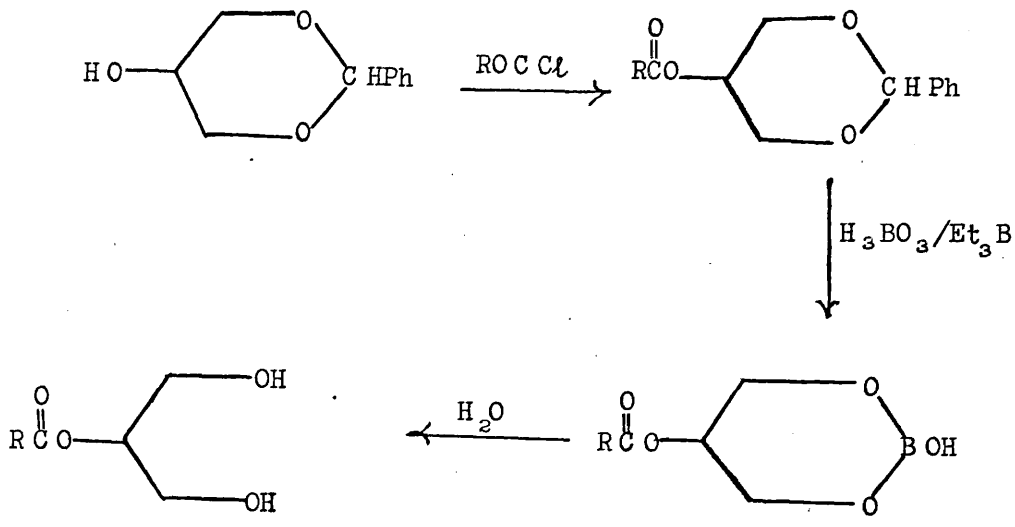


Fig.82

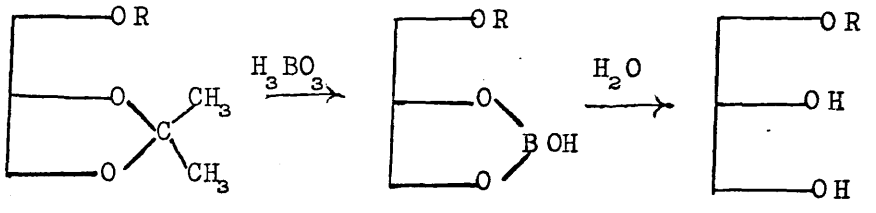


Fig.83

The primary objective therefore, was to utilise the cyclic benzeneboronate grouping as a protective entity in the synthesis of carbohydrate derivatives which otherwise require long tedious routes with a small overall yield. Initial ideas were directed at the possible employment of the previously studied pentitol boronates, in which the remaining unsubstituted hydroxy group could be converted into a potentially useful derivative. For example, the synthesis of 6-O-methyl-D-mannose was an important step in the elucidation of the products from hydrolysis of the glycosidic antibiotic curamycin[121,122], and was also prepared from 1,2,3,4-tetra-O-acetyl-α-D-mannopyranose during the structural investigation of synthetically prepared oligosaccharides[123]. In a five stage synthesis, starting

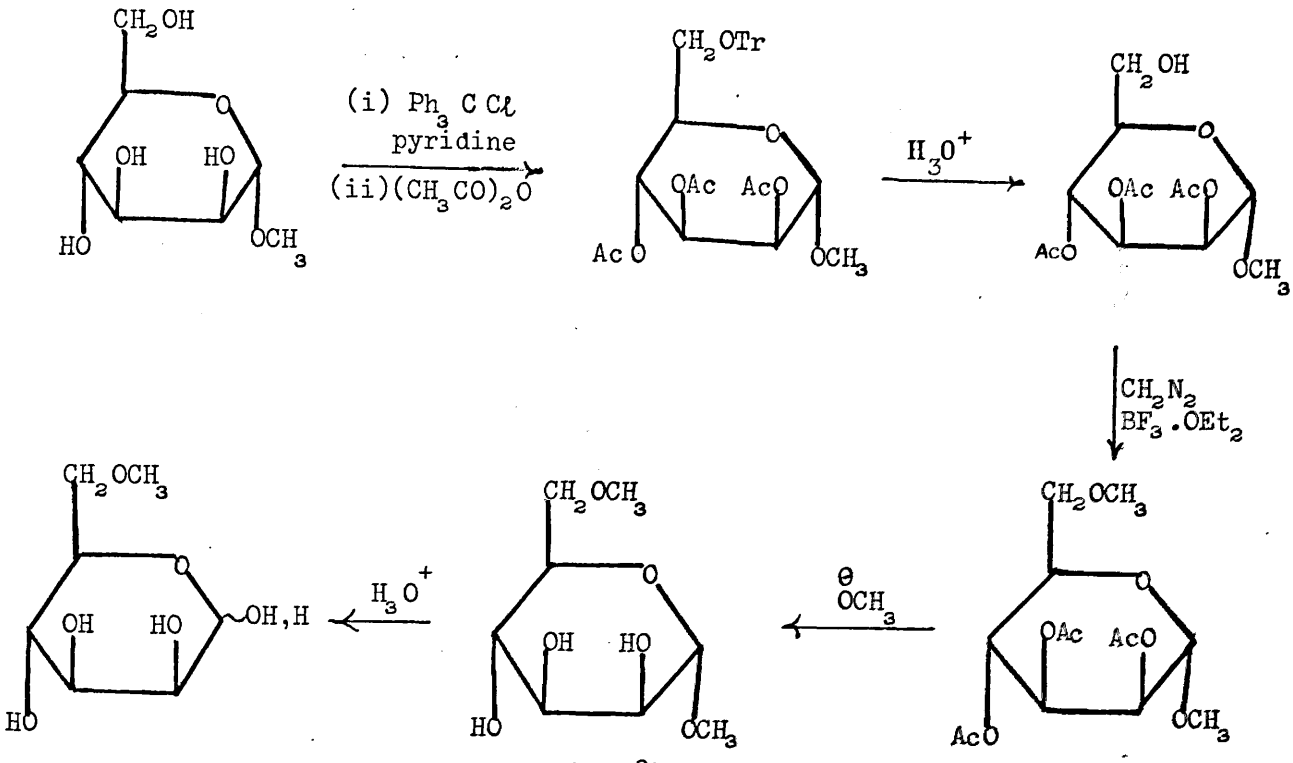
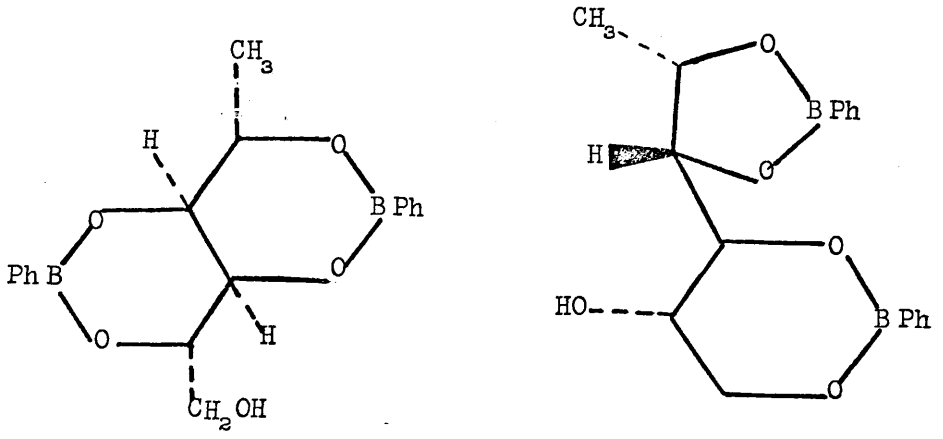


Fig.84

from methyl α-D-mannopyranoside, the 6-O-methyl-D-mannopyranose was prepared in an overall yield of 33%[122], (Fig.84), which compares favourably with the original preparation of this compound by HUDSON et al.[124], using methyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside as the starting material. The Purdie reagents for methylation were used and the overall yield was low, which, in the light of present knowledge is understandable as acetyl groups are known to migrate in this basic media.

An alternative synthesis for the preparation of this compound was envisaged, using the knowledge gained from the investigations of the pentitol bisbenzeneboronates with particular emphasis on 1-deoxy-L-mannitol bisbenzeneboronate. The bisbenzeneboronates produced from this pentitol have been shown (p. 93) to be 1-deoxy-L-mannitol 2,4; 3,5-bisbenzeneboronate and 1-deoxy-L-mannitol 2,3; 4,6-bisbenzeneboronate, in a ratio of 3.8:1 respectively, (Fig.85).



2,4 ; 3,5-bisbenzeneboronate 2,3 ; 4,6-bisbenzeneboronate
1-deoxy-L-mannitol

Fig.85

The same boronate structures, in a comparable ratio, were anticipated for the enantiomer of the pentitol, 1-deoxy-D-mannitol, since the relative spatial orientations of the hydroxyl groups are the same. Also, replacing the terminal methyl group with a diethyldithioacetal function was not expected to significantly change the ratio or the type of bisboronate structures. Therefore, D-mannose diethylmercaptal was expected to form the two isomeric bisbenzeneboronates of the same structure and in a similar ratio to the boronates of 1-deoxy-L-mannitol, (Fig.86).

Methylation, hydrolysis and demercaptalation of these boronates was anticipated to yield 6-O-methyl-D-mannopyranose and 5-O-methyl-D-mannofuranose in an approximate ratio of 4:1, (Fig.87). The proposed route was expected to afford the mono-O-methyl derivatives in good yield, since methylation with diazomethane[86], hydrolysis of the benzeneboronates[39] and demercaptalations of sugars[125,126] are known to proceed efficiently. However, when the product from methylation of the D-mannose diethyldithioacetal bisbenzeneboronate was demercaptalated with mercuric chloride and cadmium carbonate, a significant amount of a third product, besides the expected 6-O-methyl and 5-O-methyl-D-mannose derivatives, was detected by paper chromatography. Difficulty was experienced in attempts to isolate

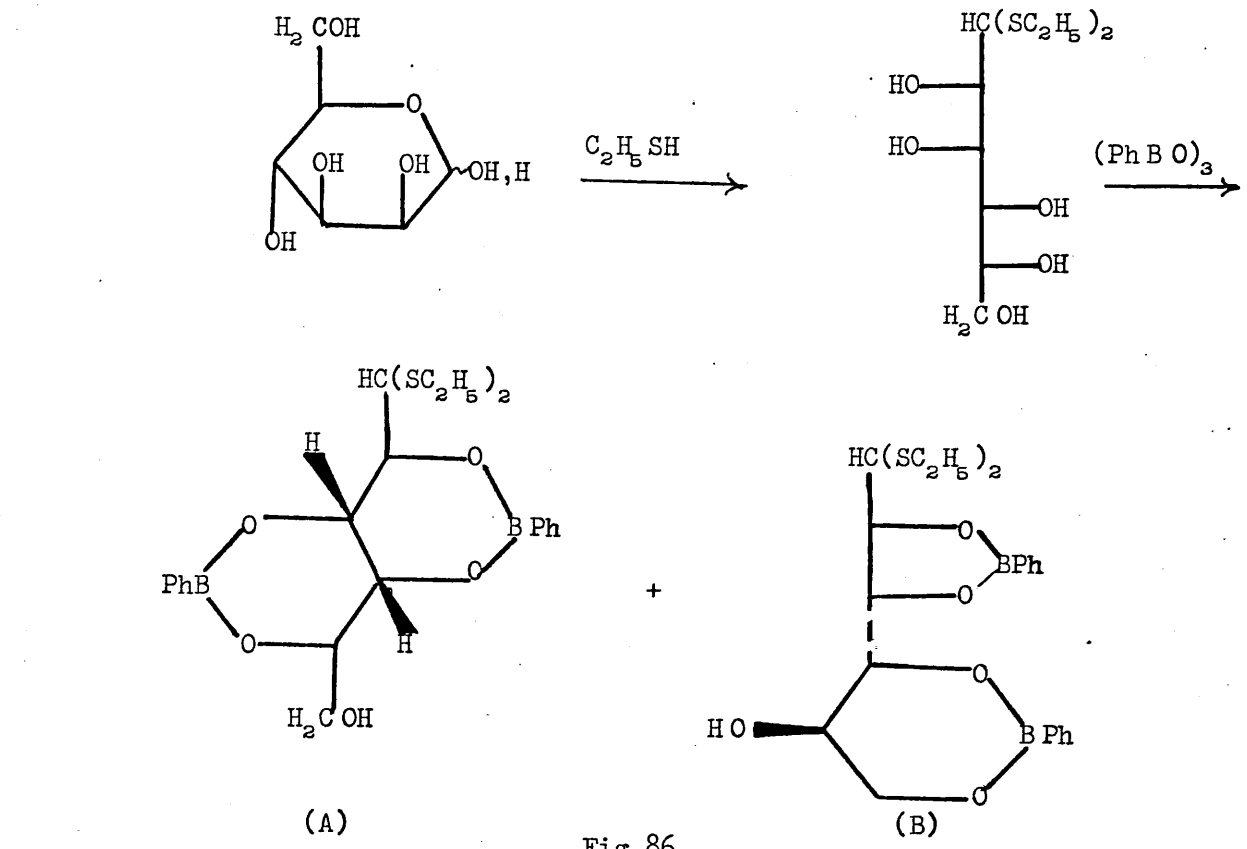

H_2COH
OH
OH
OH,H
OH
C_2H_5SH
$HC(SC_2H_5)_2$
HO
HO
OH
OH
H_2COH
$(PhBO)_3$
$HC(SC_2H_5)_2$
H
O
B Ph
O
PhB
O
O
H
H_2COH
(A)
+
$HC(SC_2H_5)_2$
O
BPh
O
O
HO
B Ph
O
(B)

Fig.86

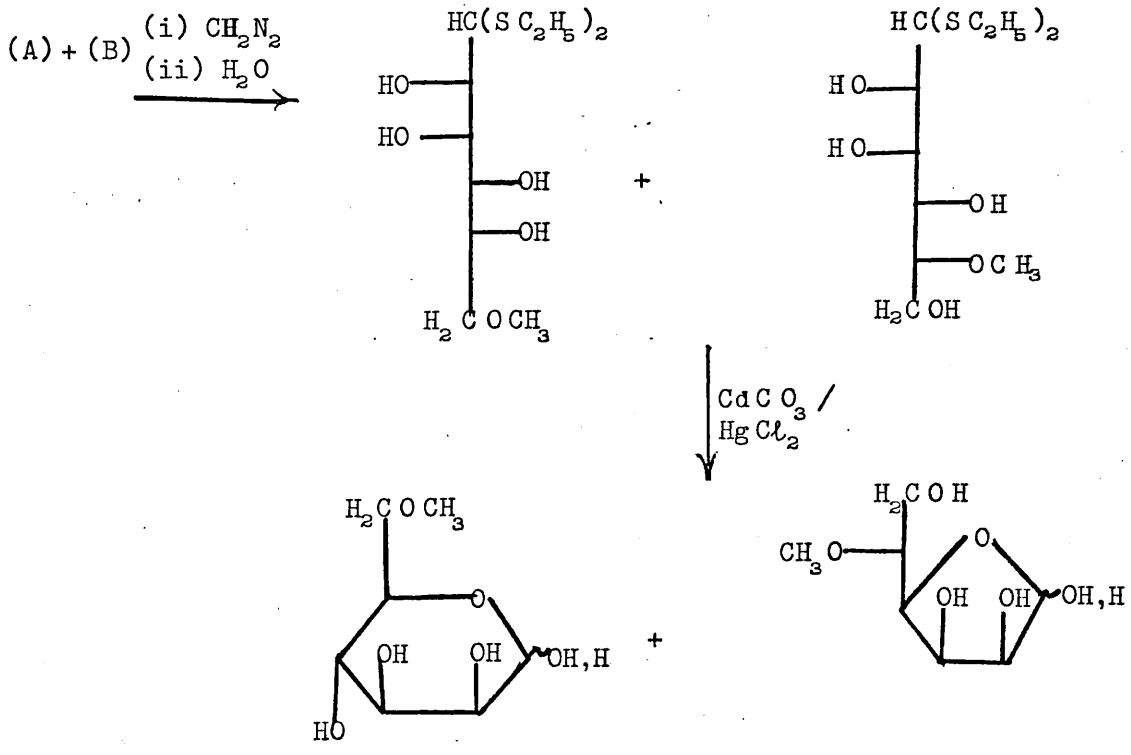
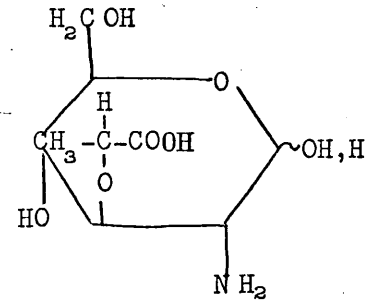

(A) + (B)
(i) CH_2N_2
(ii) H_2O
$HC(SC_2H_5)_2$
HO
HO
OH
OH
H_2COCH_3
+
$HC(SC_2H_5)_2$
HO
HO
OH
OCH_3
H_2COH
$CaCO_3$ /
$HgCl_2$
H_2COCH_3
O
OH
OH
OH,H
HO
+
H_2COH
CH_3O
O
OH
OH
OH,H

Fig.87

this compound, and its identity remains in doubt. The apparent low yields of the monomethylated derivatives together with this undesirable side product indicated that this synthesis would be unprofitable.

Attention was then directed towards the preparation of partially protected benzeneboronate derivatives of amino sugars, with a view to the synthesis of important intermediates which normally involve long synthetic routes.

The chemistry of amino sugars has received enormous attention since the isolation of the most abundant of these naturally occurring compounds, by LEDDERHOSE, in 1876, who isolated glucosamine from crustacean chitin[127]. The majority of this research has been carried out in the last few decades after such discoveries as the presence of 2-deoxy-2-methylamino-L-glucose as a structural component of streptomycin[128] and muramic acid, (Fig.88), as a constituent of bacterial cell walls[129]. Reviews on the chemistry[130-132] and distribution[133] of amino sugars give ample evidence for their ubiquity and indicate that research will continue to flourish in this field.



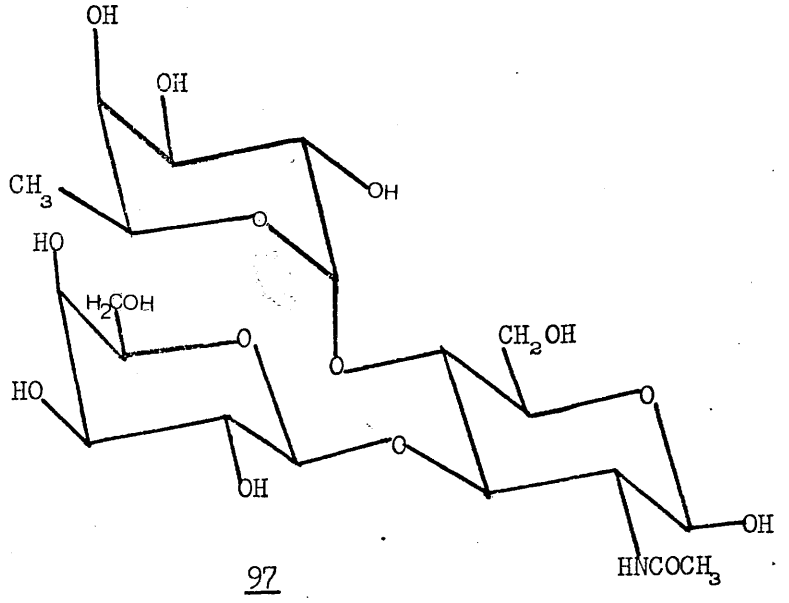
2-amino-3-O-(D-1-carboxyethyl) 2-deoxy-D-glucose. (Muramic Acid)

Fig.88

Intensive interest has generated in the field of naturally occurring glycoproteins and mucopolysaccharides which contain a variety of amino sugar derivatives. A large volume of work has been concerned with the structure elucidation and synthesis of the carbohydrate determinants in blood group substances[134-136]. These blood group specific substances are largely carbohydrate in nature, which were first isolated, in low yield, from erythrocytes. Later workers discovered that human blood group substances occurred in the tissue fluids and secretions of certain individuals, in particular from saliva[137], human urine[138]

and gastric juices[139]. The blood group substances having A activity were later isolated from the more convenient sources of pig gastric mucin[140] and ovarian cyst fluids[141].

Human blood was first classified into groups by LANDSTEINER in 1901[142], based on the presence or absence of agglutinable substances, A and B within the erythrocyte. Since then, a variety of substances with specific antigenic activity have been isolated, but the composition of the majority is still unknown. Recent advances have been made towards the structure elucidation, and synthesis of a wide variety of oligosaccharides which determine the type of antigenic activity in the blood group substances. A typical example is illustrated by the blood group substance which shows Le^a activity, discovered by MAURANT in 1946[143]. Through a series of experiments to determine the inhibitive effects of various oligosaccharides on agglutination of Le^a erythrocytes, a branched trisaccharide 97, 2-acetamido-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-(β-D-galactopyranosyl)-β-D-glucopyranose, was proposed as one of the specific determinant



97

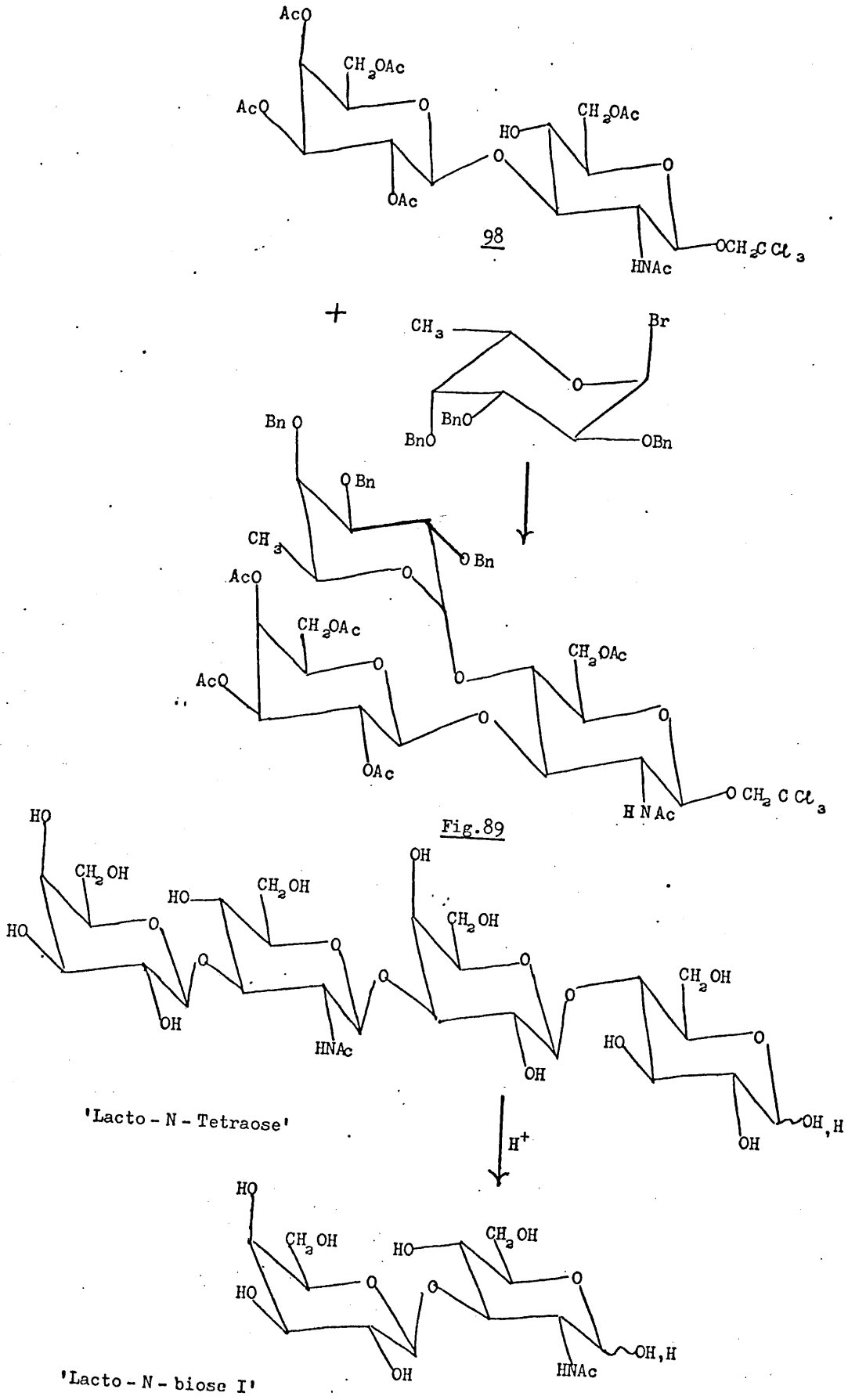


Fig.89

Fig.90

groupings[144]. This branched oligosaccharide was later isolated from human blood group Le^a substance[145]. Recently, this trisaccharide was synthesised[146] by reacting tri-O-benzyl-α-L-fucopyranosyl bromide with 98, 2,2,2-trichloroethyl 2-acetamido-2-deoxy-6-O-acetyl-3-O-(tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside, (Fig.89). The disaccharide from which 98 is derived was originally isolated from the partial hydrolysis of 'lacto-N-tetraose', a constituent of human milk[147], (Fig.90). Its synthetic preparation[148] involved a Konigs-Knorr type reaction of 99, tetra-O-acetyl-α-D-galactopyranosyl bromide with 100 benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside, (Fig. 91), with an overall yield of 28%. The same disaccharide was prepared in comparable overall yield by LEMIEUX[146] using a similar technique, but employing a 2,2,2-trichloroethyl glycoside to protect the reducing end of the sugar, which apparently, is more appropriate for the synthesis of higher oligosaccharides.

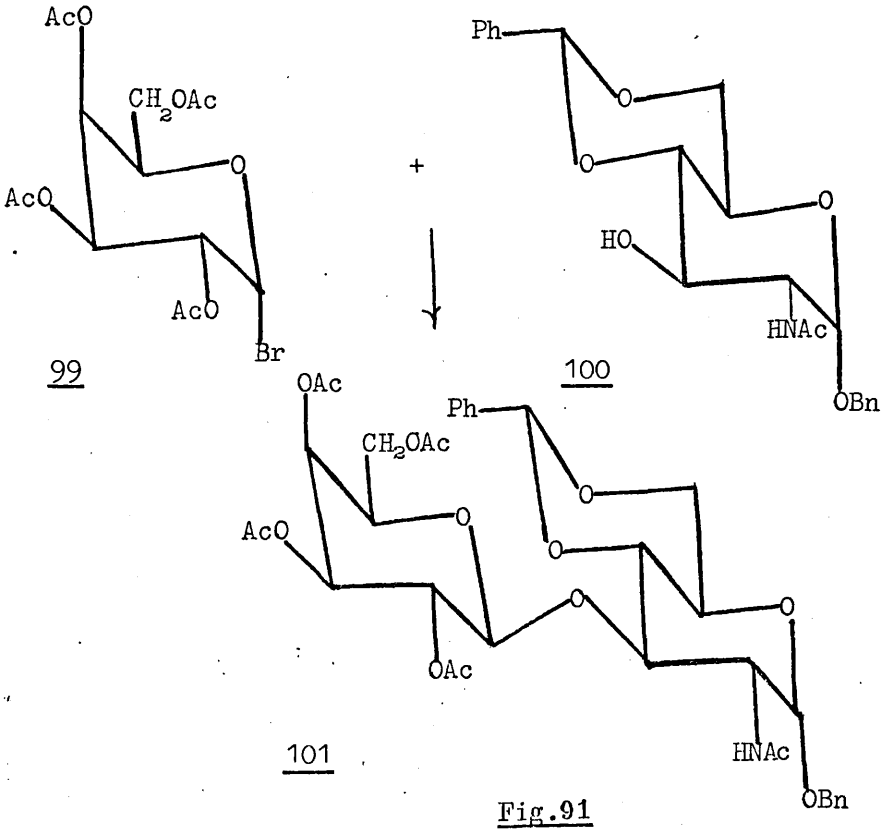


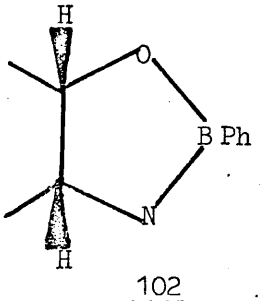
Fig.91

An important feature in the preparation of this and similar disaccharides and oligosaccharides, is the need for protective groups which can be attached and removed easily, and in the latter case, under mild conditions in order to safeguard the glycosidic linkage. It was with these considerations in mind that the preparation of benzeneboronate esters of amino sugars was undertaken.

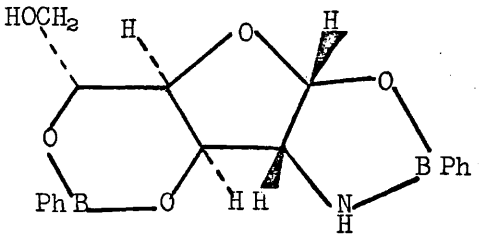
III.2 SYNTHETIC ROUTE FOR THE PREPARATION OF BIOLOGICALLY IMPORTANT DISACCHARIDES

A. Synthesis and structure determination of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate

Preliminary investigations were directed towards the preparation of cyclic benzeneboronate esters of 2-amino-2-deoxy-D-glucose, in order to determine the possibility of cyclic mono- or bisbenzeneboronate ester formation. Vicinal cis-amino-alcohol groupings are known to form 2-phenyl-1,3,2-oxazaborolidine ring systems 102 , with benzeneboronic anhydride[149].



102



103

Thus, bisbenzeneboronates of D-glucosamine such as 103, containing a single unesterified hydroxyl group were a distinct possibility. However, interaction of this sugar with benzeneboronic anhydride did not produce any isolatable esters. Similar observations have been recorded for the reaction of this sugar with butaneboronic acid[49].

An anomeric mixture of methyl glycosides of 2-acetamido-2-deoxy-D-glucopyranoside was prepared in an approximate ratio of 85% α and 15% β [150]. Reaction of this mixture with benzeneboronic anhydride afforded a crystalline derivative in 82% yield. Paper chromatography and polarimetry

of this compound indicated that the β-glycoside had been removed during the recrystallisations. In later experiments, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was separated from the β-anomer by column chromatography. The physical properties of the benzeneboronate ester produced from this pure glycoside were identical with those of the boronate isolated from the mixture of methyl glycosides.

The percentage composition of this benzeneboronate indicated a mono benzeneboronate containing one boron atom in the ring. The mass spectrum supplied evidence for the presence of a six-membered boronate ring, spanning the oxygens on C-4 and C-6. The highest observable ion in the spectrum occurred at $\frac{m}{e}$ 322, corresponding to the $(M+1)^+$ ion, generated by abstraction of a hydrogen atom from a neighbouring molecule in the ion source, a process typical of molecules containing hydroxyl groups[113]. Fission of the bond between C-1 and the methoxy aglycon, a feature characteristic of many glycosides, produced the ion 104, $\frac{m}{e}$ 290, $C_{14}H_{27}BNO_5$, (Fig.92). Elimination of water

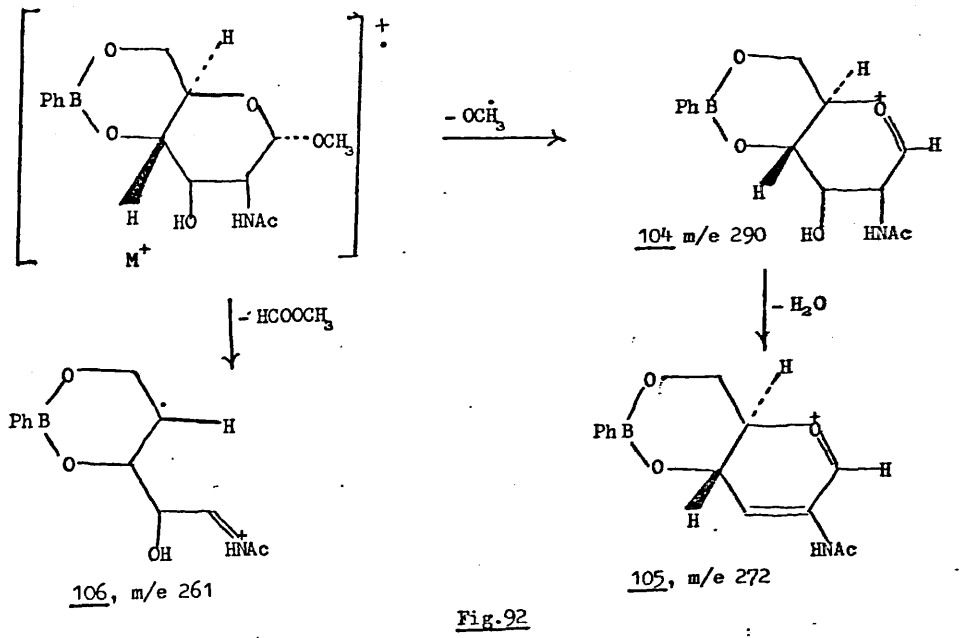


Fig.92

from this oxonium ion, afforded the ion 105, $\frac{m}{e}$ 272, $C_{14}H_{15}BNO_4$. An alternative fragmentation pathway from the molecular ion, beginning with fission of the C-1 - C-2 bond, leads to elimination of a molecule of methyl formate, producing the ion 106, $\frac{m}{e}$ 261, $C_{13}H_{16}BNO_4$. This fragmentation was supported by a weak metastable ion $\frac{m}{e}$ 212. A similar fragmentation mode occurs in the mass spectrometry of fully methylated methyl glycosides[151] and in the breakdown of the molecular ion of permethylated 2-acetamido-2-deoxy-β-D-glucopyranoside [152].

The ion 106 was shown to be a precursor of 107, $\frac{m}{e}$ 243, $C_{13}H_{14}BNO_3$, through the presence of an abundant metastable ion $\frac{m}{e}$ 226. The ion 107 was indicated to breakdown further by loss of a molecule of ketene, to provide the ion 108, $\frac{m}{e}$ 201, $C_{11}H_{12}BNO_2$, evidenced by a metastable ion $\frac{m}{e}$ 166.3. Further fragmentation of this ion provided the ion 109, $\frac{m}{e}$ 159, $C_9H_8BO_2$, indicated by a weak metastable ion, $\frac{m}{e}$ 125.8, a possible fragmentation sequence is shown, (Fig.93).

The presence of the ion, 109, $\frac{m}{e}$ 159 and $\frac{m}{e}$ 160 provide strong evidence for a six-membered boronate ring structure, bridging the oxygens on C-4 and C-6. The alternative five-membered ring system involving C-3 and C-4 oxygens would give rise to the ion $\frac{m}{e}$ 146, (Fig.94). This latter structure for the boronate ester was not anticipated to be present since vicinal hydroxyl groups in a trans-equatorial configuration on a pyranose ring, give rise to 2,4-diphenyl-1,3,5-trioxa-2,4-diborepane ring systems[18,20,68].

Further proof of this structure was provided by methylation of the unprotected hydroxyl group. Hydrolysis of the product, followed by acetylation, in a manner analogous to the procedure used for the pentitol bis-benzeneboronates, provided a product which could be analysed by combined g.c.-m.s.

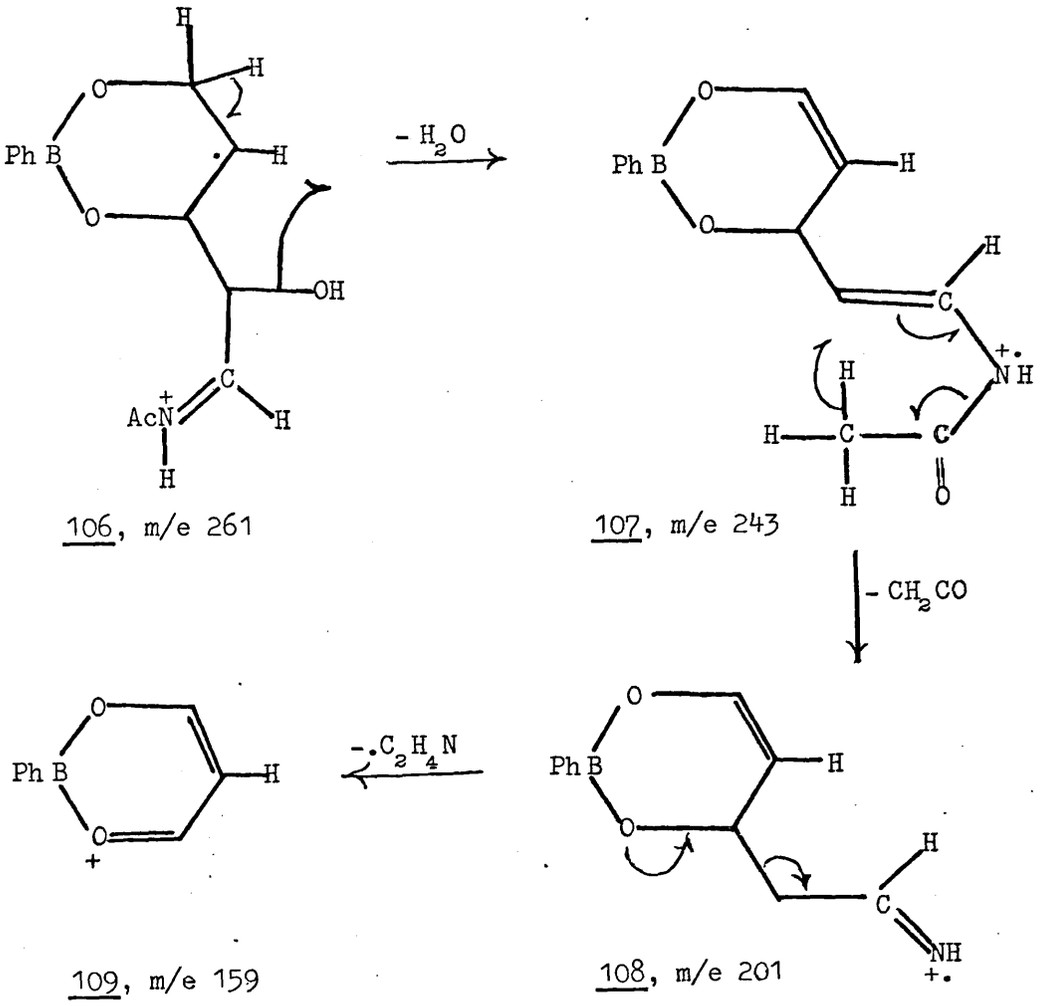

- H_2O
- CH_2CO
- C_2H_4N
106, m/e 261
107, m/e 243
109, m/e 159
108, m/e 201

Fig.93

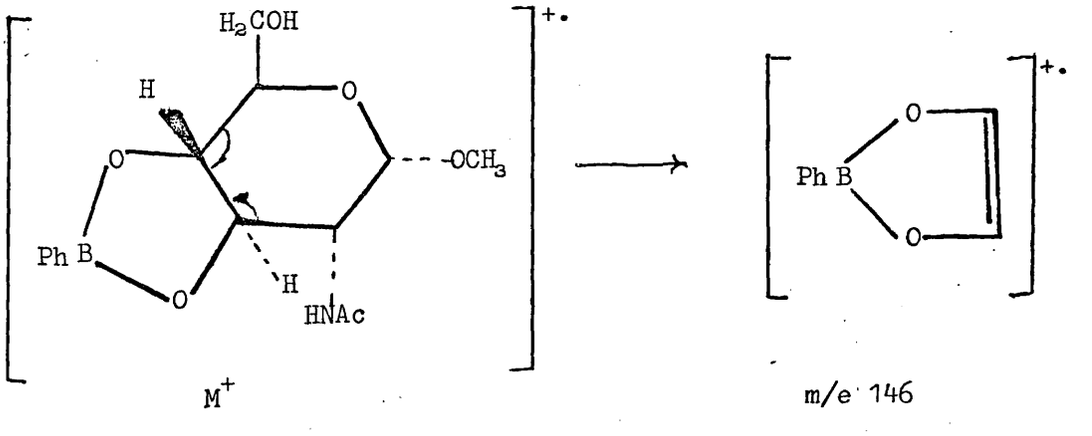
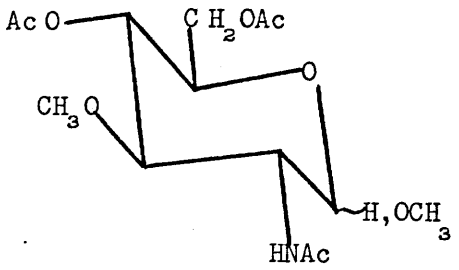
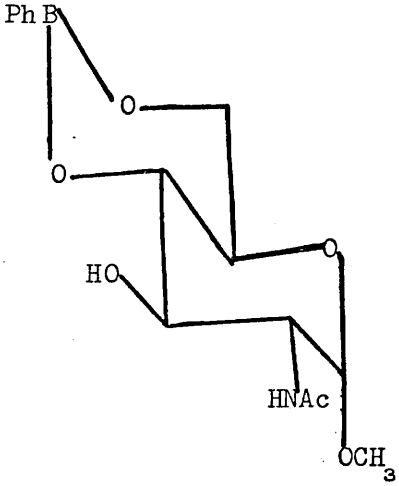

M^+
m/e 146

Fig.94

The mass spectrum of the product provided proof for the structure 109, methyl 2-acetamido-2-deoxy-4,6-di-O-acetyl-3-O-methyl-D-glucopyranoside. The gross features of the spectrum resembled the spectrum cited for methyl 2-acetamido-2-deoxy-4,6-di-O-acetyl-3-O-methyl-α-D-galactopyranoside [153]. As anticipated, the stereochemistry at C-4 did not significantly change the relative peak intensities, (see appendix). The evidence obtained provides conclusive proof for the boronate formed from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside to be 110, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate.



109



110

III.2.B Synthesis of a Partially Protected Disaccharide

The method envisaged for the preparation of the β-linked galactosyl-glucosamine disaccharide involved the use of a modified Konigs-Knorr reaction [154]. Several methods have been devised, based on the original Konigs-Knorr synthesis for the stereoselective synthesis of glycosides and disaccharides [155-158]. A method was chosen which would provide a β-linkage from 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide, involving Walden inversion at the anomeric carbon. Such a method was devised several years ago which produced the β-D-glycoside from acetylated α-D-glycosyl halides, using mercuric cyanide as a catalyst in nitromethane [159].

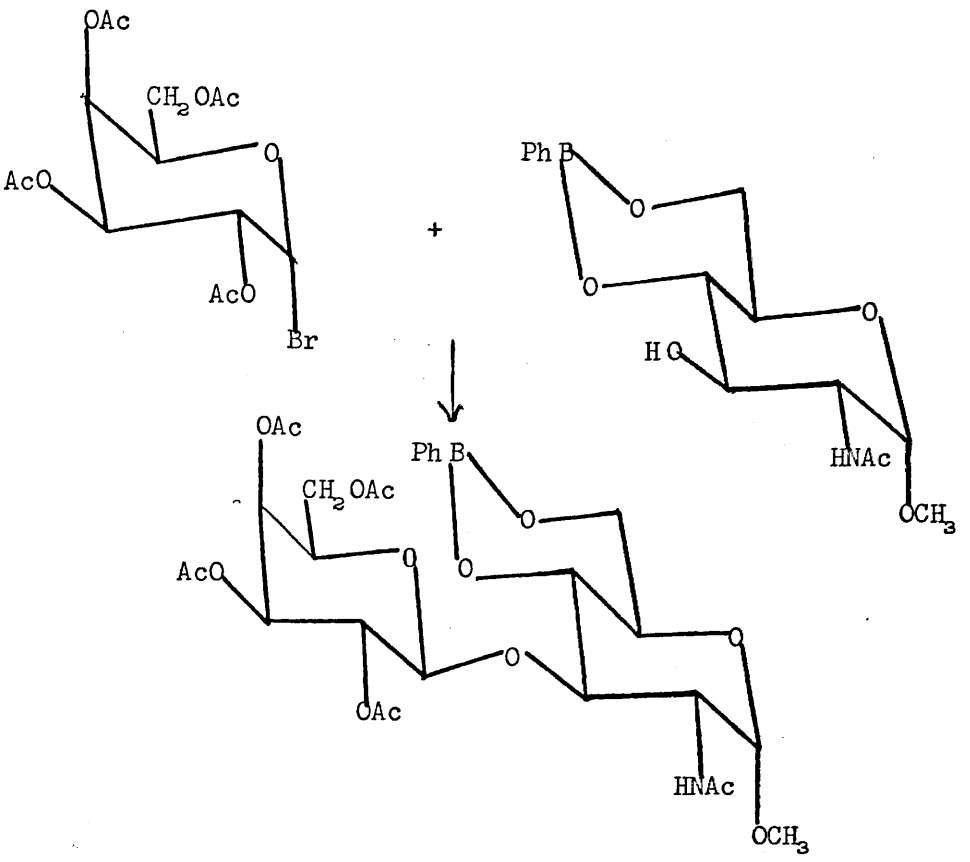
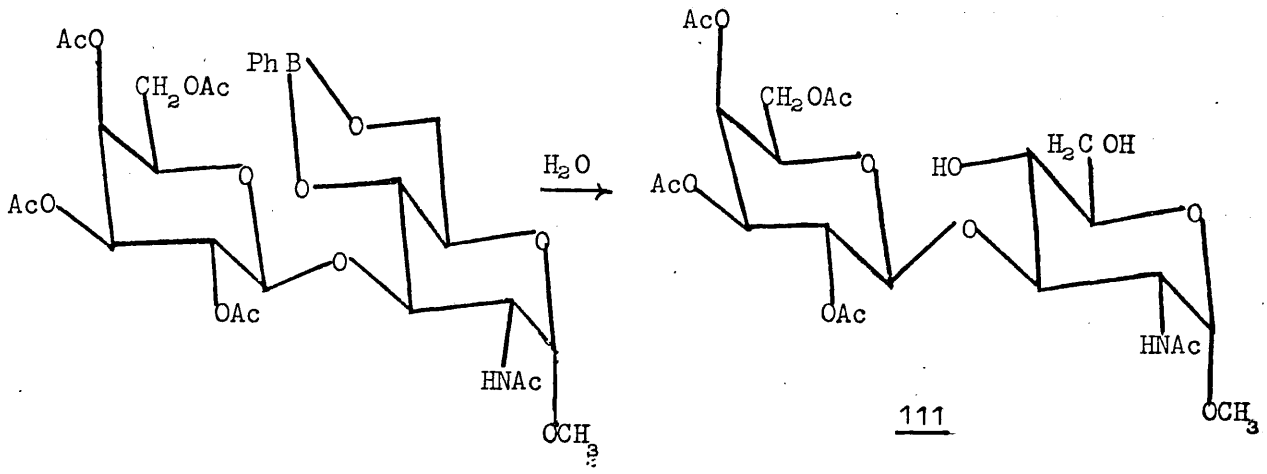


Fig.95

The method devised for the synthesis was therefore a reaction between 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate in nitromethane using mercuric cyanide as catalyst, (Fig.95).

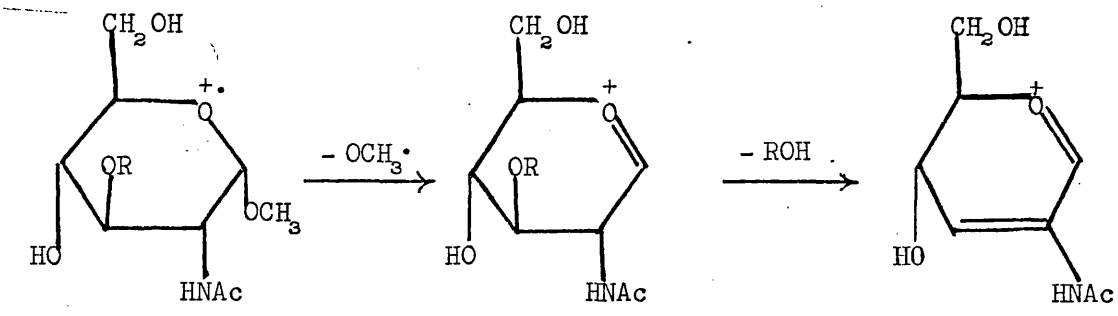
2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide was prepared from the penta-O-acetate of galactose by reaction with hydrogen bromide in glacial acetic acid. Some difficulty was encountered in dissolving the boronate in nitromethane, and at first, this solvent appeared unsuitable. However, the initial suspension of the ester in the reaction mixture finally disappeared after three days at room temperature. Analysis of the solution by t.l.c. showed complete absence of starting material. On passing the reaction product through a chromatographic column, containing silicic acid, a fraction was collected, which on examination by t.l.c., contained only a slow running disaccharide, but no benzeneboronic acid or boronate. It



111

Fig.96

was therefore concluded that the labile ester grouping on the amino sugar unit had been hydrolysed, and the subsequently formed benzeneboronic acid was separated, (Fig.96). The yield, 88%, of 111 from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside compares favourably with the



M^+ 112, m/e 534 113, m/e 186

Fig 97. R = 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl

yield of 63% using the benzylidene grouping, starting from the trichlorethyl glycoside.(146) The material thus obtained had a percentage composition in agreement with the proposed disaccharide 111. The mass spectrum of 111 contained an ion $\frac{m}{e}$ 534, with the atomic composition $C_{22}H_{32}NO_{14}$, corresponding to loss of a methoxy radical from the molecular ion, (Fig.97), and supported by a metastable ion $\frac{m}{e}$ 504.5. An abundant ion 113, $\frac{m}{e}$ 186, $C_8H_{10}O_5$, was considered to have arisen from the ion 112, by elimination of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranose, from the observation of

a metastable ion, $\frac{m}{e}$ 64.8. This type of elimination has also been shown to occur in methyl 2-acetamido-2-deoxy-3-O-methyl-α-D-galactopyranoside, (Fig.98) [153]. This compound lost methanol from the oxonium ion, $\frac{m}{e}$ 218,

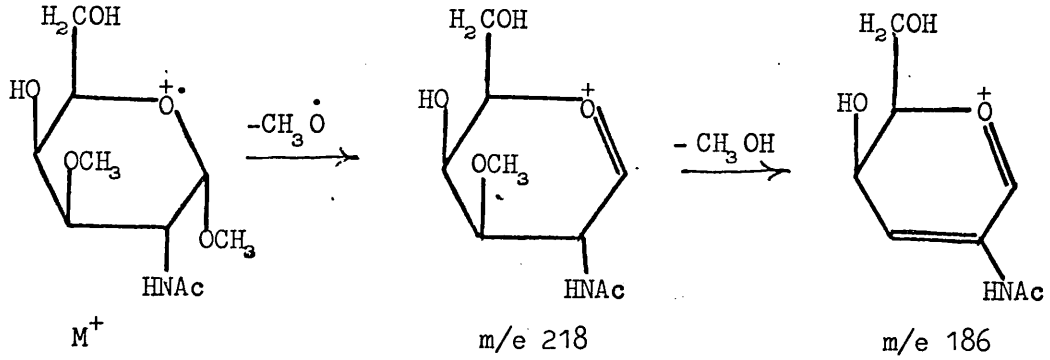


Fig.98

to produce the same ion, $\frac{m}{e}$ 186. The base peak in the disaccharide spectrum, accounting for 8% of the total ion current, occurred at $\frac{m}{e}$ 331, $C_{14}H_{19}O_9$, and was indicated to have fragmented from the molecular ion, evidenced by a metastable ion $\frac{m}{e}$ 193.6. This mode of fragmentation is also observed in the mass spectra of permethylated disaccharides, in which it was observed [160,161] that the two monosaccharide units break down independently. A similar series of ions were observed for the tetra-O-acetyl-D-galactosyl ion, $\frac{m}{e}$ 331, to those exhibited by fully acetylated hexoses [162], (Fig.99).

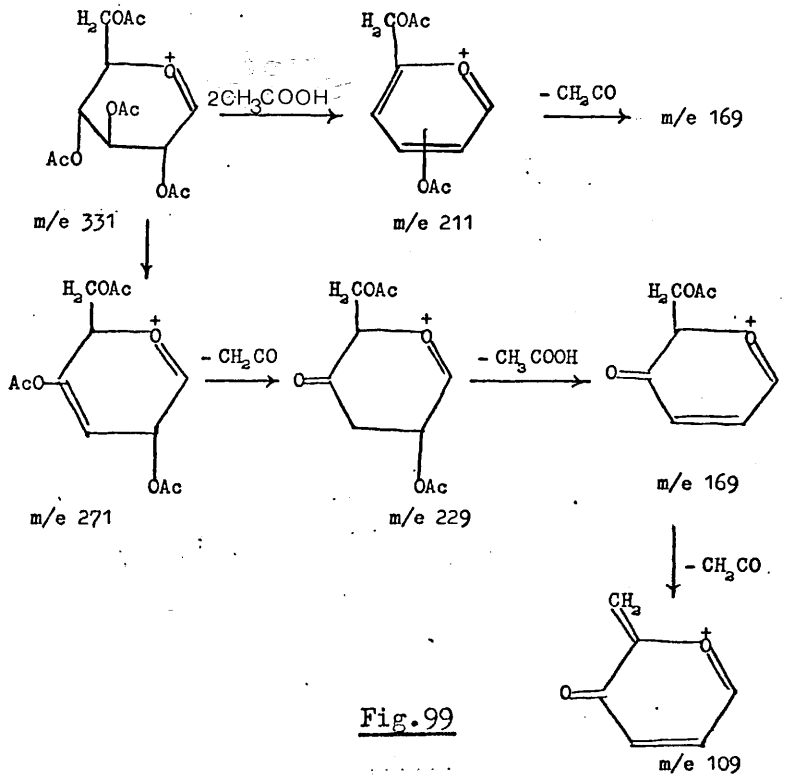


Fig.99

III.2.C Determination of Configuration of Disaccharide Linkage

The method of synthesis of the partially protected disaccharide intuitively suggests that the linkage between the sugar moieties has the β-D-(1→3) configuration. However, further evidence is required before this structure can be confidently assigned the β- configuration.

A method which has been applied[163-166] successfully to determine the configuration of the linkage in many disaccharides makes use of Hudson's isorotation rules[167]. These rules were based on an hypothesis formulated by van't Hoff which in principle states, that in optically active compounds that have more than one asymmetric centre, the rotation of each compound might be considered as the sum of the partial rotations of the asymmetric centres. However, this principle as applied directly to all substances is significantly incorrect, and cannot be applied indiscriminantly. Its application to carbohydrates however, as outlined by Hudson, is acceptable to a first approximation and can be used to obtain useful information concerning the structure and configuration.

The two classical rules of isorotation as outlined by Hudson are:

(1) The rotation of carbon 1 in the case of many substances of the sugar group is affected in only a minor degree by changes in the structure of the remainder of the molecule.

(2) Changes in the structure of carbon 1 in the case of many substances in the sugar group affect in only a minor degree the rotation of the remainder of the molecule.

In effect, the molecular rotation of a glycoside can be divided into two parts, A and B. Part A will comprise the partial rotation due to the anomeric carbon, and part B the rotatory contribution of the remainder of the molecule. It has, therefore, been possible to obtain a reasonably good estimate for the molecular rotation of the disaccharide if the specific rotation values for the methyl glycosides of the corresponding monosaccharides are known.

TABLE VII

OPTICAL ROTATIONS OF METHYL GLYCOSIDES AND THE DISACCHARIDE (111)

	$[\alpha]_D$ (degrees)	$[M]_D \times 10^{-3}$	Reference
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (A)	+ 131	+ 30.81	150
Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (B)	- 14	- 5.060	168
Methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranoside	+ 140·2	+ 79·21	(calculated)
(A) + (B)	+ 45·6	+ 25·75	
Disaccharide, 111	+ 48·5	+ 27·40	

Table VII gives the values for the specific and molecular optical rotations for the corresponding methyl glycosides, together with the measured and calculated values for the disaccharide 111, (Fig.96). The measured value of the optical rotation for the disaccharride is seen to be in close agreement with the calculated value, giving strong support for the β- configuration of the D-galactosyl residue.

Proton Magnetic Resonance Spectroscopy

The determination of the disposition of the proton on C-1 of the tetra-O-acetyl-D-galactosyl moiety of the disaccharide, provides immediate evidence for the configuration of the glycosidic link. The use of nuclear magnetic resonance (n.m.r.) spectroscopy is now considered a routine technique for the determination of the configuration of many glycosides, using the knowledge gained through studies on the effect of configuration of ring protons on the chemical shift and coupling constants[169]. These studies have also aided the assignment of proton signals in the spectra of disaccharides in attempts to correlate coupling constants and chemical shifts with the position of the protons and hence linkage configuration[170].

The proton magnetic resonance spectrum of the disaccharide, 111, was accordingly obtained using a 60 MHz spectrometer with trimethylsilane as an internal reference. The spectra of the constituent sugar entities,

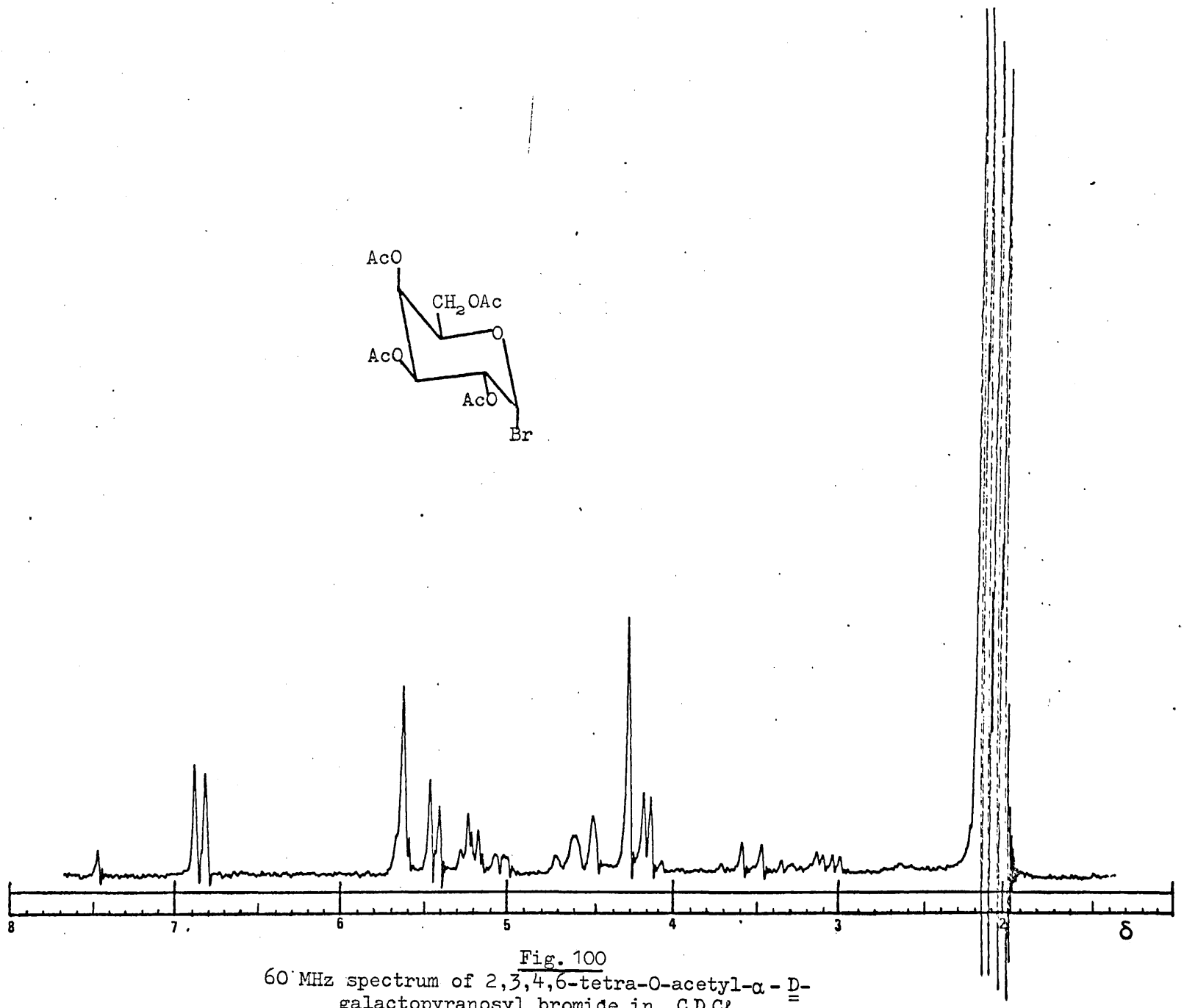


Fig. 100
60 MHz spectrum of 2,3,4,6-tetra-O-acetyl-α - D-galactopyranosyl bromide in $CDCl_3$

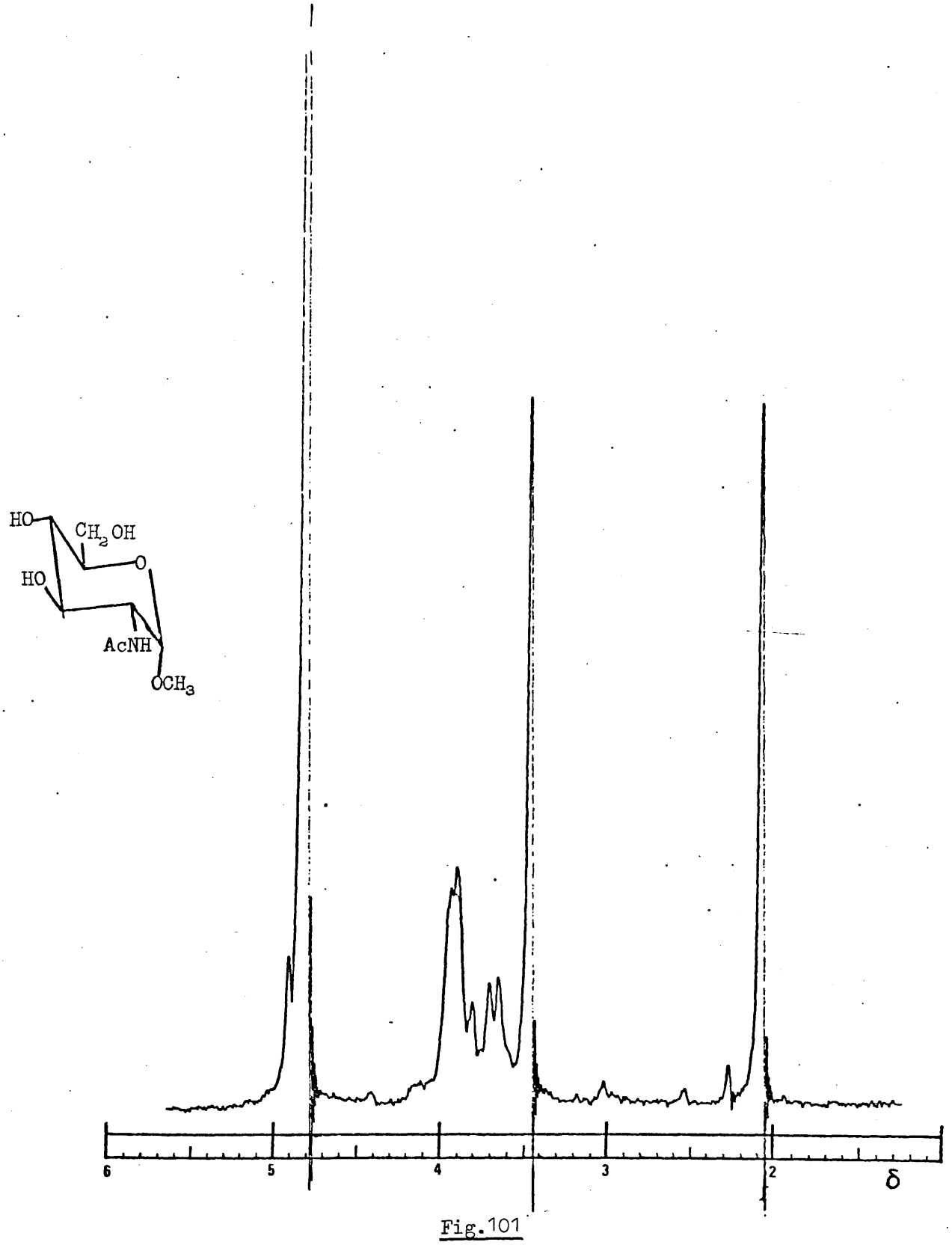


Fig.101

60 MHz spectrum of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside in D_2O

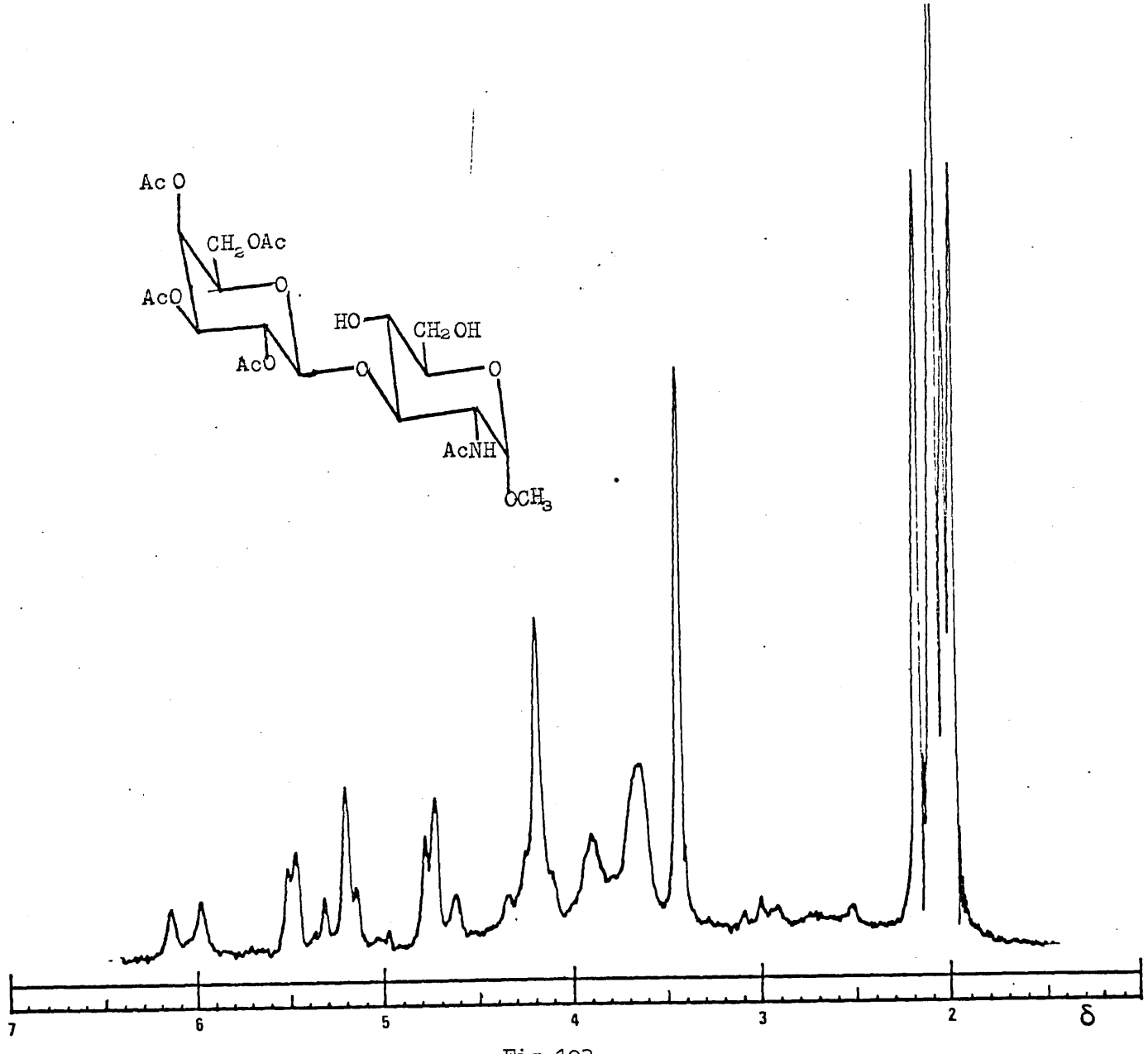


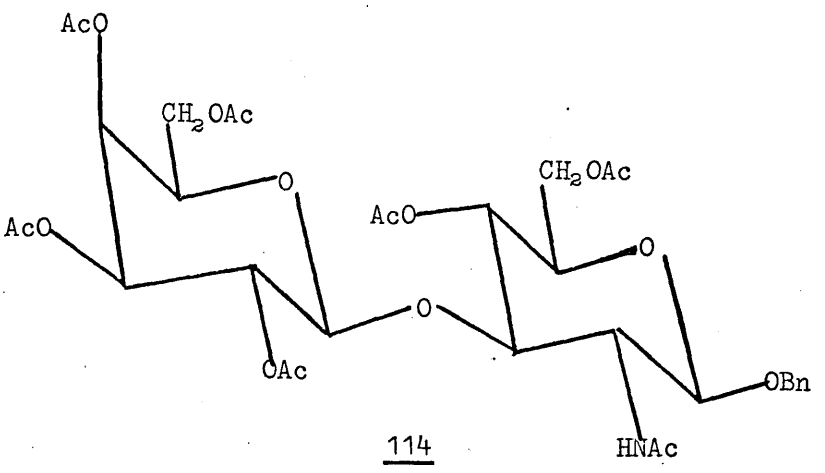
Fig.102

60 MHz spectrum of methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β - D-galactopyranosyl)-α - D-glucopyranoside in $CDCl_3$

methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide were also recorded for comparison, the spectra are shown, (Figs.100-102).

The spectra obtained for these compounds agreed with the data cited by other workers. Thus, the n.m.r. spectrum obtained for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, in deuterium oxide, showed a doublet at δ4.86, having a splitting of 3.2 c.p.s., not resolved in the spectrum shown, which was assigned to the anomeric proton occupying an equatorial configuration. The methyl protons on the acetamido group appeared as a singlet at δ2.08, and a similar line at δ3.45 was assigned to the glycosidic methyl protons, in agreement with the values given for the per-O-acetyl derivative of this sugar[171]. The n.m.r. spectrum for 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide in deuterochloroform showed a doublet at δ6.84, due to the anomeric proton. The splitting of 3.5 c.p.s. indicated that the C-1 hydrogen was occupying an equatorial position[172,173].

The n.m.r. spectrum of the disaccharide, in deuterochloroform contained a broad doublet at δ6.06 having a splitting of 10.5 c.p.s. assigned to the proton attached to the nitrogen of the acetamido group. A similar signal, for the N-H proton was noted for the disaccharide, 114, and its derivatives[174]. The anomeric proton of the galactosyl unit appeared as a broadened doublet, partially obscured by the anomeric hydrogen on the amino glycoside unit. This latter doublet appeared at δ4.76, with a splitting of 3.5 c.p.s. The galactosyl anomeric proton occurring at δ4.65, had a splitting of 8.5 c.p.s., the broadening of this signal is believed to result from second order effects, from the protons on C-3 and C-5[173,175]. The position and splitting of this signal resembled that of the anomeric proton on methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside[176]. The large splitting of the signal, due to coupling with the

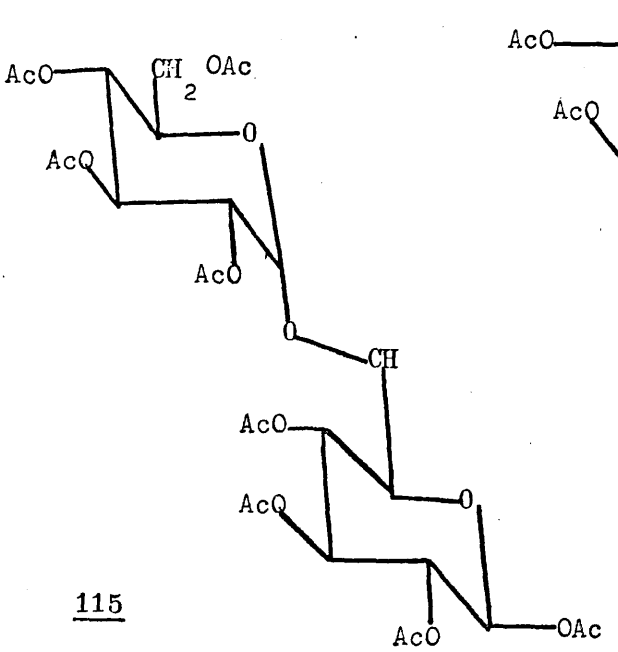


114

C-2 proton, provides evidence for the trans-configuration with respect to the latter proton [169,173], and consequently gives support for the β-D-(1→3) linkage.

Infrared Spectroscopy

The characterisation of carbohydrates through analysis of their infrared spectra has been the theme of a great deal of study[177-179], since the first report that very small differences in the stereochemistry of sugars produced quite different spectra[180]. Several reports have dealt specifically with that part of the fingerprint region, 960-730 cm^{-1}, which appears to produce evidence concerning the type of anomeric configuration[181-183]. This region was divided up into areas which appeared to be characteristic of the various types of absorption bands. It was discovered that monosaccharides having a glucopyranose structure in an α-configuration generally absorb at 917 ± 13 cm^{-1}, designated type 1a. This structure also gave rise to bands at 844 ± 8 cm^{-1} and 766 ± 10 cm^{-1}, termed type 2a and 3a respectively. The β-anomers gave a similar set of bands centred at 920 ± 5 cm^{-1}, type 1b; 891 ± 7 cm^{-1}, type 2b; and 774 ± 9 cm^{-1}, type 3b. The conclusions of this research[181-183] indicated that it was only the type 2a band which could be applied with confidence for diagnosing the α-anomeric form, and absence of this band, together with the 2b type band, provided evidence for the β-configuration.



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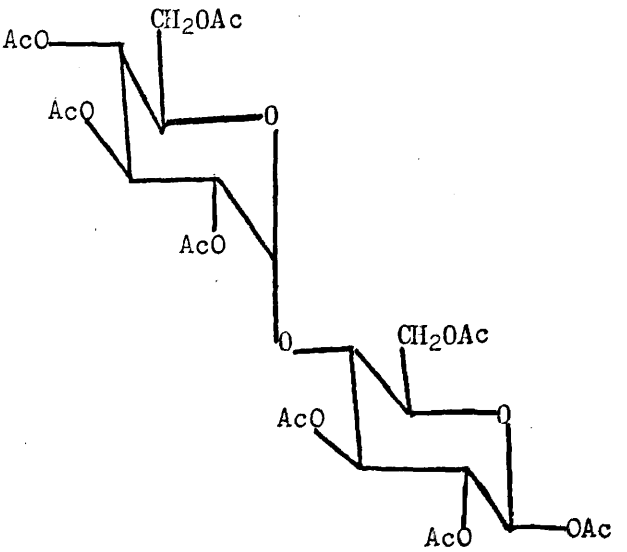

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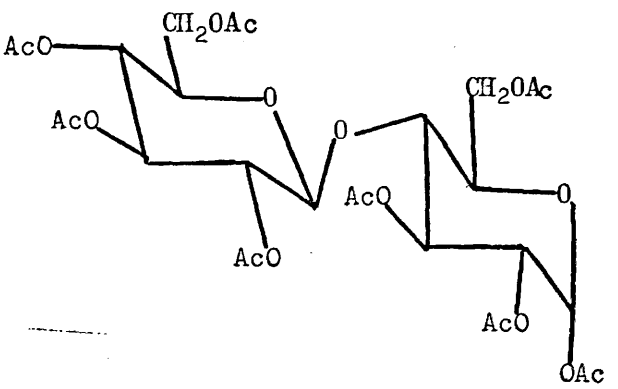

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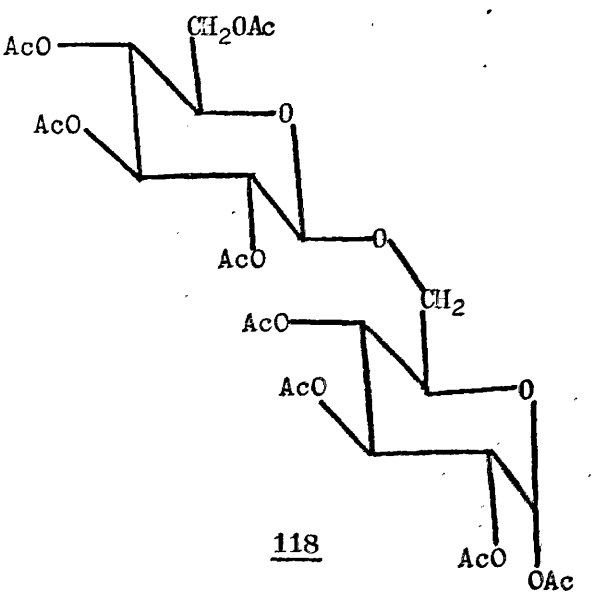


118

The 2a and 2b type absorptions were also found to be independent of the position of the pyranose unit in question. For example, the 2a absorptions for β-isomaltose octa-acetate 115, and β-maltose octa-acetate 116, occur at 825 cm^{-1} and 834 cm^{-1} respectively. In these examples the α-linkage is at the non-reducing end of the disaccharide. For α-cellobiose 117, and α-gentiobiose 118, the α-configuration is at the reducing end of the disaccharide, and both have 2a absorptions at 840 cm^{-1} (182). The anomeric configuration of sugars having the mannopyranose and galactopyranose structures can also be characterised by the presence or absence of

type 2a absorptions. These two sugars have a further absorption at 880 ± 8 cm^{-1}, designated type 2c.

From these and other considerations it was anticipated that infrared spectroscopy would provide further evidence concerning the configuration of the disaccharide linkage. Consequently the infrared spectra of the disaccharide and the individual monosaccharide units were recorded, using the potassium bromide pellet and nujol mull techniques. The spectra for the disaccharide, 111, 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate are given in the appendix. The main features of interest in the region 960-730 cm^{-1} were the bands corresponding to type 2a and 2b absorptions, which hopefully would furnish information concerning the configuration at the anomeric carbon atoms.

The absorption bands which occurred in the spectrum of the disaccharide and the corresponding monosaccharide units are given in Table VIII, together with the bands observed by BARKER et al[182] for methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside.

The spectrum of the disaccharide contained a band at 845 cm^{-1} and was assigned as a type 2a absorption, almost certainly due to the α- configuration of the methyl algycon on the amino sugar moiety. The band at 880 cm^{-1} in the disaccharide spectrum, which also appeared in the galactosyl bromide spectrum but not in the methyl glucosaminide spectrum, was assigned as a type 2c absorption, indicative of the galactopyranosyl unit[182]. The region which supplies information concerning the type 2b frequencies unfortunately overlapped with a strong band due to the methyl glucosaminide residue at 901 cm^{-1}, designated as a type 1a band. However, this band was noticeably stronger and broader in the disaccharide spectrum, indicating

TABLE VIII

INFRARED BANDS IN THE REGION 960-730 cm^{-1}

Compound	Frequency (cm^{-1})	Assignment
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside	952 (S) 929 (W)	OCH_3 etc.
	901 (S)	Type 1
	859 (W)	not identified
	845 (M)	Type 2a
	860 (M)	Type 3
Disaccharide, 111	952 (S)	OCH_3 etc.
	947 (M)	C-O-C
	910 (S)	Type 1 or Type 2b
	901 (S)	Type 1a or Type 2b
	880 (M)	Type 2c (galactose)
	845 (M)	Type 2a (glucose)
	772 (M)	Type 3 (galactose)
2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide	945 (S) 912 (VS) 899 (S)	C-O-C vibration Type 1 etc.
	873 (M)	Type 2c
	858 (M)	not identified
	841 (M)	Type 2a
	760 (M) 747 (M)	Type 3
Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside *	952 (VS) 905 (VS)	C-O-C, type 1, etc.
	896 (S)	Type 2b
	879 (W) 868 (S)	Type 2c
	727 (S)	Type 3

* Taken from Barker et al.(182)

two or more overlapping absorptions which could be assigned to the type 2b absorptions of the hydrogen attached to the C-1 carbon of the galactosyl unit. The 2b absorption thus indicates the β-configuration at this position. Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside also has the expected absorption bands at 905 and 896 cm^{-1}, Table VIII, which have been assigned[182] to type 1 and type 2b bands respectively.

The most convincing evidence for the β-D-(1→3) linkage, however, was the absence of a 2a absorption for the galactopyranosyl unit. The 2a type absorption for galactopyranose derivatives occurs in the region 825 ± 11 cm^{-1}. Absence of absorption bands in this region is a good indication for galactopyranose derivatives having a β-configuration[179]. Consequently, the absence of absorption bands in the spectrum of the partially protected disaccharide afforded striking evidence for the proposed configuration.

The evidence accumulated from the described measurements conducted on the synthesised disaccharide give sufficient proof of its structure as that shown, 111, (Fig.96). The high yield, 91%, of this disaccharide indicates that this method could provide an attractive alternative for the preparation of specific di- and trisaccharides related to blood group substances. Thus, the branched trisaccharide, 119, which is thought in part to confer specific Le^a activity[144,145] on the blood group substance, could be prepared quite simply from a disaccharide of this structure. Thus, selective acetylation of C-6 of the amino sugar moiety of 111, using N-acetylimidazole[184], followed by reaction with tri-O-benzyl-α-L-fucopyranosyl bromide would conceivably yield the required trisaccharide 119, (Fig.103).

However, the acidic conditions used for the hydrolysis of the methyl glycoside to regenerate the reducing sugar, would conceivably involve a certain amount of trisaccharide degradation. Use of the benzyl algycon

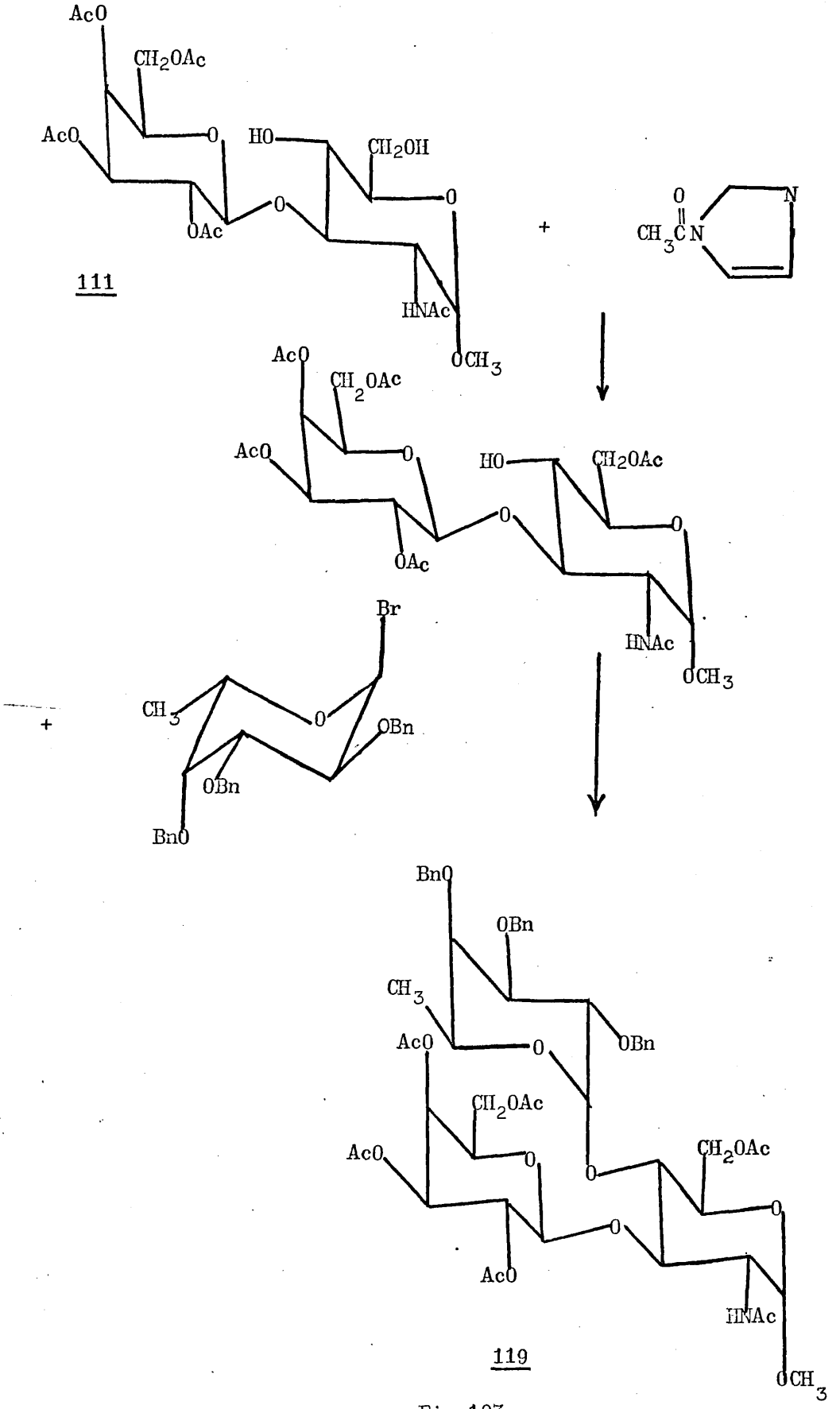

AcO
CH2OAc
AcO
O
HO
CH2OH
O
OAc
O
HNAc
OCH3
111
+
O
N
CH3C
N
AcO
CH2OAc
AcO
O
HO
CH2OAc
O
OAc
O
HNAc
OCH3
Br
CH3
O
OBn
OBn
BnO
+
BnO
OBn
CH3
AcO
O
OBn
CH2OAc
AcO
O
O
CH2OAc
O
O
AcO
HNAc
OCH3
119

Fig.103

would involve catalytic hydrogenation for its removal, a process which would involve relatively little degredation.

Preparation of the benzeneboronate of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside would therefore be the initial step in the synthesis of the di- and trisaccharides. The boronate grouping would intuitively span the 4,6-position, leaving the C-3 hydroxyl group available for reaction with acetobromo galactose. Following a similar procedure employed for the disaccharide having a methyl algycon, the corresponding benzyl glycoside derivative could be synthesised.

III.3 KETOLYSIS OF BENZENEBORONATES

Relative stabilities of five- and six-membered cyclic benzeneboronates have been the subject of many investigations, and a variety of methods have been devised to compare the behaviour of these esters and rationalise the results on the basis of ring strain. One method employed to illustrate the relative stabilities of such rings involved measurement of the rate of uptake of water by the esters[26]. These experiments indicated the greater lability of the 1,3,2-phenyldioxaborolane rings towards hydrolysis than the 1,3,2-phenyldioxaborinane. A corollary from this suggests that compounds containing five- and six-membered boronate rings would selectively hydrolyse the five-membered ring. However, attempts to partially hydrolyse certain boronate esters by addition of water to a solution of the boronate in an organic solvent, leads to complete hydrolysis of the boronate[98]. These experiments indicated that selective hydrolysis of benzeneboronate groupings, in a manner analogous to cyclic acetal derivatives, was not a viable proposition.

Attention was therefore directed towards alternative methods for selectively removing boronate esters based on the relative stabilities of the five- and six-membered rings. Experiments were carried out to determine the viability of selectively removing a five-membered ring in the

presence of six-membered rings, using acidified acetone solution, (Fig.104). The conditions used were essentially those employed for the preparation of isopropylidene ketals. Thus, the boronate was dissolved in dry acetone and acidified with a few drops of concentrated sulphuric acid.

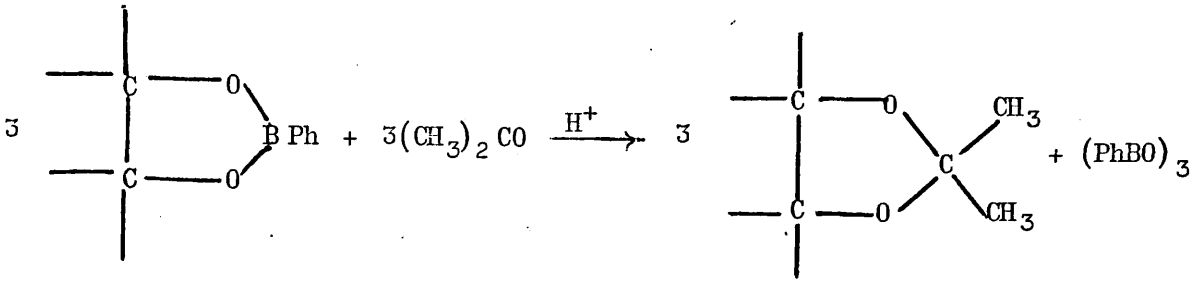


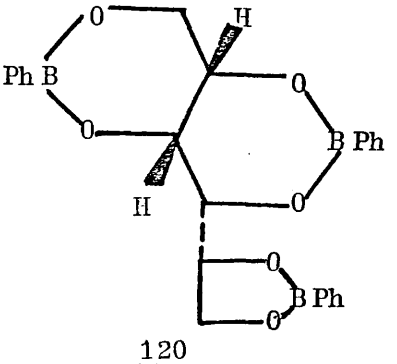
Fig.104

The proposal to selectively remove a 1,3,2-phenyldioxaborolane ring was based on its inherent instability over the 1,3,2-phenyldioxaborinane structure and also on the preferential formation of the 2,2-dimethyl-1,3-dioxolane ring over the 2,2-dimethyl-1,3-dioxane structure. Initial experiments were therefore directed towards the ketolysis of benzeneboronates of alditols of known structure, which contained six- and five-membered rings.

III.3.A Hexitol trisbenzeneboronates

(a) D-Glucitol: novel synthesis of 1,2-O-isopropylidene-L-gulitol.

D-Glucitol forms a trisbenzeneboronate on addition of an aqueous solution of the hexitol to the stoichiometric amount of benzeneboronic anhydride in methanol[17]. The structure of the ester has been proposed as 120, D-glucitol 1,3; 2,4; 5,6-trisbenzeneboronate[58]. A solution of this compound in dry acetone was acidified with concentrated sulphuric acid and allowed to react at room temperature. On neutralising the solution, a paper chromatogram showed the presence of a compound of high mobility, in



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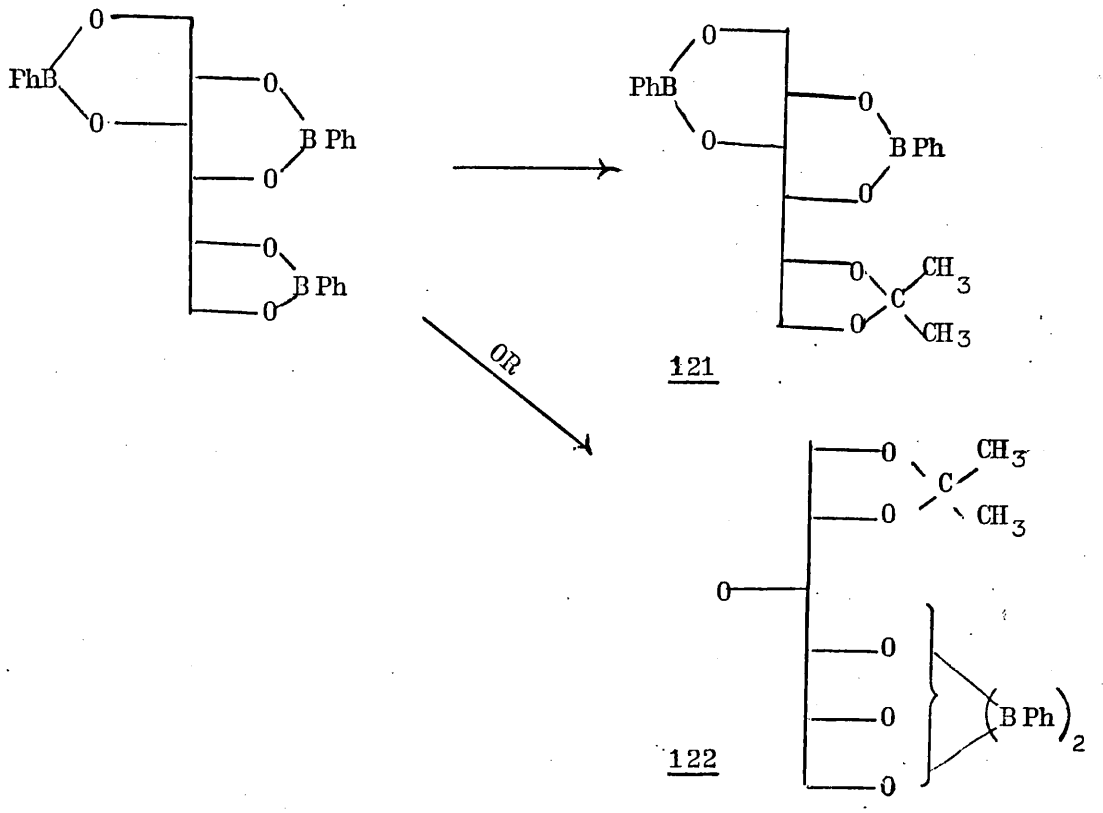
D-glucitol 1,3; 2,4; 5,6-trisbenzeneboronate

addition to unreacted D-glucitol. The homogeneity of this material was confirmed by chromatographing the neutralised reaction mixture on a preparative paper chromatogram, and rechromatographing the separated compound on an electrophoretogram, using borate buffer as the conducting medium. The product was therefore concluded to be a mono- or di-O-isopropylidene derivative of D-glucitol. A triketal was thought unlikely since the fully substituted hexitol would not be detected with silver nitrate - sodium hydroxide on paper, or have any appreciable mobility in borate buffer. A number of experiments were carried out with D-glucitol trisbenzeneboronate to find the optimum time and temperature which gave the best yields. It was found, that with the same acid strength conditions, a solution left at room temperature for 24 hours gave approximately the same quantity of suspected ketal as a solution heated at 60° for 6 hours, as estimated from paper chromatograms.

The next step was to determine the number and size of isopropylidene rings attached to the hexitol, and consequently the complete structure. A neutralised solution of the reaction mixture was evaporated down to a white solid, and its high and low resolution mass spectra were recorded. Superimposed upon the normal mass spectrum for D-glucitol 1,3; 2,4; 5,6-tribenzeneboronate was a series of peaks which provided evidence for the presence of a mono-O-isopropylidene ketal, having a 1,3-dioxolane structure attached at the end of the hexitol chain, with two cyclic benzeneboronate groupings still intact, 121 or 122, (see appendix for spectrum).

The mass spectrum of the reaction mixture contained in ion $\frac{m}{e}$ 394, which had an atomic composition $C_{21}H_{24}B_2O_6$, in agreement with a mono-O-isopropylidene bisbenzeneboronate. However, a more abundant ion appeared at $\frac{m}{e}$ 395, corresponding to the (M+1) ion. Such ions are frequently encountered in the spectra of cyclic acetals and ketals[186]. Indeed,



these ions are useful as an indication of the molecular weight of such compounds[186]. In addition, an abundant ion $\frac{m}{e}$379, $C_{20}H_{21}B_2O_6$, indicated loss of a methyl radical, a typical feature of an isopropylidene ketal[186], (Fig.105). The base peak in the spectrum occurred at $\frac{m}{e}$101, $C_5H_9O_2$, an ion which could only have arisen from the 2,2-dimethyl-1,3-dioxolane structure in a terminal position, (Fig.106).

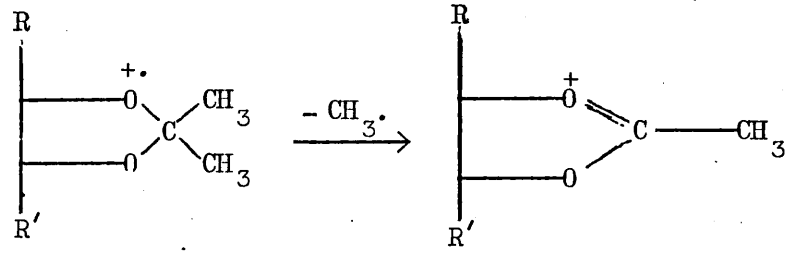


Fig.105

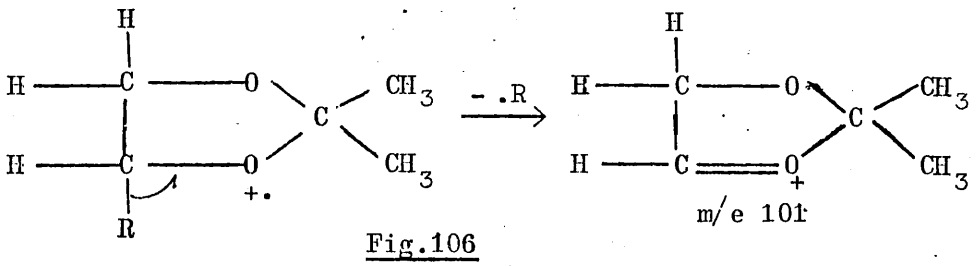


Fig.106

Consequently, mass spectrometry of the reaction mixture provided evidence which strongly supported the presence of a 2,2-dimethyl-1,3-dioxolane ring attached at the end of the hexitol chain. However, the mass spectrum could not distinguish between a 1,2-linked or a 5,6-linked ketal. Intuitively one would expect the latter to have formed, since formation of the former would involve the rupturing of two benzeneboronate rings and the subsequent recyclisation to form a 3,4-boronate ring, to account for the mass spectrum.

Samples of ketolysed D-glucitol trisbenzeneboronate were hydrolysed and chromatographed on preparative paper chromatograms to remove the boronate ester groupings and separate the product from unreacted glucitol and benzeneboronic acid, (Fig.107). The polar hydroxyl groups were then

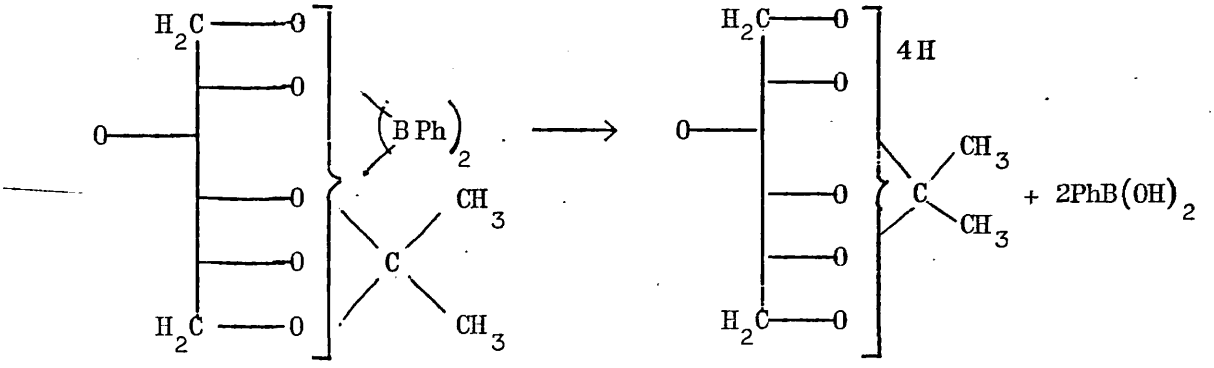


Fig.107

converted into their trimethylsilyl ether derivatives to confer sufficient volatility upon the compound for g.l.c. analysis. The chromatogram showed one main peak together with two smaller ones on either side, representing approximately 6.0% and 2.0% of the main peak. Analysis by g.c.-m.s. afforded a spectrum having the general features of a fully trimethylsilylated alditol, in addition to peaks characteristic of the ketal structure. Alditol trimethylsilyl ethers fragment in a similar fashion to methyl ethers of polyols[110,187], and as such, do not give rise to molecular ions. The primary ions anticipated from a hexitol having a terminal isopropylidene grouping, with trimethylsilyl groups on the remaining oxygens are outlined in Fig.108.

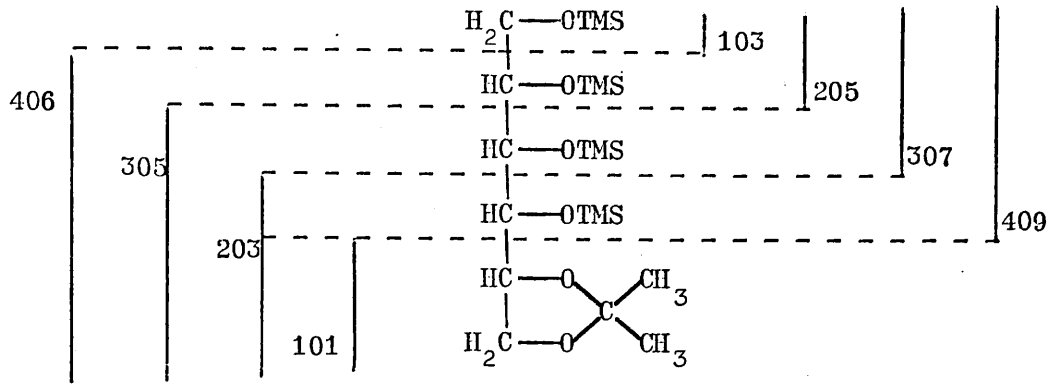
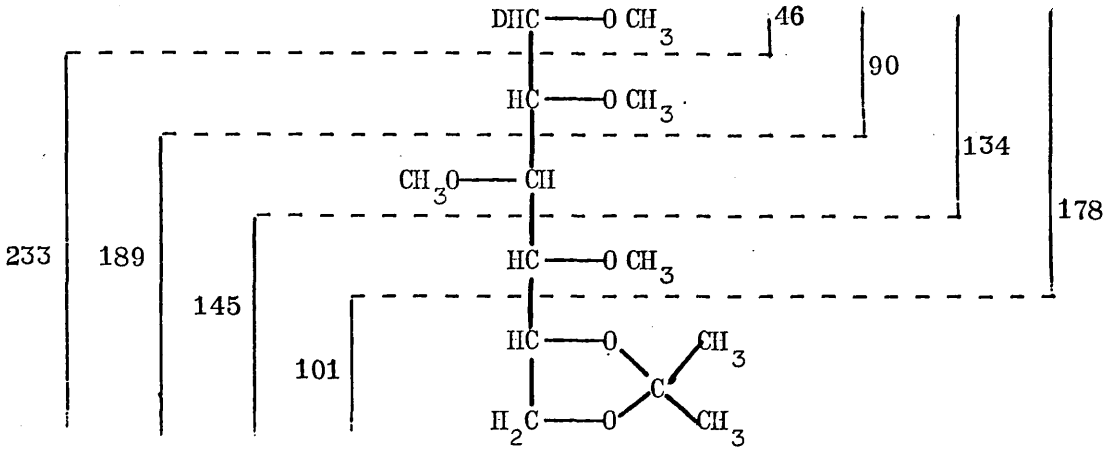


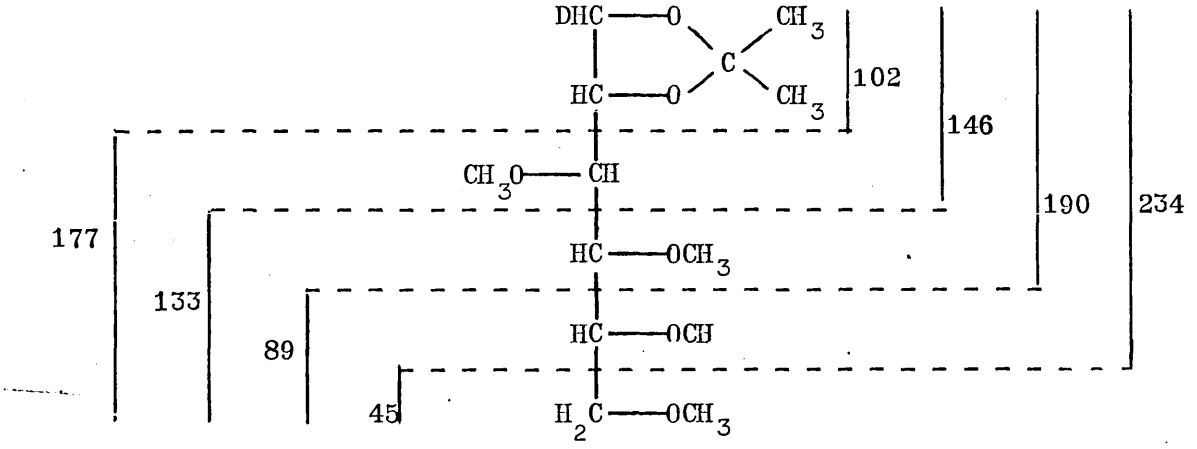
Fig.108

The resolution and sensitivity of the mass spectrometer were insufficient to observe ions of high mass number, which might be expected for alditol trimethylsilylated derivatives. However, sufficient ions were observed which indicated an isopropylidene ketal ring on the end of the hexitol chain. The most convincing evidence being the second most abundant ion in the spectrum occurring at $\frac{m}{e}$ 101. The base peak, as anticipated, occurred at $\frac{m}{e}$ 73, ascribed to the trimethylsilyl ion $(CH_3)_3Si^+$ (188).

As stated previously, the five-membered dioxolane ring should form across the C-5 and C-6 oxygens, since ketolysis involving the C-1 and C-2 oxygens would have disrupted two boronate rings, one of which would be removed completely and the other would recyclise to account for the mass spectrum of the reaction mixture. This unlikely process was excluded by performing the ketolysis reaction on 1-d_1-D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate. The hydrolysed product was methylated in dimethylformamide with silver oxide and methyl iodide at room temperature. The product was examined as a chloroform solution on g.l.c. and combined g.c.-m.s.. A non-deuterated sample was also prepared for comparison. The primary ions expected for 5,6-O-isopropylidene-1,2,3,4-tetra-O-methyl-1-d_1-D-glucitol 123, and 1,2-O-isopropylidene-3,4,5,6-tetra-O-methyl-1-d_1-D-glucitol, 124. are shown, (Fig.109).

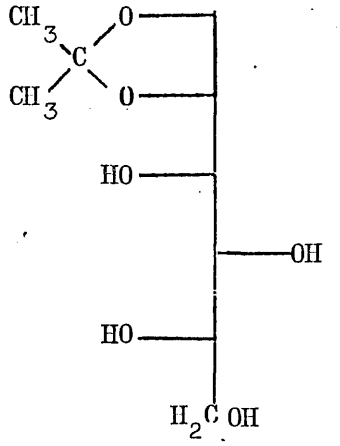


123



124

Fig.109



125

1,2-O-isopropylidene-L-gulitol
(5,6-O-isopropylidene-D-glucitol)

The spectrum exhibited all the primary ions shown for 123, the ion, $\frac{m}{e}$ 101, forming the base peak. Table IX gives the relative abundances of the primary ions in the deuterated and non-deuterated methylated glucitol derivatives. The m/e values of the primary ions which would appear if the ketal grouping was attached across the C-1 and C-2 oxygens are also included

for completeness. The figures clearly show that the isopropylidene grouping is attached to the unlabelled end of the molecule. The ketal therefore has the structure, 125.

The yield of the ketal was estimated by g.l.c.. A small quantity of trisbenzeneboronate was ketolysed, and the neutralised solution lined out on a preparative paper chromatogram. After development, the band corresponding to the fast-moving benzeneboronic acid was removed, the remaining compounds being eluted off together. The mixture was acetylated and its chloroform solution injected onto a g.l.c. column, where the acetylated ketal separated as a single component from the unreacted hexitol acetate. Peak areas were measured and from this the yield of ketal could be estimated, and found to be 43%.

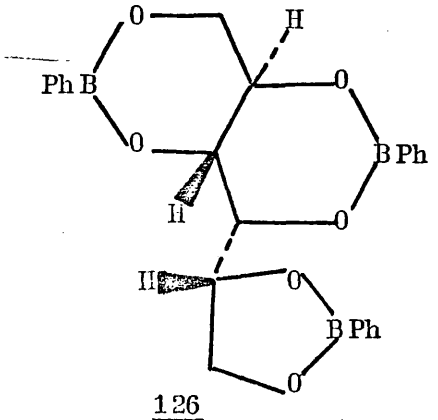
TABLE IX

PRIMARY IONS IN THE MASS SPECTRA OF LABELLED AND UNLABELLED D-GLUCITOL KETAL

Primary ion (m/e)	Percentage of the total ion current $\% \Sigma_{40}$	
	Deuterated	Non-Deuterated
234	0	0
233	0.3	0.3
190	0	0
189	0.8	0.8
178	2.8	0
177	0.0	4.3
146	0	0
145	1.5	6.4
134	3.6	0
133	0	3.4
102	0	0
101	20.0	26.6
90	7.2	0
89	1.1	7.9
46	4.4	0
45	4.6	6.9

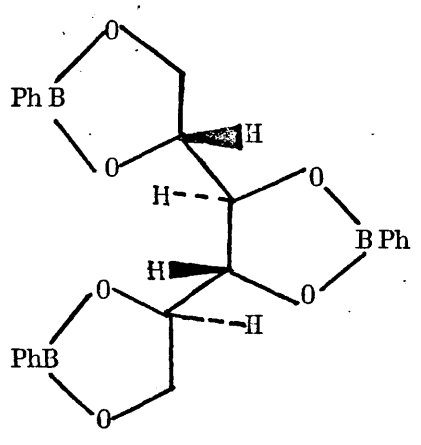
D-Mannitol: novel synthesis of 1,2-O-isopropylidene-D-mannitol.

The trisbenzeneboronate of mannitol has been known for many years[17], but the disposition of the boronate rings is not known with certainty. Two fused six-membered rings with one five-membered ring, 126, may be anticipated, in a similar manner to D-glucitol. However, this configuration would involve unfavourable steric interaction between the axially disposed five-membered ring and the axial hydrogen on C-2. The possibility of the three five-membered ring structure, 127, was indicated from its mass spectrum, on the basis of absence of evidence for fused six-membered rings[58]. However, definitive proof for the structure of D-mannitol trisbenzeneboronate is lacking. It was therefore of considerable interest to discover the effect of acidified acetone on this compound with regard to structure elucidation and selective ketolysis.



126

1,3; 2,4; 5,6-trizbenzeneboronate



127

1,2; 3,4; 5,6-trisbenzeneboronate

D-Mannitol trisbenzeneboronate was subjected to the same conditions as outlined for D-glucitol trisbenzeneboronate. Paper chromatography revealed a single component, besides unreacted hexitol, which appeared to have formed in good yield. Methylation of a sample of this material followed by combined g.c.-m.s. analysis showed that a 1,3-dioxolane ring had formed at the terminal position of the mannitol chain.

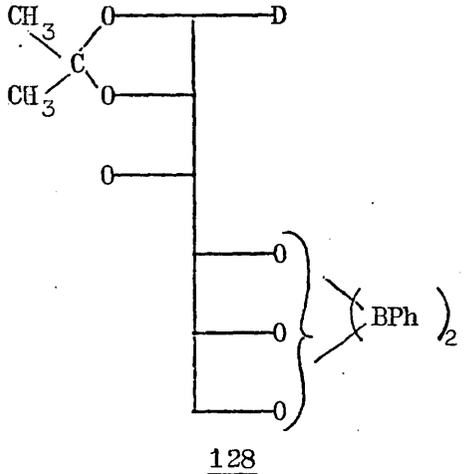
Formation of an isopropylidene ketal on either end of the mannitol chain does not afford two different products, since 1,2- and 5,6-O-isopropylidene-D-mannitol are the same compound. However, deuterium labelling at C-1 can be used to determine which end of the mannitol trisbenzeneboronate is taking part in the ketolysis. If the structure of the trisboronate consists of two trans-fused six-membered rings and one five-membered ring, then a similar result to the D-glucitol case would be anticipated. A triple five-membered ring structure would intuitively involve ketolysis at both ends of the hexitol chain. In addition, a product containing two isopropylidene groups would be expected, i.e. 1,2; 5,6-di-O-isopropylidene-D-mannitol. The absence of this latter compound initially suggested the fused six-membered ring structure, 126.

TABLE X

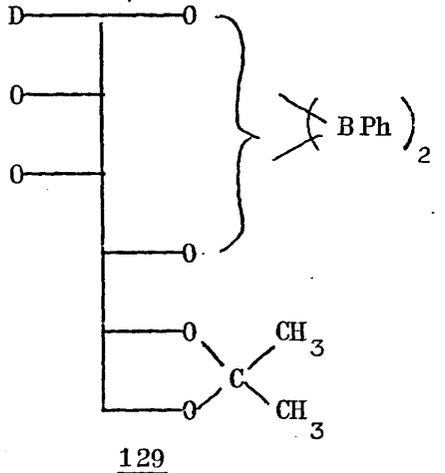
PRIMARY IONS IN THE MASS SPECTRA OF LABELLED AND UNLABELLED MANNITOL KETAL

Primary ion (m/e)	Percentage of total ion current $\% \Sigma_{40}$	
	Deuterated	Non-deuterated
234	0.5	0
233	0.5	0.8
190	0.5	0
189	0.5	0.8
178	1.7	0
177	1.7	4.6
146	3.4	0
145	4.5	10.2
134	2.3	0
133	2.8	4.6
102	8.5	2.1
101	13.5	20.0
90	3.4	0
89	3.9	6.2
46	3.9	0
45	9.0	3.8

The trisbenzeneboronate of 1-d_1-D-mannitol was prepared and ketolysed and the product analysed as described above for the non-deuterated material. The primary ions expected for a terminal isopropylidene group attached to mannitol are the same as those for D-glucitol described above, (Fig.109). The ions observed for the deuterated and non-deuterated samples are given in Table X. The figures demonstrate that 1,2-O-isopropylidene-1-d_1-D-mannitol, 128, and 5,6-O-isopropylidene-1-d_1-D-mannitol, 129, are formed on ketolysis of the trisbenzeneboronate. These results together with the mass spectrometric results for D-mannitol trisbenzeneboronate[58], which did not produce any evidence for the presence of a two-fused six-membered ring structure, lend strong support for the trisboronate structure, 127. Estimation of the yield of 1,2-O-isopropylidene-D-mannitol, using the method outlined for the estimation of 5,6-isopylidene-D-glucitol, gave a value of 78%, suggesting that this method could be a useful alternative for the preparation of this compound.



128



129

Previous preparations of 1,2-O-isopropylidene-D-mannitol utilised the fortuitous formation of a 1,2-O-isopropylidene-4,5-borate complex of mannitol on reacting mannitol with boric acid and acetone, acidified with concentrated sulphuric acid[189], (Fig.110). Removal of the borate complex by methanolysis afforded the ketal in a 15% overall yield.

This compound has also been synthesised from the acetonation of D-mannose diethyldithioacetal[190]. Demercaptalation of the dithioacetal

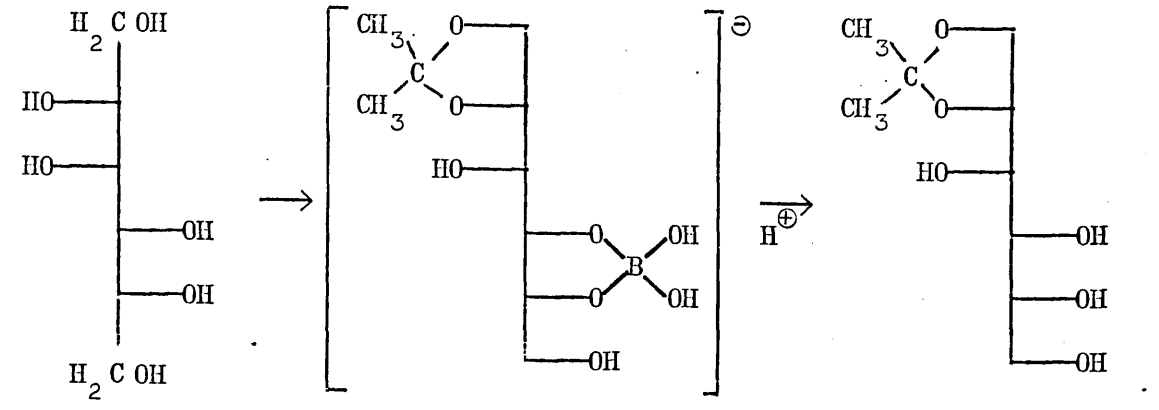


Fig.110

produced the isopropylidene derivative of D-mannofuranose, which could then be reduced to provide 1,2-O-isopropylidene-D-mannitol, (Fig.111).

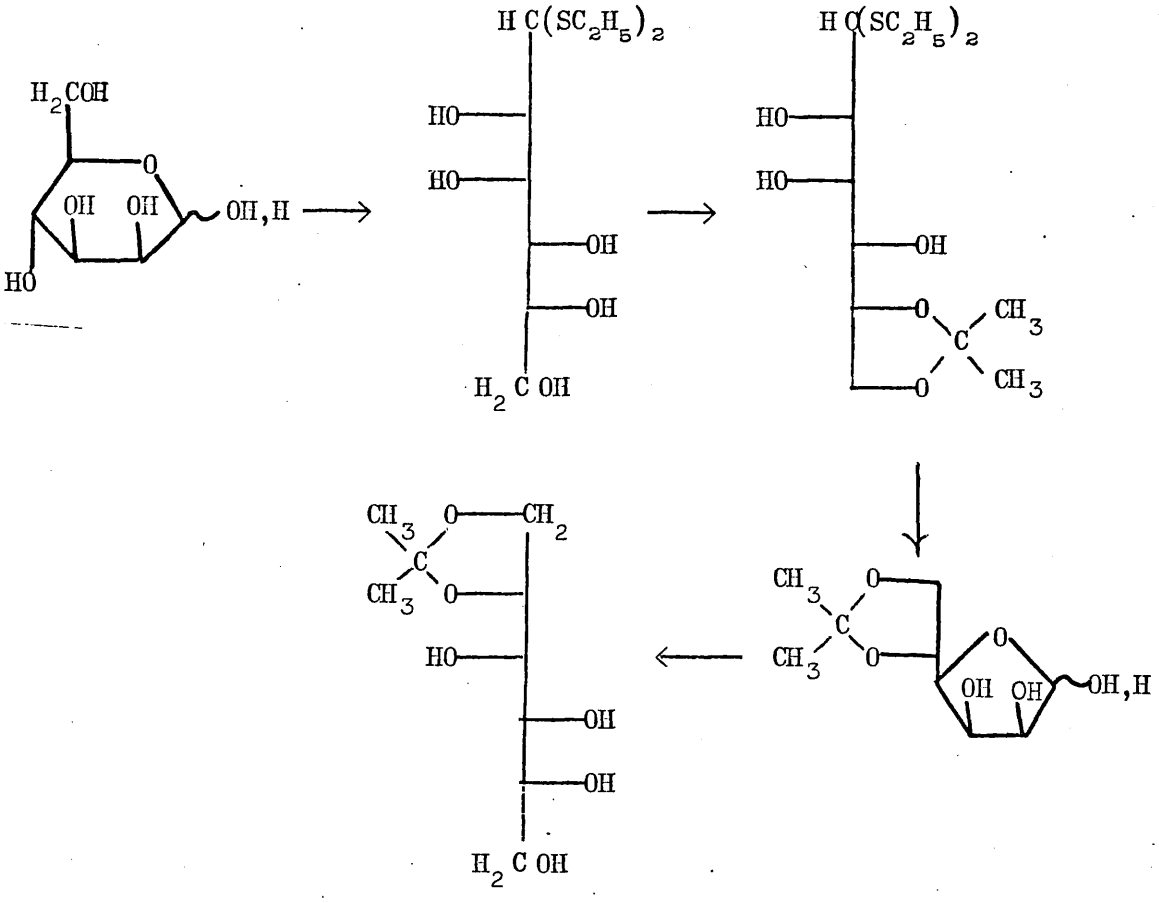


Fig.111

A simple alternative for the preparation of this compound was devised which involved the ketolysis of D-mannitol trisbenzeneboronate. Removal of the remaining boronate ester groupings was then expected to yield the desired compound, (Fig.112). Hydrolysis of benzeneboronate groupings

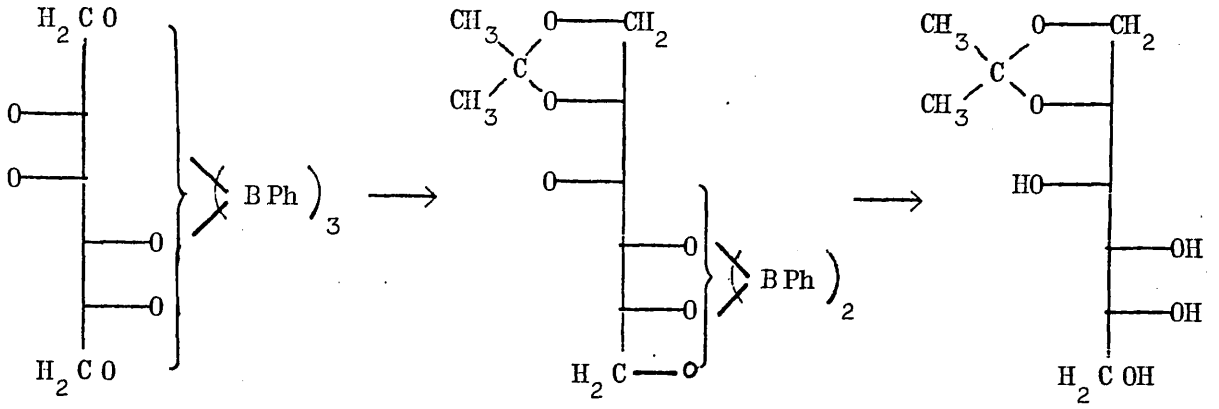


Fig.112

is accomplished easily during chromatography on cellulose paper, eluting with a neutral solvent such as butanol, ethanol and water mixture. The removal of the residual boronate entities from the mono-isopropylidene product was consequently expected to proceed easily by passing the reaction mixture down a cellulose column, eluting with the same solvent. However, this procedure did not secure hydrolysis and separation of benzeneboronic acid, and therefore an alternative procedure was sought.

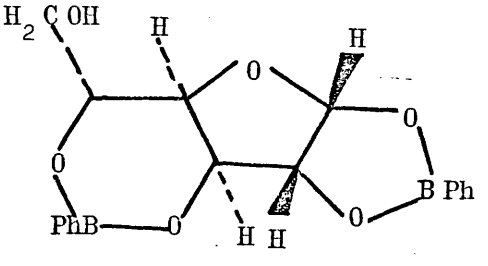
Previous workers have applied a transesterification reaction for the removal of benzeneboronate esters[64] based on the ready formation of propane 1,3-diol benzeneboronate. The method is particularly useful, since the reaction proceeds at room temperature under neutral conditions. Furthermore the propane diol 1,3-benzeneboronate is a liquid which can be distilled from the reaction mixture under low vacuum. This latter method proved successful, and the majority of the benzeneboronic acid was easily removed. The remaining traces of acid and unreacted mannitol were separated from the ketal derivative by passing the residue through a strongly basic resin column. Although the overall yield was 31%, it is believed that this could have been considerably improved with a more efficient use of the resin column. In conclusion, this method has been shown to be an extremely simple one for the preparation 1,2-O-isopropylidene-D-mannitol and it may prove a good general procedure for the preparation of other similar compounds.

A second and equally important feature of this ketolysis reaction concerns the information obtained pertaining to the structure of the original benzeneboronate ester. These experiments suggested that the conditions for ketolysis may provide a method for the selective removal of five-membered ring boronates. If true, then this would provide an extremely valuable method for structure elucidation of boronates which are comprised of both five- and six-membered rings. In an attempt to illustrate the generality of this hypothesis, an investigation of the ketolysis products of two hexose bisbenzeneboronates was carried out.

III.3.B Hexose Benzeneboronates

(a) D-Glucose

The structure of the boronate formed when D-glucose reacts with benzeneboronic anhydride has been shown to be 130, α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate[21]. The presence of a five- and six-membered boronate ring therefore provides another opportunity to test the selectivity of the ketolysis reaction. The primary objective therefore, was not to devise a simple method for the synthesis of important intermediate compounds, but rather to investigate the general applicability of the ketolysis reaction. Concomitant formation of synthetically useful intermediates would, however, be an important additional advantage.



130

When α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate was left overnight at room temperature in acidified acetone, very little reaction occurred. Refluxing for 12 hours produced a compound, which, by paper chromatography, appeared to be a mono-O-isopropylidene derivative. Hydrolysis

of the boronate and removal of the benzeneboronic acid, followed by acetylation with pyridine and acetic anhydride, afforded a product which could be analysed by combined g.c.-m.s.

The anticipated product from the above procedure, 1,2-O-isopropylidene 3,5,6-tri-O-acetyl-α-D-glucofuranose, was anticipated to give rise to an ion $\frac{m}{e}$ 331 on electron impact mass spectrometry, through loss of a methyl radical from the 2,2-dimethyl-1,3-dioxolane ring[186], (Fig.113). This ion was observed to constitute almost 7% of the total ion current, thus providing evidence for this structure. Continued fragmentation of this ion is expected to afford the ions $\frac{m}{e}$ 271, 229 and 169, through loss of acetic acid and ketene. The ion $\frac{m}{e}$ 169 constituted 8% of the total ion current and was the second most abundant ion in the spectrum, the base peak being the acetylium ion, $\frac{m}{e}$ 43.

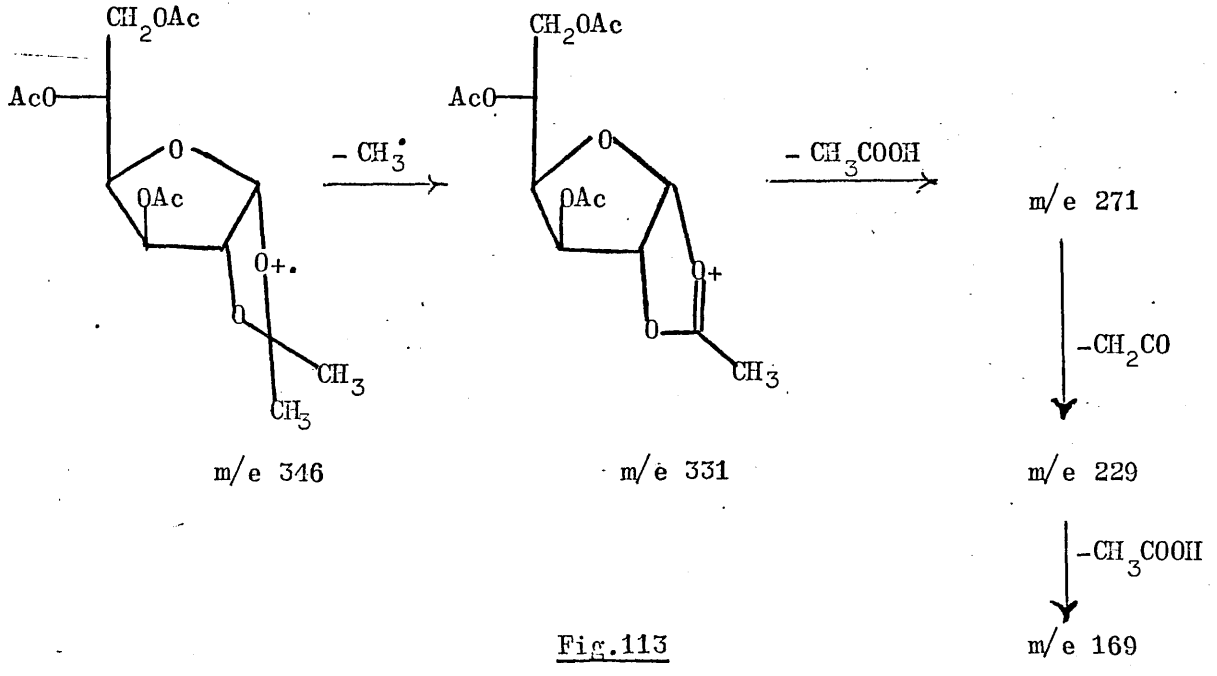


Fig.113

Support for the isopropylidene grouping spanning the C-1 and C-2 oxygens came from the presence of an abundant ion $\frac{m}{e}$ 201, which conceivably arose from fission of the C-4 - C-5 bond, (Fig.114), a process characteristic of aldofuranose isopropylidene derivatives[186]. This ion was believed

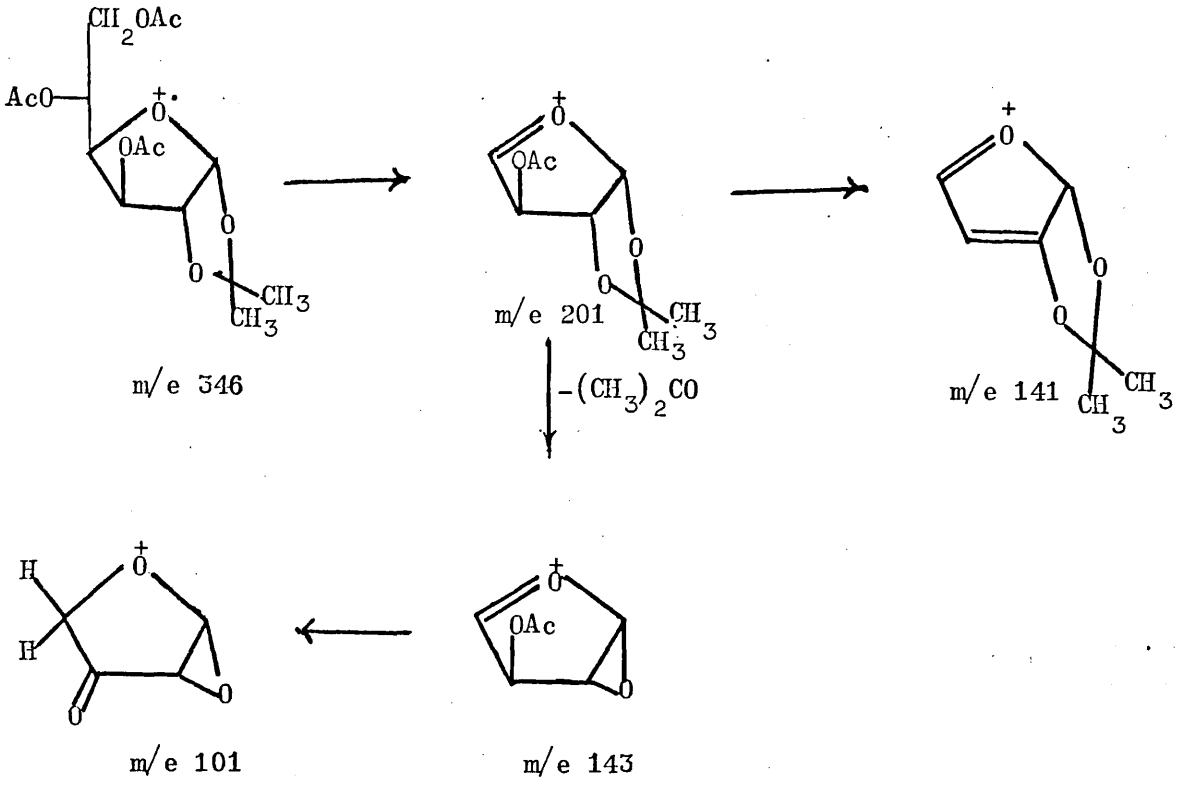


Fig.114

to be responsible for the ions at $\frac{m}{e}$ 141 and 143, as a result of acetic acid and acetone elimination, (Fig.114). The 5,6-O-isopropylidene and 3,5-O-isopropylidene structures were not supported by the mass spectrum.

Additional evidence for the assignment of the 1,2-O-isopropylidene arrangement for the product of ketolysis reaction was obtained by attempted reduction of the product with sodium borohydride. Involvement of the C-1 oxygen would render the product inactive to borohydride reduction. Alternatively, if the ketal involved oxygens other than the C-1 oxygen, reduction to the alditol would occur. The product obtained after borohydride treatment was acetylated. Analysis by g.c.-m.s. afforded a single product which provided a mass spectrum identical to the untreated ketolysis product. The involvement of the C-1 oxygen was therefore verified.

It is proposed, therefore, that ketolysis of α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate involves the replacement of the 1,2-boronate ring with a 1,2-O-isopropylidene ketal as the major reaction. A small

shoulder adjacent to the main peak on the gas-liquid chromatogram indicated a second product. However, because of its very low yield and difficulty experienced in resolving it from the main product, it was not identified.

TABLE XI

ANALYSIS OF KETOLYSIS PRODUCTS

Benzeneboronate	G.c.-m.s. of ketolysis product after partial hydrolysis and acetylation			
	T (a)	Abundance of (M-15) ion (b)	Yield (%)	Identity (c)
D-glucitol 1,3; 2,4; 5,6	2.67	2.5	43	5,6
D-mannitol 1,2; 3,4,5,6	2.15	3.8	78	1,2
α-D-glucofuranose 1,2; 3,5	1.90	6.6	63	1,2
D-mannopyranose 2,3; 4,6	2.16	10.0	-	2,3

(a) Retention time relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol

(b) Expressed as $\% \Sigma_{40}$

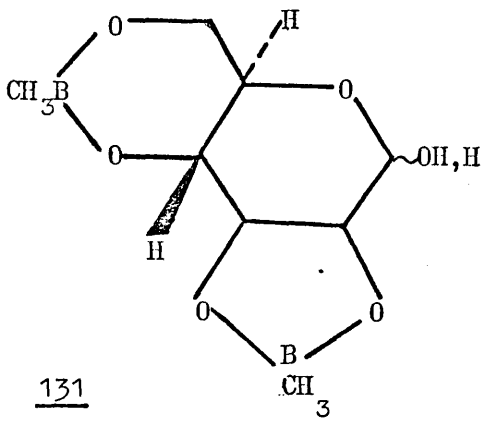
(c) Refers to position of C atoms involved in cyclic ketal.

The yield of 1,2-O-isopropylidene α-D-glucofuranose from the ketolysis of the bisbenzeneboronate was estimated by g.l.c., as outlined for 5,6-O-isopropylidene-D-glucitol, (p.137). The results from g.c.-m.s. analysis of the ketolysis reactions for the four boronates investigated are summarised in Table XI.

III.3.B

(b) D-Mannose

The structural elucidation of D-mannose bisbenzeneboronate has not been reported in theliterature, but a crystalline derivative is known to form when D-mannose is heated in 2-methoxyethanol with benzeneboronic anhydride[191]. Methane-[50] and butaneboronic[47,48] acids have been mixed with D-mannose, followed by trimethylsilylation for analysis on g.l.c. Results showed[49] that small amounts of other products in addition to the main bisboronate may be present. The methaneboronate derivative was



131

concluded[50], on the basis of g.c.-m.s. analysis of the trimethylsilylated product, to have the structure 131, D-mannose 2,3 ; 4,6-bismethaneboronate. D-Mannose bisbenzeneboronate therefore provided an opportunity for the application of the ketolysis reaction to structure determination and a further demonstration of selective ketolysis of five-membered boronate rings.

Refluxing D-mannose bisbenzeneboronate in acidified acetone overnight produced a material having a paper chromatographic mobility approximately three times that of D-glucitol. This material was eluted from the paper and acetylated for g.c.-m.s. analysis. The g.l.c. trace showed the main component was mixed with at least two minor components and also a significant amount of a compound with a short retention time, believed to be the di-O-isopropylidene ketal.

The mass spectrum of the major component of the mono-O-isopropylidene derivative contained several features similar to the 1,2-O-isopropylidene-α-D-glucofuranose acetate. However, several prominent peaks indicated that the ketal grouping was not spanning the C-1 and C-2 oxygens. The highest observable ion in the spectrum, which was in fact the second most abundant, appeared at $\frac{m}{e}$ 331, caused by the loss of a methyl radical from the molecular ion, affording evidence for a mono-O-isopropylidene group, together with three acetate groups.

A strong peak at $\frac{m}{e}$ 287, absent in the glucose derivative, indicated the presence of an acetate group on C-1. Loss of an acetoxy radical from C-1 is a common feature of peracetylated sugars, and gives rise to a series of ions characteristic of these derivatives[162]. This resonance - stabilised carbonium ion continues to breakdown by loss of acetone, 58 m.u. and

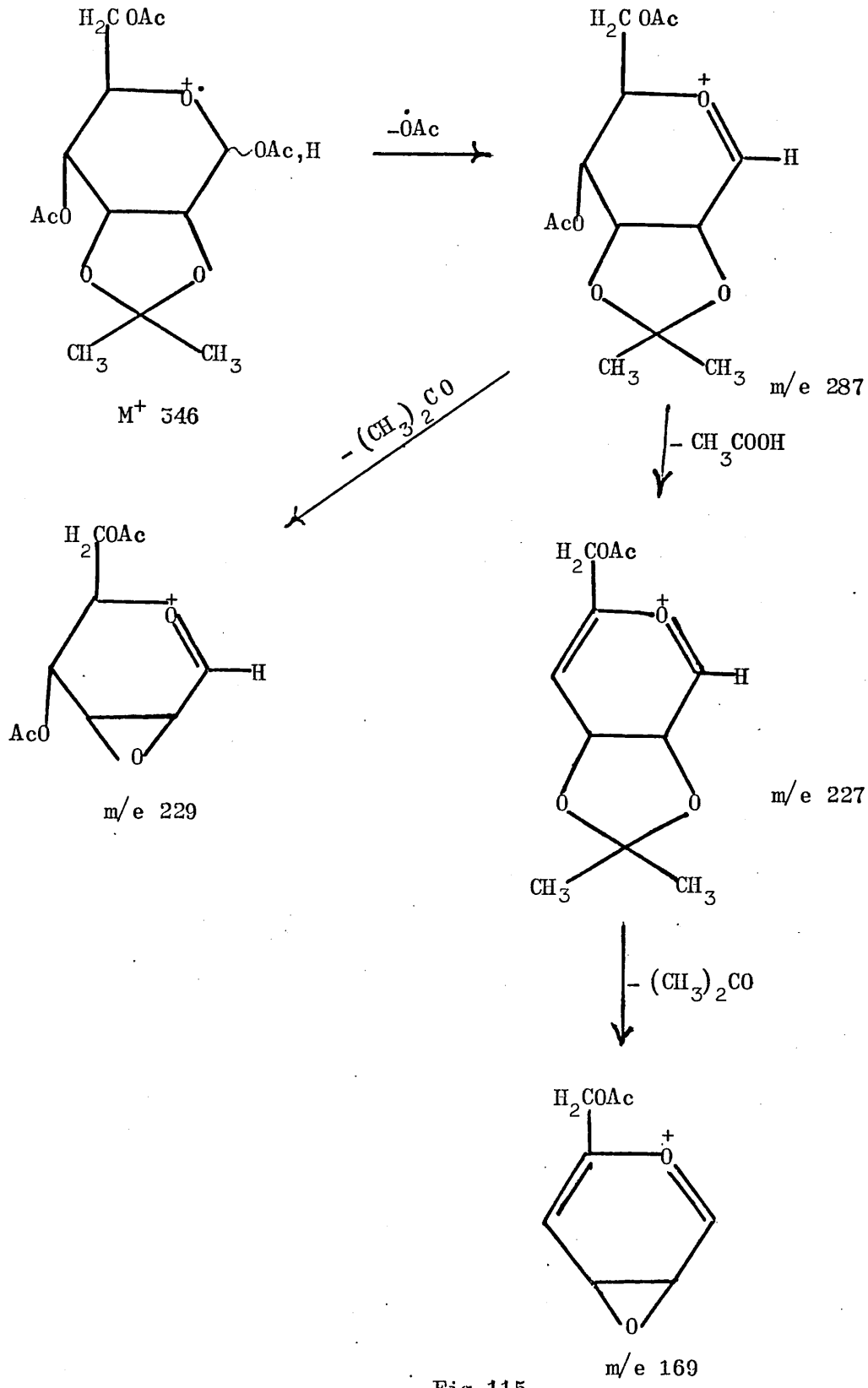

H2C OAc
OAc,H
AcO
CH3
CH3
M+ 346
-OAc
H
m/e 287
- (CH3)2CO
- CH3COOH
H2COAc
m/e 229
m/e 227
- (CH3)2CO
m/e 169

Fig.115

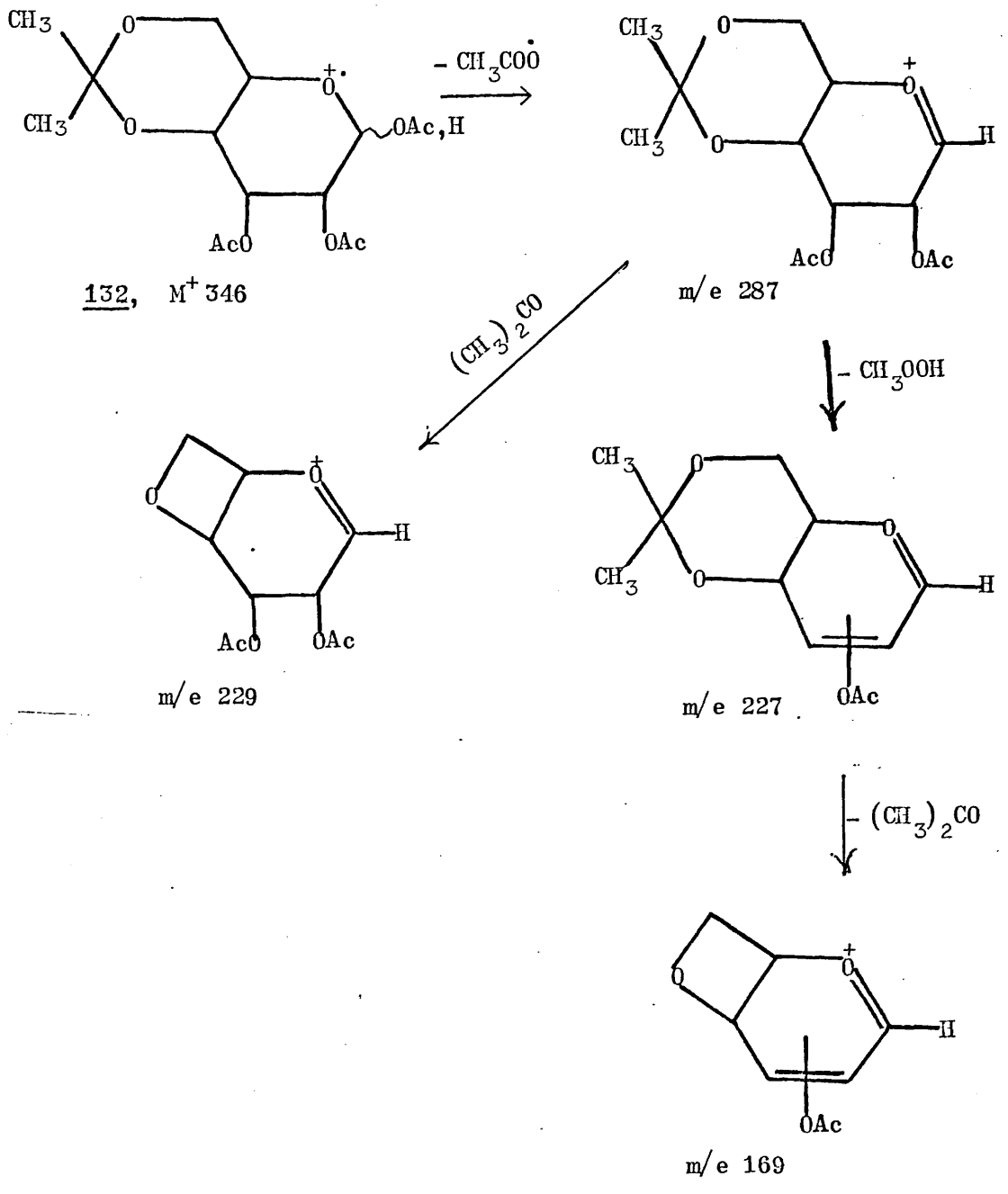
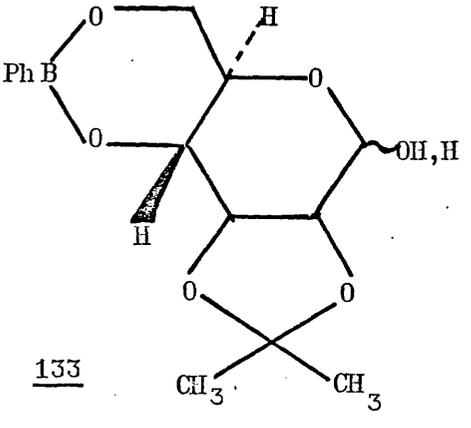

CH3
O
CH3
O
+·
O
OAc,H
AcO
OAc
- CH3COO·
132, M+ 346
CH3
O
CH3
O
+
O
H
AcO
OAc
m/e 287
(CH3)2CO
- CH3OOH
O
+
O
H
AcO
OAc
m/e 229
CH3
O
CH3
O
O
H
OAc
m/e 227
- (CH3)2CO
O
+
O
H
OAc
m/e 169

Fig.116

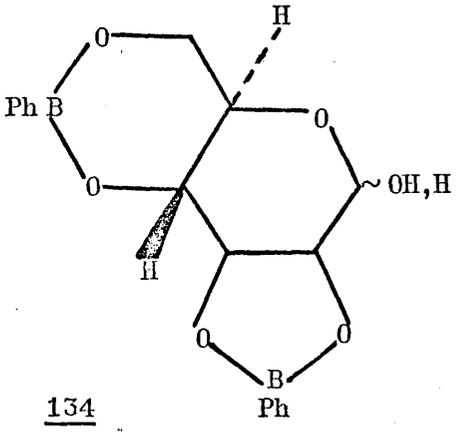
acetic acid, 60 m.u., giving rise to the ions $\frac{m}{e}$ 229 and $\frac{m}{e}$ 169, (Fig.115). Alternative loss of acetic acid followed by loss of acetone provides the ion $\frac{m}{e}$ 227.

Although the mass spectrum of the acetylated product supplied convincing evidence for a 2,3-O-isopropylidene derivative, the 4,6-O-isopropylidene, 132, could conceivably produce a similar spectrum via. the fragmentation shown, (Fig.116). Reaction of D-mannose with acetone in the presence of 1% sulphuric acid or 1% hydrogen chloride, produces 2,3 ; 5,6-di-O-isopropylidene α-D-mannofuranose[192]. The preferential formation of the 2,2-dimethyl-1,3- dioxolane over the 2,2-dimethyl-1,3-dioxane ring lends support for the proposed ketolysis to occur preferentially at the 2,3- position. However, the mass spectrum of the partially hydrolysed and acetylated product from the ketolysis of D-mannose bisbenzeneboronate does not exclude the formation of the 4,6-O-isopropylidene derivative.

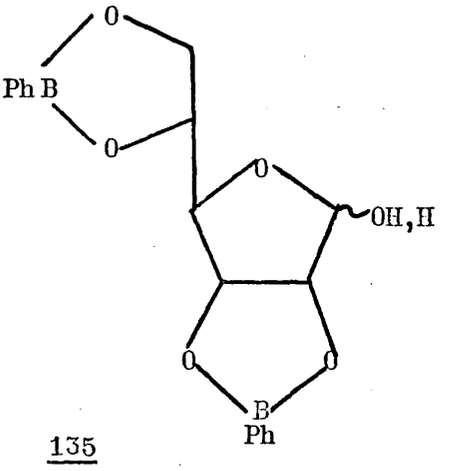
The present evidence indicates that the ketolysis of D-mannose bisbenzeneboronate involves the removal of a boronate ring spanning the C-2-C-3 positions, with the simultaneous formation of the 2,3-O-isopropylidene derivative, 133. In addition the



133

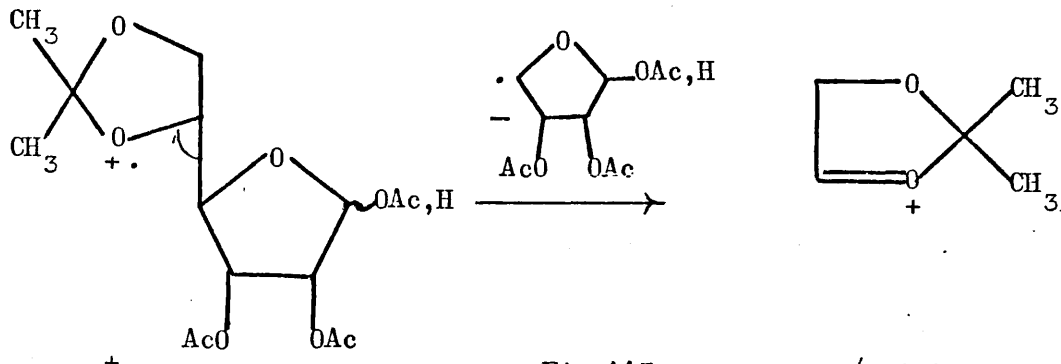


134



135

results indicate that the structure of the bisbenzeneboronate of D-mannose is 134, 2,3; 4,6-bisbenzeneboronate. Had the bisboronate consisted of two five-membered rings having the structure 135, some of the 5,6-O-isopropylidene derivate would be expected in the product, giving rise to an abundant ion, $\frac{m}{e}$ 101, in the mass spectrum, (Fig.117). However, no evidence for this derivative was observed on g.c.-m.s.. The yield for the D-mannose derivative could not be calculated with accuracy because of the presence of other minor products which could not be completely separated from the major product.



M^+ 346 Fig.117 m/e 101

CONCLUSION

The use of cyclic benzeneboronates in the field of synthetic carbohydrate chemistry has been demonstrated. The ability of boronate esters to remain intact in conditions used for disaccharide synthesis shows particular promise for its use as a protective grouping in this important area. The near quantitative yields of benzeneboronates coupled with their ability to hydrolyse completely under extremely mild conditions, clearly emphasizes the suitability of these esters to act as intermediates in processes which usually proceed in low yield.

The ketolysis of benzeneboronates has emphasized several important features concerning cyclic boronate esters. It appears that the ketolysis of polybenzeneboronates of systems which contain five- and six-membered boronate rings proceeds with selective cleavage of the five-membered ring.

This tendency for the five-membered ring to undergo ketolysis in preference to the six-membered ring is in keeping with the relative stabilities and ease of hydrolysis of these structures.

The conditions used for the ketolysis reactions indicate that water is not essential for the production of the ketal, in more than catalytic amounts. However, it is realised that on the experimental scale used in the present investigations there is sufficient water in the concentrated sulphuric acid to hydrolyse the boronate ester to the polyol and benzeneboronic acid. Nevertheless, a reaction scheme can be envisaged which requires only a catalytic amount of water, (Fig.118).

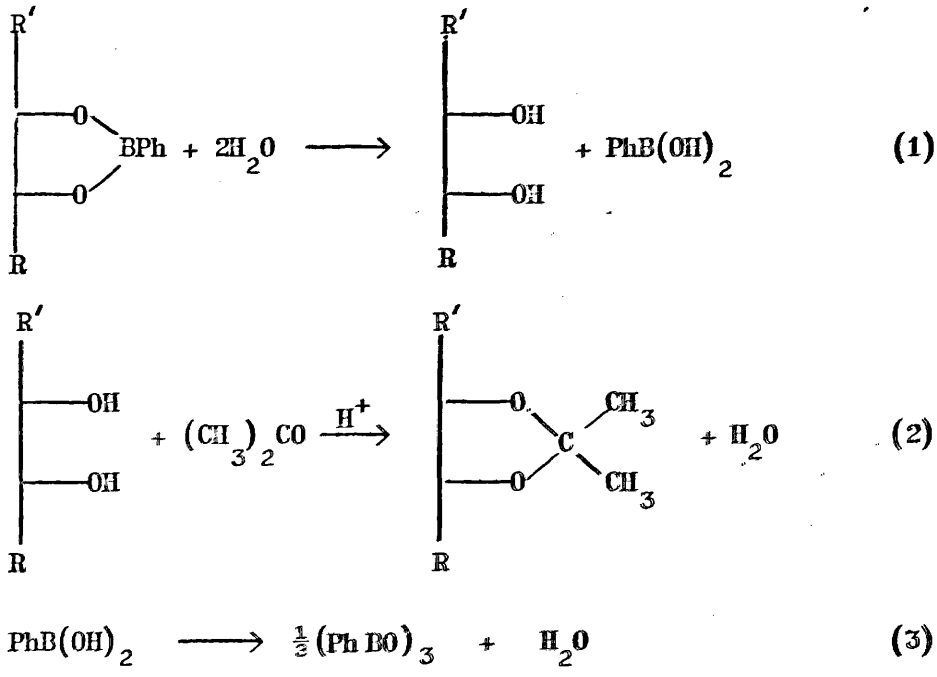


Fig.118

The initial hydrolysis of the boronate ester produces the free polyol and benzeneboronic acid, (1), requiring two moles of water for one mole of ester. The acid catalysed formation of the ketal then produces one mole of water, (2). Under the almost anhydrous conditions, the benzeneboronic acid, liberated in step (1), will form benzeneboronic anhydride, (3), with the liberation of one mole of water per mole of acid.

A further aspect of the ketolysis reaction is a direct corollory of the first phenomena. Thus, if the structure of the polybenzeneboronate is unknown, but is suspected of having rings of different sizes, i.e. five- and six-membered rings, then selective cleavage of the five-membered ring will provide a means of gaining information concerning its structure. This latter assertion, which inherently depends upon the first, has been shown to apply to four polyboronates. The fact that D-mannose bisbenzeneboronate produced additional minor products indicates that further research is necessary before ketolysis can be applied with confidence for structure elucidation of benzeneboronates.

In addition, the ketolysis of benzeneboronates provides an alternative route to the preparation of synthetically important compounds, in particular the isopropylidene ketals. These compounds are normally prepared directly from the polyol, affording the fully protected ketal derivative. Graded acid hydrolysis then provides the required partially protected polyol. Mannitol, for example, forms 1,2; 3,4; 5,6-tri-O-isopropylidene mannitol on reacting with acetone and concetrated sulphuric acid[193]. The 1,2; 5,6-di-O-isopropylidene minnitol is produced when hydrochloric acid is employed as the catalyst[194]. The latter compound is also produced in addition to the triketal when zinc chloride is used[195,196]. Graded acid hydrolysis of the triketal produces the 1,2; 3,4-di-O-isopropylidene and 3,4-O-isopropylidene-D-mannitol[193]. The 1,2-O-isopropylidene derivative is not usually prepared by graded acid hydrolysis, but by the method of VARGHA[189], with low overall yield. Ketolysis of D-mannitol trisbenzeneboronate therefore provides a simple alternative for the preparation of 1,2-O-isopropylidene-D-mannitol in reasonable overall yield.

GENERAL TECHNIQUES

Mass Spectrometry Combined with Gas-Liquid Chromatography

A Perkin Elmer F-11 chromatograph, incorporating a flame ionization detector, was coupled, via. a Watson-Biemann separator, to a Hitachi RMS-4 mass spectrometer. Helium was used as the carrier gas at a flow rate of approximately 14 $cm^3\ min^{-1}$. The glass columns (12' × 0.25" and 6' × 0.25") were packed with the following stationary phases:

Type I: OV-225 - 3% of cyanopropylmethyl-phenylmethylsilicone coated on 100-120 Gas Chrome Q .

Type II: A.P.K. - 7.5% Apiezon-K coated on Chromasorb W, pre-treated with dimethyldichlorosilane.

Low and high resolution mass spectra data of the benzeneboronates were obtained by the University of London Intercollegiate Research Service using an A.E.I. MS-902 mass spectrometer. A direct insertion method was used with an ionization potential of 70 eV and a trap current of 100 μA.

Gas-Liquid Chromatography

A Pye 104 dual column chromatograph equipped with flame ionization detectors and glass columns, (9' × 0.25"), was used for the majority of analyses. Nitrogen was employed as the carrier gas at a flow rate of 40 $cm^3\ min^{-1}$. Stationary phases were of type I and II. Retention times and peak areas were measured by a Hewlett Packard 3370B/71B integrator, connected in series between the chromatograph amplifier and recorder.

Infrared Spectroscopy

Infrared spectra were obtained using a Perkin Elmer 257 grating spectrophotometer. Compounds were examined as their potassium bromide discs of approximately 1% W/W composition.

Ultra-Violet Spectroscopy

Optical densities were measured with a Pye Unicam S.P. 1800 spectrophotometer. Boron estimations were carried out in 1:1, ethanol/water, solutions, using U.V. cells with the ethanol/water mixture as a reference.

Nuclear Magnetic Resonance Spectroscopy

Proton magnetic resonance spectra were obtained at 60 MHz on a Varian E.M. 360 instrument. Chemical shifts were measured relative to the internal reference signal of tetramethylsilane, (T.M.S.), and are expressed in parts per million (p.p.m.) relative to T.M.S. = 0, referred to as δ values. The reference for D_2O solutions was sodium 4,4-dimethyl-4-silapentane-1-sulphonate.

Polarimetry

Optical rotations were measured on a Perkin-Elmer 141 polarimeter, using 1 dm glass cells enclosed in a constant temperature water jacket.

Paper Chromatography

Descending chromatography was carried out with Whatmann No.1 and No.3MM chromatography paper. A neutral solvent system of n-butanol, ethanol, water, 40:11:19 V/V, was used. Detection of components was effected by dipping the paper through three solutions in succession:-

(a) A saturated aqueous solution of silver nitrate (5 cm^3) in acetone (1.0 ℓ) and water (20 cm^3).

(b) An ethanolic sodium hydroxide solution (NaOH:2g, EtOH: 98 cm^3, H_2O : 2 cm^3).

(c) A 10% W/W aqueous solution of sodium thiosulphate pentahydrate.

Electrophoresis

Paper electrophoresis using Whatmann No 3MM, 11 cm wide chromatography paper, was carried out with a Shandon High Voltage Electrophoresis instrument. Glycerol was used as a non-migrating marker to correct for electroendosmosis. An aqueous solution of sodium molybdate was used as the electrolyte and was prepared as follows: Sodium molybdate dihydrate (24.2g) was dissolved in water (1.0 ℓ) and the solution was adjusted to pH 5.0 by careful addition of concentrated sulphuric acid. The electrophoretograms were developed by applying 1200 V, corresponding to a current

of approximately 60 mA for $2\frac{1}{2}$ hours. The papers were subsequently dried and the components detected using the procedure outlined above. Mobilities of compounds are referred to D-glucitol as standard and are quoted as M_s values.

Thin Layer Chromatography

Silica gel coated on glass or precoated on plastic plates was used as the stationary phase in thin layer chromatography. Solvent systems used:

(A) Ethyl acetate - dichloromethane 1:1

(B) 2-Butanone saturated with water.

Detection of the components was achieved by exposing to iodine vapour for a short time. Benzeneboronic acid and benzeneboronates could be detected with this method. Spraying the plate with a 10% solution of concentrated sulphuric acid in ethanol and heating at 120° effected the detection of the remaining components.

EXPERIMENTAL

Expt. 1 - Benzeneboronic Anhydride

Benzeneboronic acid was prepared by the controlled hydrolysis of phenylboron dichloride. The preparation of the dichloride was similar to that of BIRCH et al.[7]. Boron trichloride (100 g), previously cooled in a cardice/acetone bath, was poured onto tetraphenyltin (90 g) in a 250 cm^3 flask fitted with a cold finger. After the initial reaction had subsided, the mixture was heated gently for one hour and then distilled, using a short fractionating column filled with glass helices. The fraction which distilled in the range 172-178° was collected.

After the addition of an equal volume of carbon tetrachloride, the solution was carefully added dropwise onto cracked ice (600 cm^3), whereupon a white flocculent mass of benzeneboronic acid separated. The acid was filtered and recrystallised from water after a hot filtration. Heating

at 120° for 4 hours afforded benzeneboronic anhydride (28.5 g , 32%), m.p. 218-219° , lit[197] m.p. 214-216° .

Expt.2 - L-Rhamnitol

Potassium borohydride (1.0 g) was added to a solution of L-rhamnose monohydrate (0.55 g) in water (5 cm^3) and left overnight at room temperature. Amberlite 1R-120 (H^+) resin was added to remove cations, filtered and washed with water. Evaporation of the water under reduced pressure yielded a colourless syrup, which was repeatedly dissolved in methanol (3 cm^3) and evaporated under reduced pressure. After six additions of methanol, a white sticky solid remainined, which, on boiling in acetone (30 cm^3) and cooling, gave the white crystalline product, L-rhamnitol (0.50 g , 87%), m.p.121-122°, M_s (M_o) 0.90, lit[198] m.p. 121°.

Expt.3 - D-Arabinitol

D-Arabinitol was prepared by borohydride reduction of D-arabinose (2.02 g), using the same method as outlined in expt. 2. After boiling in acetone, subsequent cooling produced D-arabinitol (1.46 g , 72%), m.p. 102-103°, lit[199] m.p. 102°.

Expt.4 - Preparation of pentitol bisbenzeneboronates

The pentitol (ca. 0.5 g) was dissolved in dry 2-methoxyethanol (10 cm^3) together with benzeneboronic anhydride (0.66 mole equivalent) and heated for one hour over steam. Evaporation of the solvent under reduced pressure, yielded either a white crystalline residue or a viscous syrup. Traces of water were removed by redissolving the product in dry toluene and evaporating. The crude bisbenzeneboronates were stored in a desiccator.

Expt.5 - Diazomethane

The method for the preparation of diazomethane was similar to that of DeBOER and BACKER[200]. A solution of potassium hydroxide (3 g) in water (5 cm^3) was mixed with methyldigol (18 cm^3) and dichloromethane

($5\ cm^3$) and heated in an oil bath to 70-75°. A solution of toluene - ρ - sulphonylmethylnitrosamide (10 g) in dichloromethane ($50\ cm^3$) was added over a period of ½ hour, with continuous stirring, effecting distillation of diazomethane together with dichloromethane. The distillate was collected in a cooled receiver, and comprised approximately of 65% diazomethane. The deep yellow solution was dried over sodium hydroxide pellets and stored at 0°.

Expt.6 - Methylation of pentitol bisbenzeneboronates

The bisbenzeneboronate of a pentitol (ca 0.10 g) was dissolved in a solution of borontrifluoride etherate in 1,2-dimethoxyethane (0.1%; $5\ cm^3$) and cooled to -10° in a methanol/ice bath. A solution of diazomethane in dichloromethane ($5\ cm^3$) was added and the mixture allowed to stand for 3 minutes, after which time the yellow colour had disappeared. A further $5\ cm^3$ portion of diazomethane solution was added, which retained its yellow colour for approximately ½ hour. Paper chromatograms of the reaction mixture indicated that the majority of the methylations were almost quantitative, very little pentitol remained.

Some difficulty was experienced with one boronate, ribitol, to secure effective monomethylation. Initial attempts resulted in the production of a variety of methylated products. Effective monomethylation was only obtained when the crude bisbenzeneboronate was prepared using ten times the stiochiometric amount of benzeneboronic anhydride.

The polymethylene which is produced as a side product of the reaction[102], was filtered off and the filtrate evaporated down under reduced pressure to a small volume and applied onto a preparative Whatmann No 3MM chromatography paper. After development of the paper, the monomethylated pentitol was eluted from the paper with water.

Evaporation of the eluate under reduced pressure left a thin syrup, which was dissolved in acetic anhydride (1.0 cm^3) and pyridine (1.0 cm^3) and heated over steam for 10 minutes. The mixture was evaporated to a yellowish syrup, which was dissolved in chloroform (1.0 cm^3) and an aliquot (1.0 $\mu\ell$) injected onto a gas-liquid chromatography column containing type I packing material, at ca. 180°. The effluent from the column was fed directly into a mass spectrometer. By this means, each component could be detected, by a flame ionization detector, and analysed, on line, by the mass spectrometer.

The mixture of methylated alditol acetates was injected onto a Pye 104 dual column gas-liquid chromatograph, linked to a Hewlett Packard integrator, which recorded the retention times and areas of each component. From the areas the mole fractions were calculated, and are given, together with the relative retention times, in Table IV on p.76.

Expt.7 - 1-d_1-D-Arabinitol

Sodium borodeuteride (0.31 g) was added to a solution of D-arabinose (1.92 g) in water (8.0 cm^3) and left at room temperature for 14 hours. After work up of the solution with Amberlite 1R-120(H^+) resin, the mixture was filtered and the filtrate evaporated to a clear syrup. Repeated evaporation of a methanolic solution of the syrup under reduced pressure, produced a crystalline mass. Recrystallisation from methanol yielded 1-d_1-D-arabinitol (1.10 g, 56%), m.p. 102°.

Expt.8 - D-Arabinose diethyldithioacetal

D-Arabinose (10.15 g) was dissolved in concentrated hydrochloric acid (12 cm^3) and cooled to 0° in ice. Ethanethiol (12 cm^3), previously cooled in a cardice/acetone bath, was added and the mixture shaken for 3 hours at room temperature. A white crystalline mass formed which was shaken for a few minutes with water (100 cm^3), filtered, and washed with

water. Recrystallisation from methanol afforded D-arabinose diethyldithioacetal (14.2 g, 79%), m.p. 126-127°, R_G 3.64, lit[201] m.p. 127-128°.

Expt.9 - D-Ribose diethyldithioacetal

D-Ribose (10 g) was dissolved in concentrated hydrochloric acid (12 cm^3) and cooled in an ice bath. Ethanethiol (12 cm^3), previously cooled in cardice/acetone, was added and shaken for 3 hours at room temperature. The residual clear brown syrup was diluted with water (100 cm^3), neutralised with sodium carbonate and extracted with dichloromethane (2 × 100 cm^3). The solvent was removed under reduced pressure, leaving a brownish crystalline mass. Recrystallisation from methanol yielded D-ribose diethyldithioacetal (10.2 g, 60%), m.p. 80-82°, R_G 3.33, lit[203] m.p. 82-83°.

Expt.10 - D-Lyxose diethyldithioacetal

Ethanethiol, (1 cm^3) cooled in a cardice/acetone bath, was added to a chilled solution of D-lyxose (1 g) in concentrated hydrochloric acid. The mixture was shaken for 4 hours at room temperature and then diluted with water (2 cm^3). The solution was deionised by passing through an Amberlite 1R-400 (OH^-) resin column. Evaporation of the eluate produced a crystalline product, which was redissolved in a small amount of ethanol and refrigerated. The product crystallised and was filtered and washed with ether. Yield: 0.72 g, 42%, m.p. 103-104°, R_G 3.63, lit[206] m.p. 103-104°.

Expt.11 - Preparation of Raney Nickel

The method used was based on that of VOGEL[202]. Sodium hydroxide pellets (190 g) were dissolved in water (750 cm^3) in a 2 ℓ beaker equipped with a stirrer and placed in an ice bath. An alloy of nickel and aluminium 1:1, (150 g) was added carefully, so that the temperature remained below 25°. When addition was complete, the mixture was allowed to reach room temperature

The evolution of hydrogen subsided and the suspension was heated at 80° for 1 hour. On cooling, the supernatant was decanted and a 10% solution of sodium hydroxide was added to disperse the nickel. The catalyst was washed by dispersion and decantation until the supernatant liquid was neutral to litmus. The washings were repeated with ethanol (6 × 100 cm^3), and the nickel (130 cm^3) finally stored under ethanol.

Expt.12 - 1-Deoxy-D-arabinitol

Raney nickel (8 cm^3) was added to a solution of D-arabinose diethyldithioacetal (1.0 g) in ethanol (20 cm^3) and refluxed for 1 hour. After filtration of the catalyst, removal of the solvent produced a syrup. Dissolution in a small quantity of ethanol and refrigeration gave crystalline 1-deoxy-D-arabinitol (0.21 g, 40%), m.p. 126-127°, lit[204] m.p.129-131°.

Acetylation of the product (ca. 5 mg) with acetic anhydride and pyridine, and injection onto a gas-liquid chromatography column containing stationary phase type I, produced a single peak, T = 0.197. Xylitol penta-O-acetate, T = 1.00.

Expt.13 - 1-Deoxy-D-ribitol

The procedure was similar to that of 1-deoxy-D-arabinitol outlined in expt.12. Recrystallisation from ethanol furnished 1-deoxy-D-ribitol m.p.74-80°, R_G 1.71, lit[205] m.p. 74-75°.

Acetylation of the product (ca. 5 mg) and injection onto a gas-liquid chromatography column containing stationary phase type I, produced a single peak, T = 0.195, relative to xylitol penta-O-acetate.

Expt.14 - 1-Deoxy-D-lyxitol

Reductive desulphurisation of D-lyxose diethyldithioacetal was carried out with Raney nickel as outlined for 1-deoxy-D-arabinitol, expt. 12. The product was a syrup which could not be crystallised. Acetylation with pyridine/acetic anhydride, followed by injection of a chloroform

solution onto a gas-liquid chromatography column containing phase I, produced a single peak, T = 0.208. A similar procedure for the preparation of this compound also produced a syrup[204].

Expt.15 - Bisbenzeneboronates of tetritols

The 1-deoxy-pentitols and 1,6-dideoxy-hexitols (ca. 0.02g) were dissolved in 2-methoxyethanol (3 cm^3) together with benzeneboronic anhydride (0.66 mole equivalent). The mixture was heated, with the exclusion of moisture, over steam for 1 hour, when the solvent was removed under reduced pressure. The residue was redissolved in toluene and evaporated again to remove small amounts of residual water, to yield a white crystalline residue.

Expt.16 - D-Mannose diethyldithioacetal

D-Mannose (3.0g) was dissolved in concentrated hydrochloric acid (3.0 cm^3) and cooled to 0°. Ethanethiol (3.0 cm^3), previously cooled in a cardice/acetone bath, was added and the mixture shaken for 15 minutes. Addition of water (6.0 cm^3) resulted in solidification to a whitish mass. The product was filtered and washed several times with water. The crude material was recrystallised from ethanol to yield a product of m.p. 127-128°. A second recrystallisation afforded chromatographically pure D-mannose diethyldithioacetal, R_G 3.1, m.p. 132-134°, yield 2.4g, 50.3%, lit[207] m.p. 132-134°.

Expt.17 - D-Mannose diethyldithioacetal bisbenzeneboronate

D-Mannose diethyldithioacetal (0.60 g) was dissolved in 2-methoxyethanol (10 cm^3) together with benzeneboronic anhydride (0.44g, 0.66 mole quivalent) and heated for 1 hour over steam, with the exclusion of moisture. Evaporation of the solvent under reduced pressure afforded a viscous syrup. Traces of water were removed by redissolving in dry toluene (10 cm^3) and evaporating. The crude material obtained was used in the attempted preparation of 6-O-methyl-D-mannose.

Expt.18 - Methylation of D-mannose diethyldithioacetal bisbenzeneboronate

The crude D-mannose diethyldithioacetal bisbenzeneboronate (0.06 g) was dissolved in a solution of borontrifluoride etherate in 1,2-dimethoxyethane (0.1%, 5 cm^3) and cooled to -10°. A solution of diazomethane, (expt.5), in dichloromethane (10 cm^3) was added, resulting in the liberation of nitrogen and gradual disappearance of the yellow colour after a few minutes. A further 10 cm^3 portion of diazomethane solution was added, the yellow colouration persisting for approximately 20 minutes.

Paper chromatography revealed a fast moving component, R_G = 3.20, close to unmethylated D-mannose diethyldithioacetal, R_G = 3.10. The intensities of the spots indicated that approximately 50% of the bisbenzeneboronate had been methylated.

Expt.19 - Demercaptalation of D-mannose diethyldithioacetal mono-O-methyl derivatives

The general method for demercaptalations was similar to that used by WOLFROM[208]. The products obtained from the methylation of D-mannose diethyldithioacetal bisbenzeneboronate were filtered and evaporated down to yield a clear syrup. This was dissolved in a mixture of 1,2-dimethoxyethane and water, (4:1, 5 cm^3). Cadmium carbonate (0.23 g) was added and the mixture stirred vigorously while a solution of mercuric chloride (0.22 g) in 1,2-dimethoxyethane/water (5 cm^3) was added dropwise. After 12 hours at room temperature the mixture was refluxed for $\frac{1}{2}$ hour and filtered. Paper chromatography showed a weak spot corresponding to D-mannose, a second, R_G = 1.9, a third weak spot, R_G = 2.8, and an intense fourth spot R_G = 3.6. The components R_G = 1.9 and R_G = 2.8 were believed to be the 6-O-methyl and 5-O-methyl D-mannose derivatives. However, the unexpected component, R_G = 3.6, could not be identified by paper chromatography.

Expt.20 - 2-Acetamido-2-deoxy-α-D-glucopyranose

1-Amino-2-deoxy-α-D-glucopyranose hydrochloride (22.0 g) was added to a solution of sodium (2.3 g) in methanol (100 cm^3). The mixture was agitated to ensure thorough mixing for 3 minutes and then filtered under gentle suction, washing the residue with methanol (2 × 10 cm^3). The combined filtrate was immediately treated with acetic anhydride (12 cm^3) and cooled. The solution was left overnight at room temperature and then refrigerated for 2 hours to complete the crystallisation of the product. The crude material was recrystallised by dissolving in hot ethanol/water (7:10) and adding hot 1,2-dimethoxyethane to turbidity. Refrigeration yielded crystalline 2-acetamido-2-deoxy-α-D-glucopyranose, (19.0 g, 84.2%) m.p. 207-208°, R_G = 1.27, lit[209] m.p. 205°.

Expt.21 - Methyl 2-acetamido-2-deoxy-D-glucopyranoside

2-Acetamido-2-deoxy-α-D-glucopyranose (8 g) was mixed with Amberlite 1R-120 (H^+) resin (24 cm^3) in absolute methanol (120 cm^3) and gently refluxed for 15 hours. The resin was filtered off and washed with hot methanol (3×10 cm^3). The combined filtrate was evaporated under reduced pressure to yield a crystalline mass of crude product. Recrystallisation from methanol afforded methyl 2-acetamido-2-deoxy-D-glucopyranoside with the approximate anomeric composition: 85% α, 15% β [150]. Yield: 7.6 g, 90%, m.p. 188-189°, R_G(α) 2.19, R_G(β) 1.95, lit[150] m.p. 189°.

Analysis: $C_9H_{17}NO_6$ requires: C 45.90 H 7.23 N 5.95
found: C 46.00 H 7.13 N 6.14.

Expt.22 - Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate

Methyl 2-acetamido-2-deoxy-D-glucopyranoside (0.67 g), of approximate composition 85% α-anomer and 15% β-anomer, was partially dissolved in dry toluene (50 cm^3), together with benzeneboronic anhydride (0.30 g) and

refluxed for 4 hours. The water produced was removed azeotropically with the aid of a Dean and Stark head. On cooling, the mixture produced a fine crystalline product which was filtered and washed with toluene. Recrystallisation was effected with 2-methoxyethanol to furnish methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate (0.75 g, 97%, calculated for the α-anomer), m.p. 287-288°, $[\alpha]_D^{25}$ = + 132° (C = 1, pyridine).

Analysis:	$C_{15}H_{20}BNO_6$	requires:	C 56.07	H 6.23	B 3.43	N 4.36
		found:	C 55.81	H 6.24	B 3.30	N 4.52.

Expt.23 - Boron analysis

The boron content of benzeneboronates can be estimated spectrophotometrically, by using the absorption due to the B-phenyl grouping in the U.V. spectrum at 219 nm. A calibration graph was obtained using a range of concentrations of benzeneboronic anhydride in ethanol/water, 1:1. Benzeneboronic anhydride (0.123 g) was dissolved in aqueous ethanol (100 cm^3), then diluted ten times. From this solution aliquots of 5 cm^3 and 10 cm^3 were taken and made up to 100 cm^3. From the second solution, further portions of 10, 20 and 30 cm^3 were taken and again made up to 100 cm^3.

The absorbances of these solutions were taken, using the 1:1, water/ethanol, mixture as a reference. The values obtained are given in the table, from which a calibration graph was drawn of absorbance against boron concentration.

Benzeneboronic Anhydride g/100 cm^3	Absorbance	Concentration of Boron g/100 cm^3
6.10×10^{-4}	0.575	6.45×10^{-5}
3.63×10^{-4}	0.343	3.84×10^{-5}
2.40×10^{-4}	0.229	2.56×10^{-5}
1.12×10^{-4}	0.115	1.28×10^{-5}

The sample (0.041 g) was dissolved in 50% ethanol and made up to 100 cm^3. A 10 cm^3 aliquot was diluted to 100 cm^3 and again by a factor of ten to give a final concentration of 0.41×10^{-3} g/100 cm^3. This solution gave an absorbance of 0.118 which corresponded to 1.35×10^{-5} g of boron/100 cm^3, and a boron content of 3.3% for the methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate.

Expt.24 - Methylation of methyl 2-acetamido-2-deoxy-α-D-Glucopyranoside 4,6-benzeneboronate

The methylation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate was carried out using diazomethane as the methylating reagent, in a manner as described in expt.6. The residue was applied to a preparative Whatmann No 3MM chromatography paper and developed in the neutral solvent. The component corresponding to the methylated material was cut out and eluted from the paper with water (30 cm^3). Removal of the water under reduced pressure produced a colourless syrup, which was acetylated with a pyridine/acetic anhydride mixture (1 cm^3 each), over steam for 10 minutes.

The solution was evaporated and the residue dissolved in chloroform (1 cm^3). Injection of the solution (1.0 μl) onto a gas-liquid chromatography column, containing stationary phase, type I, coupled to a mass spectrometer, afforded separation of the mono-O-methyl ether of the amino sugar glycoside from traces of fully acetylated material, and simultaneously supplied the mass spectrum.

Expt.25 - Preparation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside

The glycoside (0.8 g), prepared by the resin method containing both anomers in an approximate composition 85% α and 15% β, was passed through a Dowex-1 (OH^-), 200-400 mesh, resin column (1.5 × 85 cm) and eluted with deionized, distilled, carbon dioxide-free water from a reservoir fitted

with a soda-lime trap. The fractions (25 cm^3) were monitored by optical rotation. Evaporation of fractions 5-10 produced a white crystalline material which was recrystallised from ethanol to yield methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (0.45 g), m.p. 187-188°, $[\alpha]_D^{25}$ = + 129° (C = 1, H_2O), lit[140] m.p. 187-188°, $[\alpha]_D^{25}$ = + 131°, (C = 1, H_2O).

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate was prepared from the above α-glycoside (0.15 g), and benzeneboronic anhydride (0.07 g) in toluene (50 cm^3). Azeotropic removal of water, followed by evaporation of the solvent yielded a white residue which was recrystallised from 2-methoxyethanol. Yield: 0.20 g, 97%, m.p. 285-286°.

Expt. 26 - 1,2,3,4,6-Penta-O-acetyl-β-D-galactopyranose

D-Galactose (25 g) was added portionwise to a stirred mixture of acetic anhydride (135 cm^3) and anhydrous sodium acetate (11 g) at 100°. After 1 hour, when the addition was complete, the mixture was heated for a further hour at 100° in an oil bath, and then allowed to cool to room temperature. The solution was poured onto cracked ice (400 cm^3) and stirred for 3 hours. The heavy syrup which initially separated, crystallised and was filtered, washed with cold water (2 × 50 cm^3) and dried. Recrystallisation from ethanol yielded 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (28.7 g, 53%), m.p. 142°, lit[210] m.p. 142°.

Expt. 27 - 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide

Hydrogen bromide, prepared by the method of DUNCAN[211], was bubbled into glacial acetic acid at 0° until a saturated solution was obtained. The mixture was poured onto 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (20 g) and shaken at room temperature for 1½ hours. After diluting with chloroform (100 cm^3), the mixture was poured onto ice (400 cm^3). The chloroform layer was separated and washed with water (8 × 100 cm^3) until the washings were neutral to universal indicator paper.

After drying with anhydrous sodium carbonate, the chloroform was removed by evaporation under reduced pressure, to furnish a clear syrup. Dissolving thesyrup in dry ether and refrigerating overnight produced a white crystalline mass. Recrystallisation from ether yielded 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl boromide (18.1 g, 68%), m.p. 85°, lit[212] m.p. 85°.

Exp.28 - Methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate (0.93 g) was shaken with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1.38 g, 1.2 mol) and mercuric cyanide (0.37 g) in freshly distilled nitromethane (120 cm^3) at room temperature for two days. The initial suspension of the glycoside boronate gradually disappeared, leaving a clear solution, indicating completion of the reaction. T.ℓ.c. on silica gel, solvent system A, of the reaction mixture showed one slow moving component, R_F 0.04, with complete absence of starting material.

The reaction mixture was concentrated under diminished pressure and chromatographed on a silicic acid column, (SIL-R, 100-300 mesh, Sigma Chemical Co.), using a lyotropic series of solvents. A chromatographically pure product emerged with ethyl acetate/ethanol, 10:1. Yield of crude material: 1.49 g, 91%. Recrystallisation from ethyl acetate afforded methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside, (1.35 g, 82%). The melting point indicated that polymorphic modifications were present. Thus, at 95-100° the crystals appeared to change form. This second modification gave a sharp melting point at 189-190°. $[\alpha]_D^{25}$ = + 48.5° (C = 1, $CHC\ell_3$).

Analysis: $C_{23}H_{35}NO_{15}$ requires: C 48.84 H 6.24 N 2.48
found: C 48.58 H 6.39 N 2.68 .

Expt.30 - Ketolysis of D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate

A solution of D-glucitol 1,3;2,4;5,6-trisbenzeneboronate (0.03 g) in dry acetone (10 cm^3) was acidifed with concentrated sulphuric acid (1 drop) and left at room temperature for 14 hours. Neutralising with concentrated ammonia precipitated ammonium sulphate which was removed, and the filtrate was evaporated, under reduced pressure, to a syrup.

Paper chromatography revealed a fast moving component, R_G 2.41, together with glucitol and benzeneboronic acid. The syrup was dissolved in a small quantity of acetone and applied to a preparative Whatmann No 3MM paper. The suspected ketal component was cut out and eluted from the paper with water. Paper electrophoresis in molybdate buffer gave a single spot, M_S (Mo) 0.93. Removal of the water gave a thin syrup.

Expt.31 - Methylation of the ketolysis product from D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate

The residue obtained from the ketolysis of D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate was dried under vacuum and then dissolved in freshly distilled dimethyl formamide (0.5 cm^3). Silver oxide (0.5 g) , previously dried at 90° under vacuum for 1 day, was added, together with methyl iodide (0.5 cm^3) and shaken for 24 hours. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were reduced to a small volume and a sample (1.0 $\mu\ell$) was injected onto a gas-liquid chromatography column, containing stationary phase A, coupled to a mass spectrometer. The methylated mono-O-isopropylidene ketal was accordingly detected and its mass spectrum recorded.

A sample (1.0 $\mu\ell$) was also injected onto a dual column gas-liquid chromatograph containing columns (9' × 0.25") packed with stationary phase A, coupled to an integrator, which recorded the retention time of the methylated ketal, T (T.M.G.) = 0.191. The retention time of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol, (T.M.G.), referred to as T = 1.000.

Expt.32 - Trimethylsilylation of D-glucitol mono-O-isopropylidene ketal

D-Glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate (0.03 g) was subjected to the same procedure outlined in experiment 30, to yield a thin syrup which was dissolved in dry pyridine (1.0 cm^3). Hexamethyldisilaza ne (0.2 cm^3) and trimethylchlorosilane (0.1 cm^3) were added and the mixture shaken for 1 minute and allowed to stand for 5 minutes. The white precipitate of ammonium chloride was removed by centrifugation, and the solution (1.0 $\mu\ell$) injected onto a gas-liquid chromatography column (12' × 0.25"), containing stationary phase B, connected to a mass spectrometer. This afforded the mass spectrum of the trimethylsilylated compared, T = 0.93, referred to the retention time of 1,2,3,4,5,6-hexa-O-trimethylsilyl-D-glucitol, T = 1.000.

Expt.33 - 1-d_1-D-Glucitol

Sodium borodeuteride (0.078 g) was added to a solution of D-glucose (0.498 g) in water (5.0 cm^3) and left for 14 hours at room temperature. Removal of the sodium ions was effected with Amberlite 1R-120 (H^+) resin (3 cm^3). After filtering and washing the resin with water, the solution was evaporated down under reduced pressure to yield a syrup, which was repeatedly dissolved in methanol and evaporated to remove the boric acid as the volatile methyl borate. The white crystalline residue was recrystallised from ethanol to afford 1-d_1-D-glucitol (0.43 g , 86%), M_S (Mo) = 1.00.

Expt.34 - D-Mannitol 1,2 ; 3,4 ; 5,6-trisbenzeneboronate

Addition of D-mannitol (0.316 g) in water (4.0 cm^3) to a warm suspension of benzeneboronic anhydride (0.553 g) in methanol (4.0 cm^3) produced a crystalline product on standing at room temperature overnight. Recrystallisation from toluene/hexane, 1:1, afforded D-mannitol 1,2 ; 3,4 ; 5,6-trisbenzeneboronate (0.649, 85%), m.p. 135-136°, lit[17] m.p. 134-135°.

Expt.35 - Ketolysis of D-mannitol 1,2; 3,4; 5,6-trisbenzeneboronate

The ketolysis of D-mannitol 1,2; 3,4; 5,6-trisbenzeneboronate was accomplished in the same manner as outlined for D-glucitol trisboronate. The fast moving product, $R_G = 3.4$ was obtained on a milligram scale from a preparative paper chromatogram, M_s (Mo) 0.93.

Expt.36 - G.c.-m.s. investigation of the ketolysis product of D-mannitol 1,2; 3,4; 5,6-trisbenzeneboronate

The product obtained from the ketolysis of D-mannitol trisboronate was methylated in a manner as described for the product obtained from D-glucitol trisboronate, expt.31. The methylated material was examined by combined g.c.-m.s. for purity and structure determination.

A second sample of the ketolysis product was trimethylsilylated as outlined for D-glucitol and examined by g.l.c. for purity. Using an integrator in conjunction with the dual column chromatograph enabled retention times, relative to a standard compound, to be recorded. The results of the g.l.c. analysis of the acetylated, methylated and trimethylsilylated derivatives of D-mannitol and D-glucitol are shown in Table XII.

TABLE XII

G.l.c. OF D-GLUCITOL AND D-MANNITOL KETAL DERIVATIVES

Derivative of hexitol ketal	Retention Time	Stationary Phase	Temperature
D-glucitol acetate	2.670 [a]	A	173°
D-mannitol acetate	2.148 [a]	A	173°
D-glucitol methyl ether	0.261 [a]	A	173°
D-mannitol methyl ether	0.213 [a]	A	173°
D-glucitol T.M.S. ether	0.930 [b]	B	162°
D-mannitol T.M.S. ether	0.889 [b]	B	162°

[a] Retention times are related to the retention time of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.

[b] Related to 1,2,3,4,5,6-hexa-O-trimethylsilyl-D-glucitol.

Expt.37 - α-D-Glucofuranose 1,2; 3,5-bisbenzeneboronate

D-Glucose (2.42g) was partially dissolved in dry 2-methoxyethanol (50 cm^3), together with benzeneboronic anhydride (2.82g) and heated over steam for 40 minutes. The resulting clear solution was evaporated under reduced pressure to a clear syrup, which was taken up with a small quantity of dry toluene. Refrigeration overnight furnished crystalline α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate (4.21g, 89%), m.p. 161-162°, lit[21] m.p. 161-162°.

Expt.38 - Ketolysis of α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate

A solution of α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate (0.10g) in dry acetone (10 cm^3) was acidified with concentrated sulphuric acid (2 drops) and refluxed for 15 hours. Neutralising with concentrated ammonia, filtering and evaporating produced a thin syrup. Paper chromatography detected a fast moving component, R_G 2.61, together with unreacted glucose.

The faster moving component was separated on a preparative Whatmann No 3MM paper, and acetylated with pyridine (1.0 cm^3) and acetic anhydride (1.0 cm^3). Evaporation under reduced pressure yielded a syrup which was dissolved in chloroform and analysed on g.c.-m.s., using column packing I at 200°. A further sample was analysed on a dual column chromatograph, affording one major peak, T = 1.90, T(T.M.G.) = 1.00, together with smaller peaks at longer retention times, T ~ 6.0, which were later identified as glucose penta-O-acetates.

Expt.39 - Attempted reduction of mono-O-isopropylidene-α-D-glucofuranose

A second sample of ketolysed α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate was chromatographed on paper. The eluted material was dissolved in water (2 cm^3) to which potassium borohydride (0.05g) was added and left at room temperature for 14 hours. The solution was worked up with Amberlite

1R-120 (H^+) resin (1.0 cm^3), filtered and washed. Boric acid was removed by adding methanol (4 × 4 cm^3) and evaporating under reduced pressured.

The residue was dissolved in dry pyridine (1 cm^3) and acetic anhydride (1 cm^3) and heated over steam for ten minutes. Evaporation of excess reagents yielded a yellow syrup which was dissolved in chloroform and analysed on the g.c.-m.s. system.

Expt.40 - Estimation of yields by gas-liquid chromatography

The procedure for the ketolysis of α-D-glucofuranose 1,2; 3,5-bisbenzeneboronate (ca. 60 mg) was carried out as described in expt.38. The preparative paper chromatogram was developed and the fast-moving benzeneboronic acid was cut off. The remaining components on the chromatogram were eluted from the paper with water. The eluent was evaporated under reduced pressure to a syrup, and acetylated with pyridine/acetic anhydride.

The residue obtained after evaporation under reduced pressure was dissolved in chloroform and a sample injected onto a gas-liquid chromatography column containing stationary phase type I. Separation of 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-glucofuranose from the D-glucopyranose and furanose acetates was achieved easily and peak areas were recorded. An estimation of the yields of the mono-O-isopropylidene ketals was then calculated from the total area under the peaks, making the necessary adjustments for molecular weight differences.

Expt.41 - 1,2-O-Isopropylidene-D-mannitol

D-Mannitol 1,2; 3,4; 5,6-trisbenzeneboronate (1.0 g) in dry acetone (30 cm^3) was acidified with concentrated sulphuric acid (3 drops) and left at room temperature for two days. After neutralising with concentrated ammonia and filtering, the solution was evaporated under reduced pressure to yield a yellow syrup, which was left overnight in an evacuated desiccator. The residue was dissolved in dry acetone (20 cm^3) and propane-1,3-

diol (0.6 g) added and left at room temperature for 3 hours. The solvent was removed under reduced pressure and the benzeneboronate ester derived from propane-1,3-diol distilled at 90°/1 mm.

T.l.c, using solvent B, of the residue, revealed a trace of benzeneboronic acid. The above procedure was repeated using a further quantity of propane-1,3-diol (0.3 g), affording a residue free from benzeneboronic acid. The ketal was extracted with boiling acetone (30 cm^3). Traces of mannitol were removed by passing an aqueous solution of the extracted material through a Dowex-1×8, (OH^-), 200-400 mesh, resin column (1.5 × 85 cm), eluting with water. Evaporation of fractions (10 cm^3), 15-19, afforded a crystalline material. Recrystallisation from acetone furnished 1,2-mono-O-isopropylidene-D-mannitol, (0.22 g, 30%), m.p. 167-168°, lit[189] m.p. 167°.

Analysis	$C_9H_{18}O_6$	requires:	C 48.64 H 8.16
		found:	C 48.54 H 8.30

Expt.42 - Ketolysis of D-mannose 2,3; 4,6-bisbenzeneboronate.

D-Mannose bisbenzeneboronate (0.10 g) was dissolved in dry acetone (10 cm^3) and acidified with concentrated sulphuric acid (2 drops), and refluxed for 12 hours. The solution was neutralised with concentrated ammonia and the precipitated ammonium sulphute was removed by filtering. Paper chromatographic examination of the filtrate detected a fast-moving component, R_G 2.78, in addition to the hydrolysed starting material, D-mannose.

A preparative chromatographic paper, Whatmann No 3MM, of the product afforded separation of the suspected mono-O-isopropylidene derivative. The material obtained was acetylated, using pyridine (1.0 cm^3) and acetic anhydride (1.0 cm^3) and evaporated, under reduced pressure, to a thin syrup. A solution of this product in dry chloroform was examined on g.c.-m.s. and on a dual column chromatograph, using column packing I at 192°. The main peak, T(T.M.G.) = 2.16 was identified as a mono-O-isopropylidene derivative. Smaller peaks adjacent to the main product were not completely resolved.

APPENDIX I

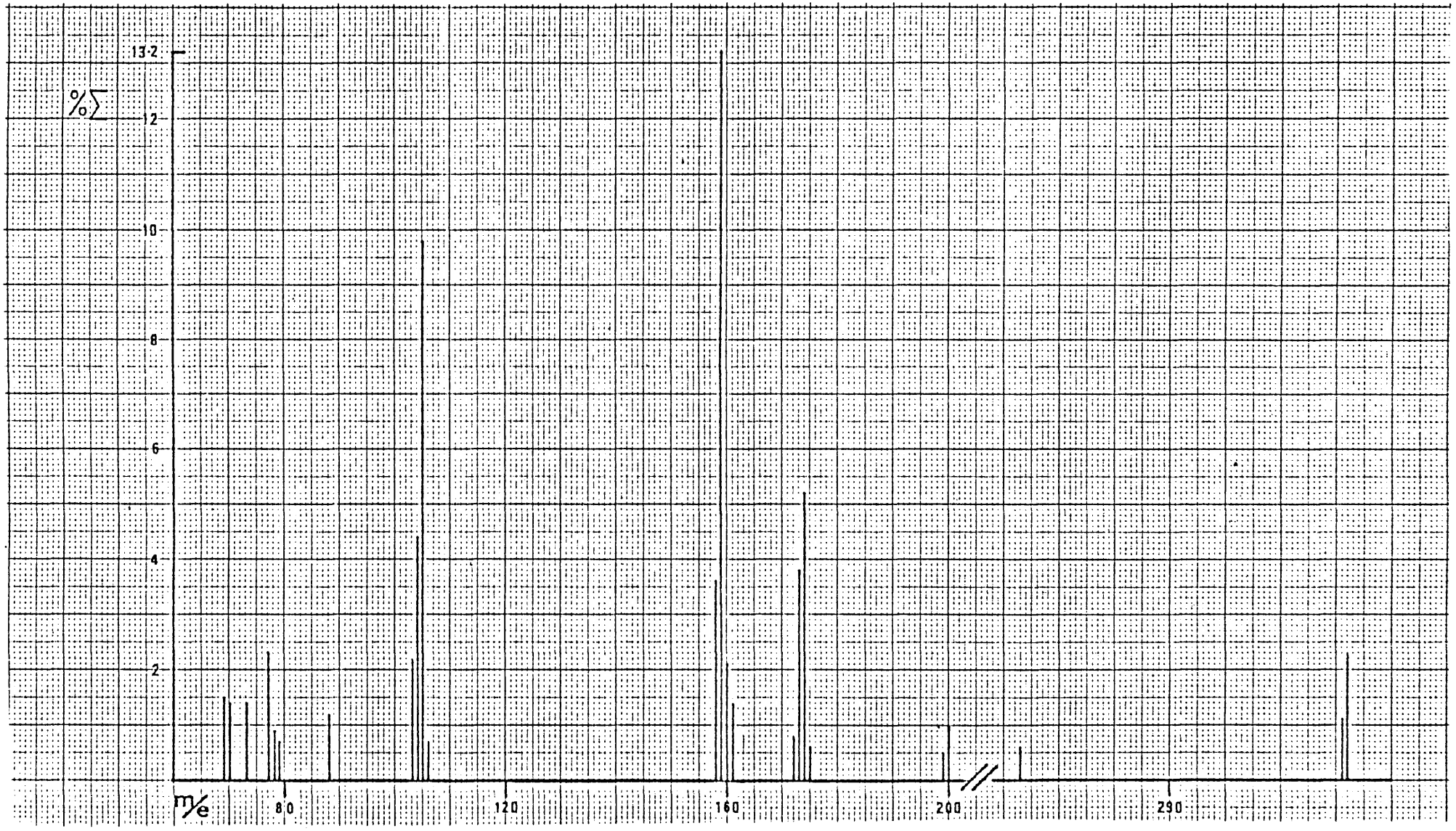
MASS SPECTRAL DATA

The following mass spectra data is presented in three parts:

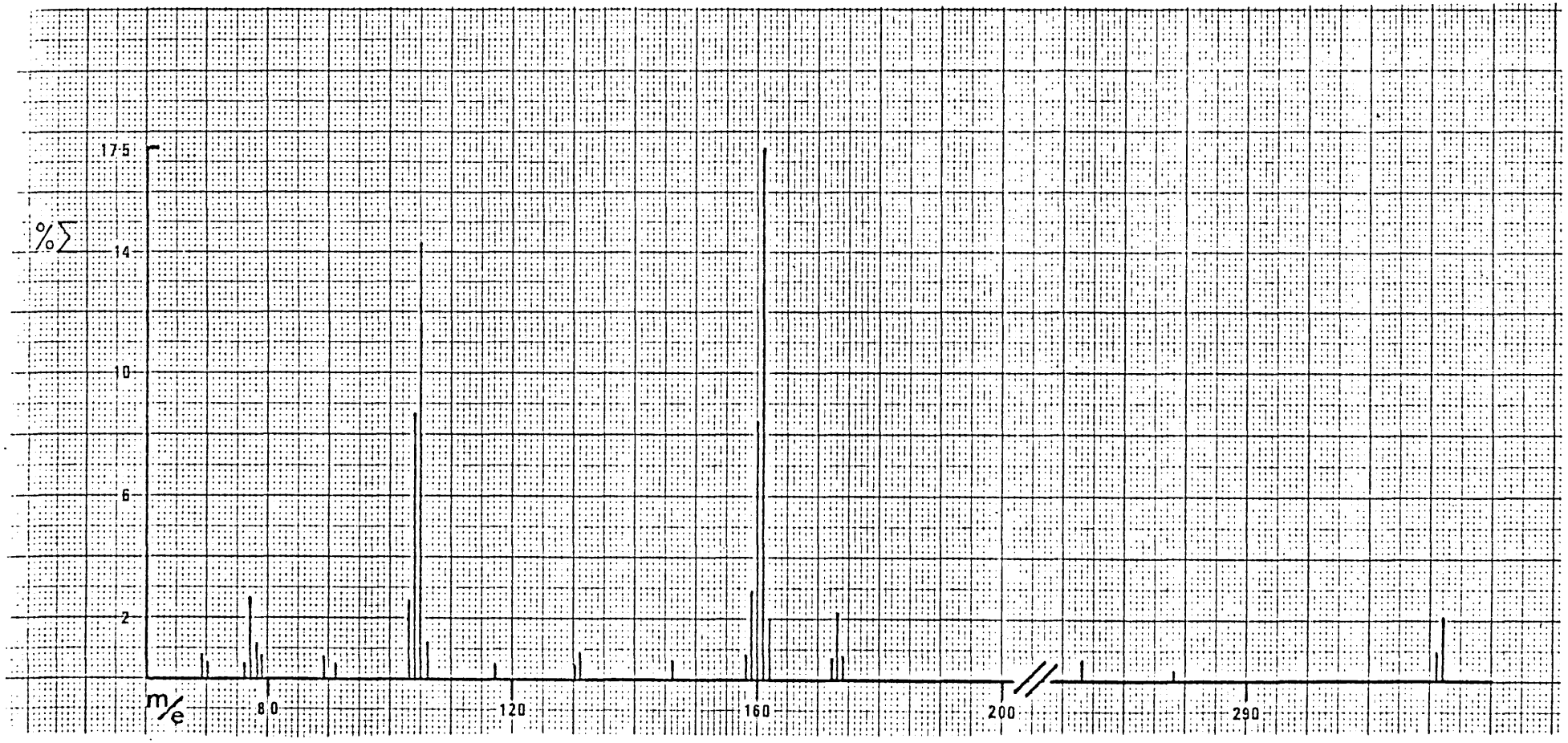
A. Low resolution mass spectra of all the benzeneboronates cited in the text, together with those of the partially protected disaccharide 111, methyl 2-acetamido-2-deoxy-4,6-di-O-acetyl-3-O-methyl-α-D-glucopyranoside, 109, and the ketolysis reaction mixture of D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate. The abundances of the ions are expressed as a percentage of the total ion current.

B. High resolution mass spectral data of the benzeneboronates, the partially protected disaccharide, 111, and the ketolysis reaction mixture of D-glucitol 1,3 ; 2,4 ; 5,6-trisbenzeneboronate. The precise mass measurements, together with the calculated masses, are given to four decimal places.

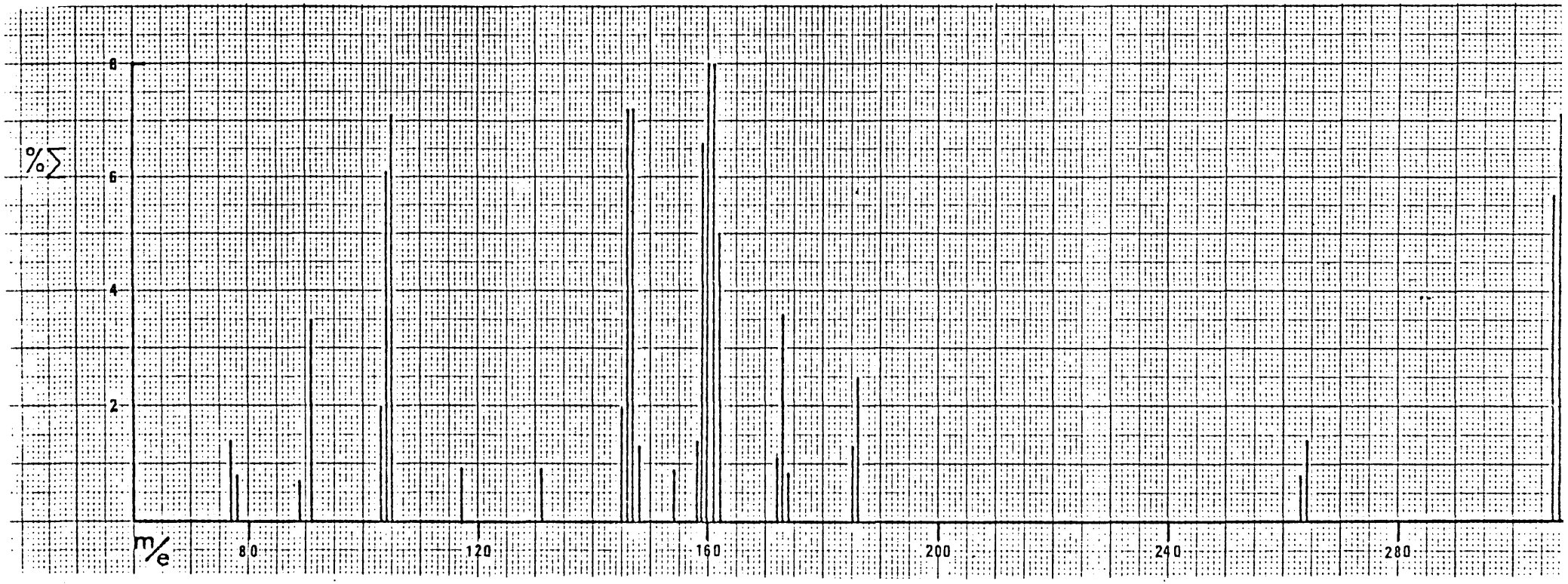
C. The metastable ions which appeared in the enhanced metastable ion spectra, together with the parent and daughter ions thought to be responsible.



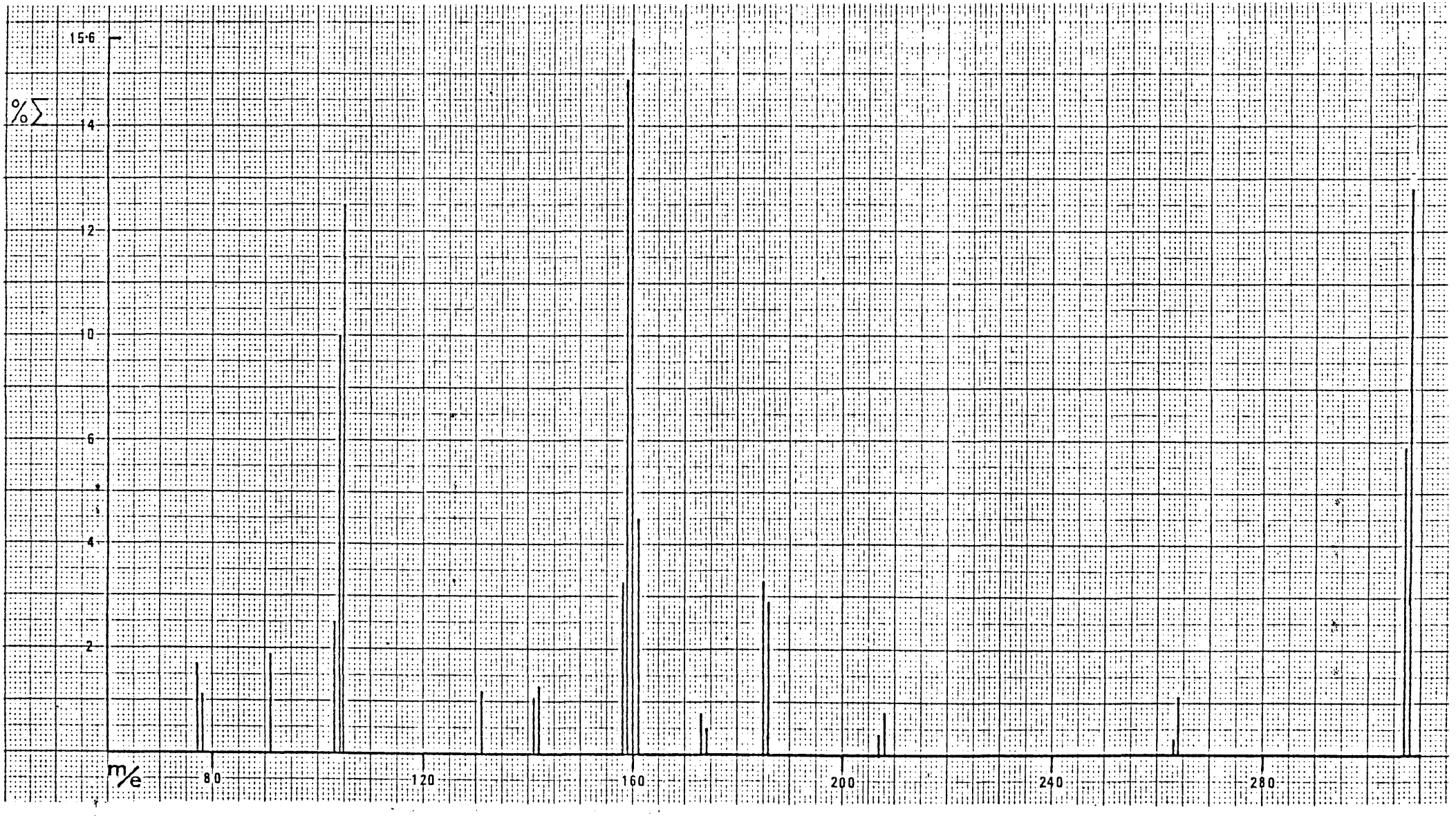
1,6-DIDEOXY-L- MANNITOL BISBENZENEBORONATE (110°)



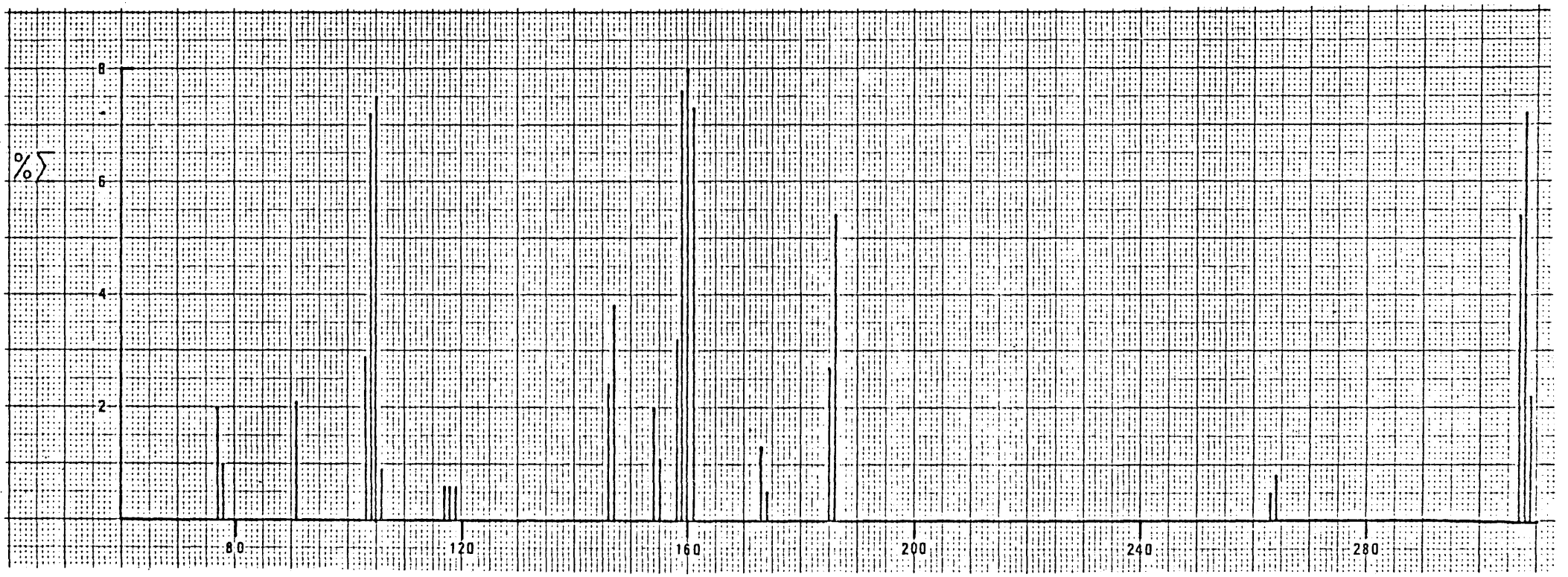
1,6-DIDEOXY-GALACTITOL BISBENZENEBORONATE (105°)



1-DEOXY-D-ARABINITOL BISBENZENEBORONATE (135°)

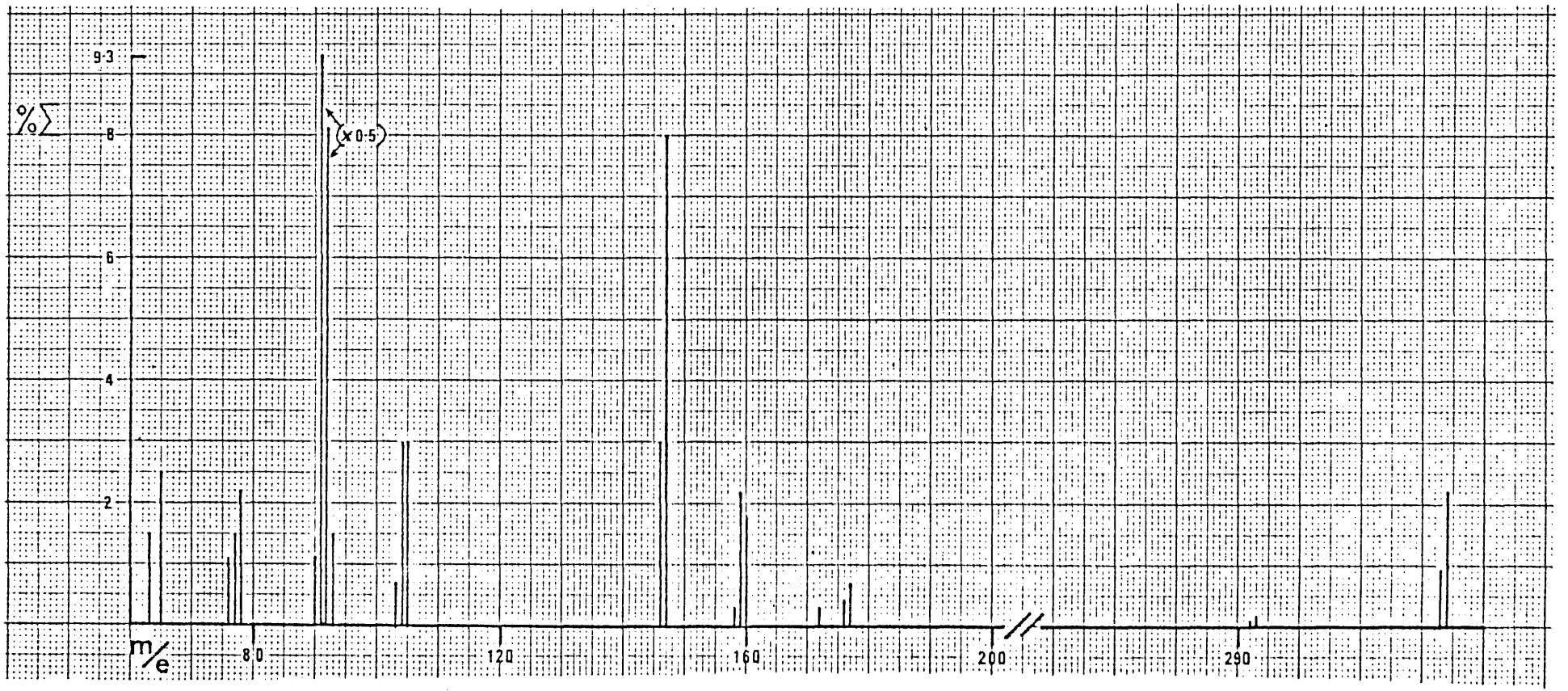


1-DEOXY-D-LYXITOL BISBENZENEBORONATE (120°)

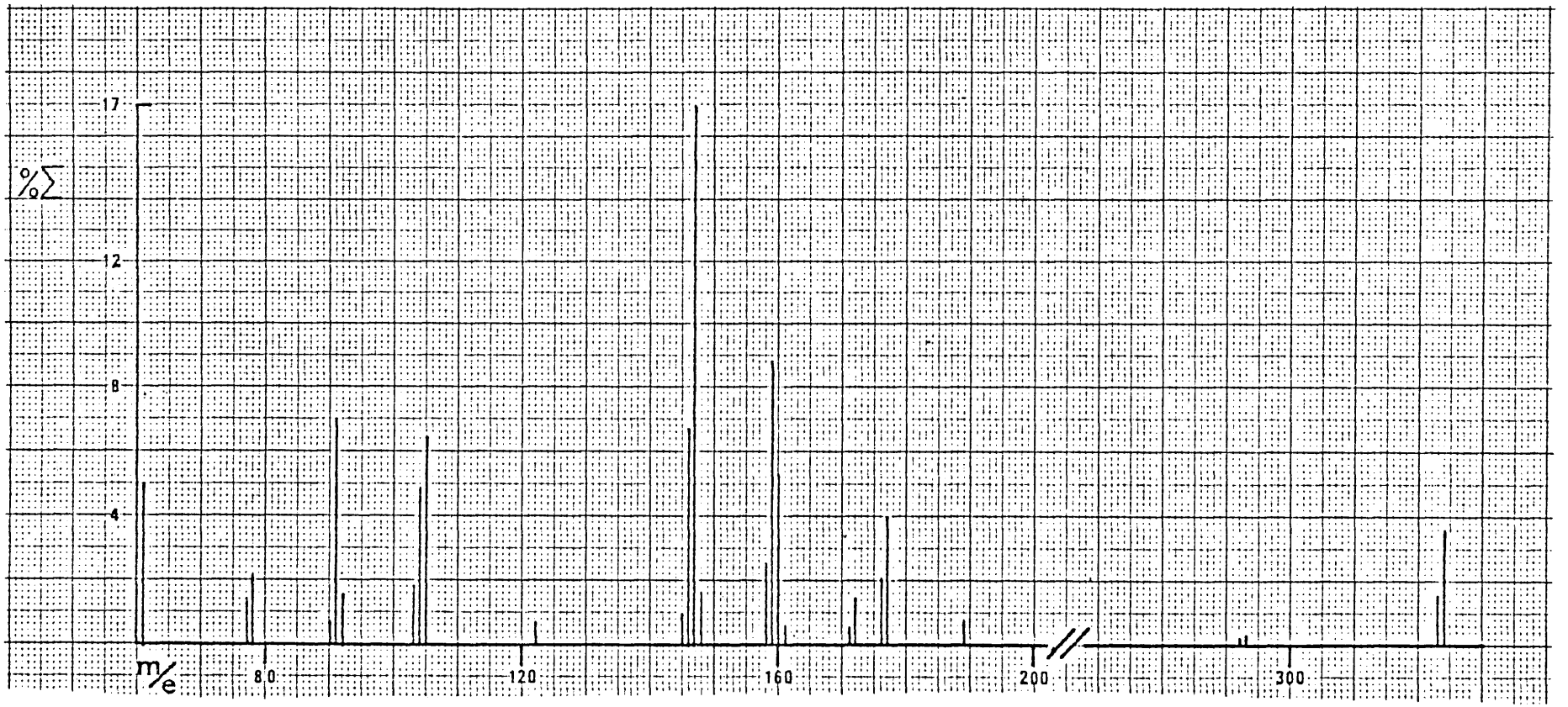


1-DEOXY-D-RIBITOL BISBENZENEBORONATE (135°)

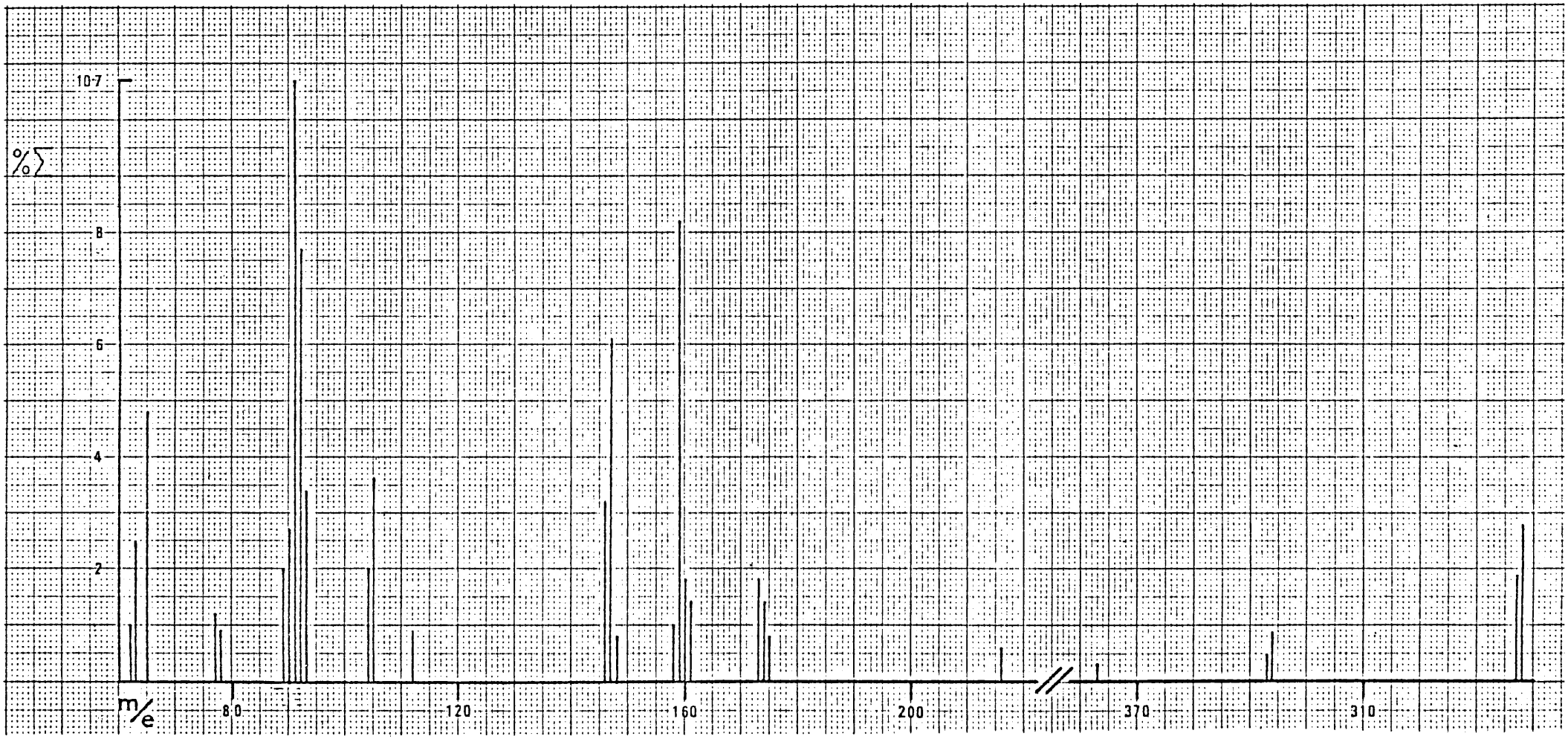
RIBITOL BISBENZENEBORONATE (125°)



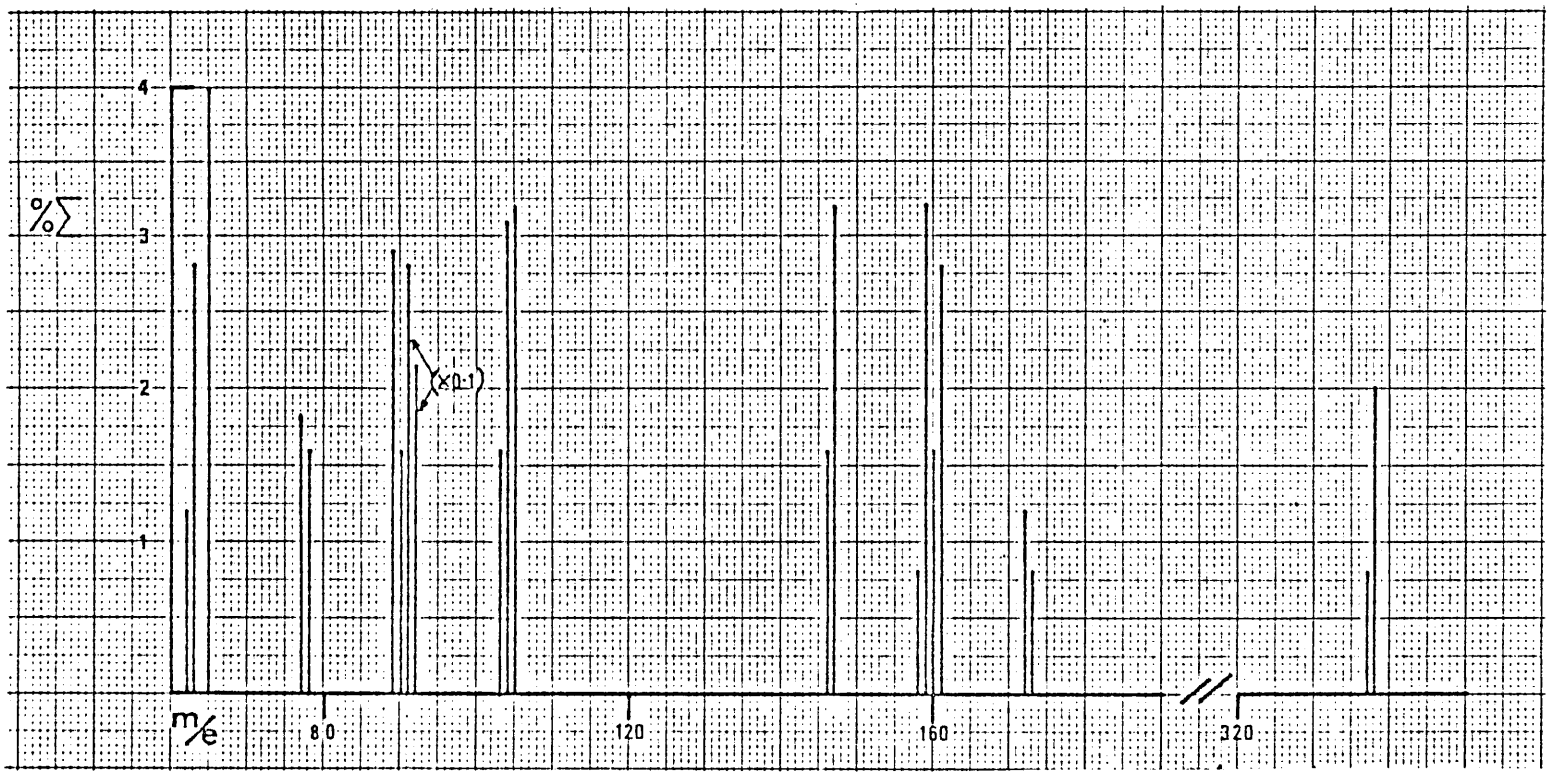
D-ARABINITOL BISBENZENEBORONATE (110°)



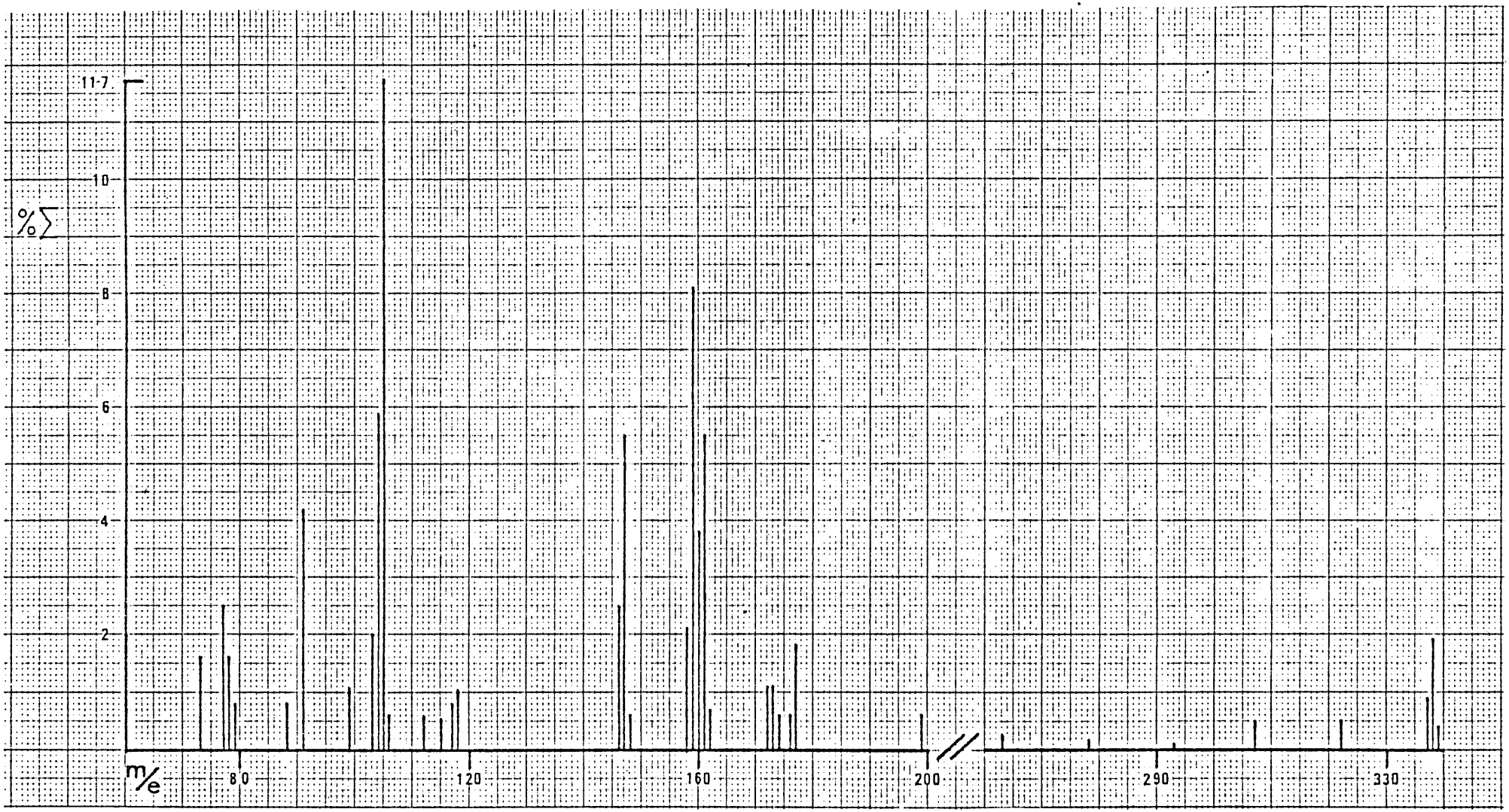
XYLITOL BISBENZENEBORONATE (115°)



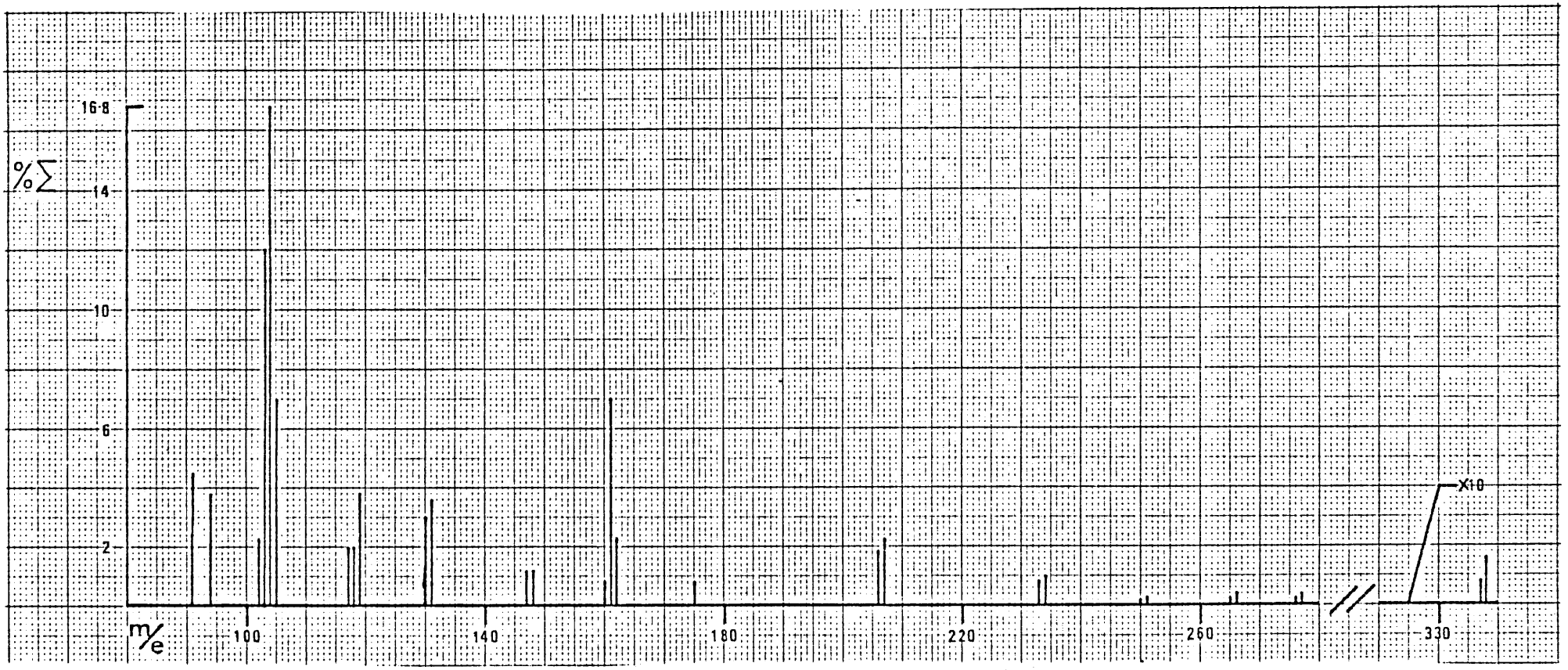
1-DEOXY-L-MANNITOL BISBENZENEBORONATE (110°)



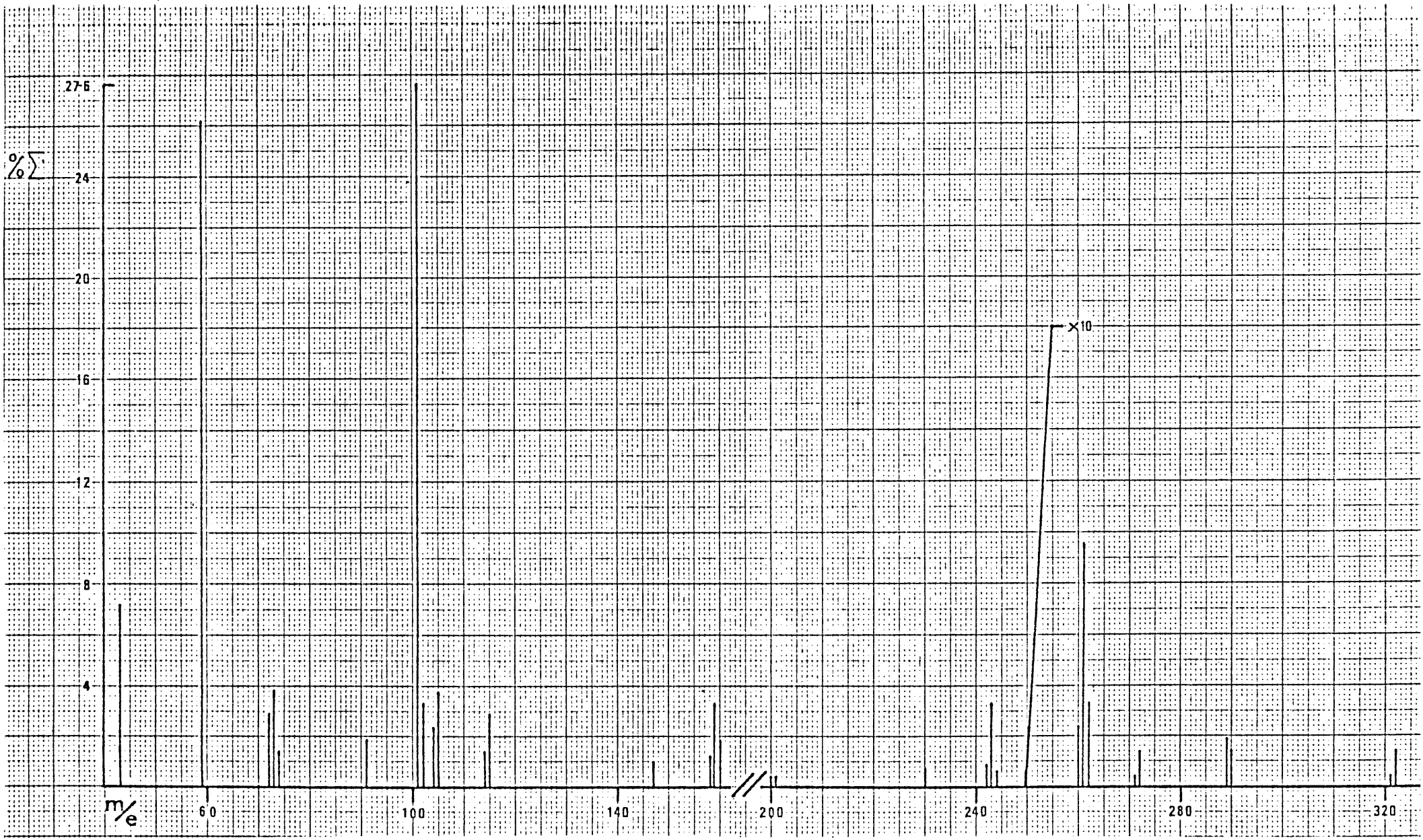
1-DEOXY-D-GLUCITOL BISBENZENEBORONATE (110°)



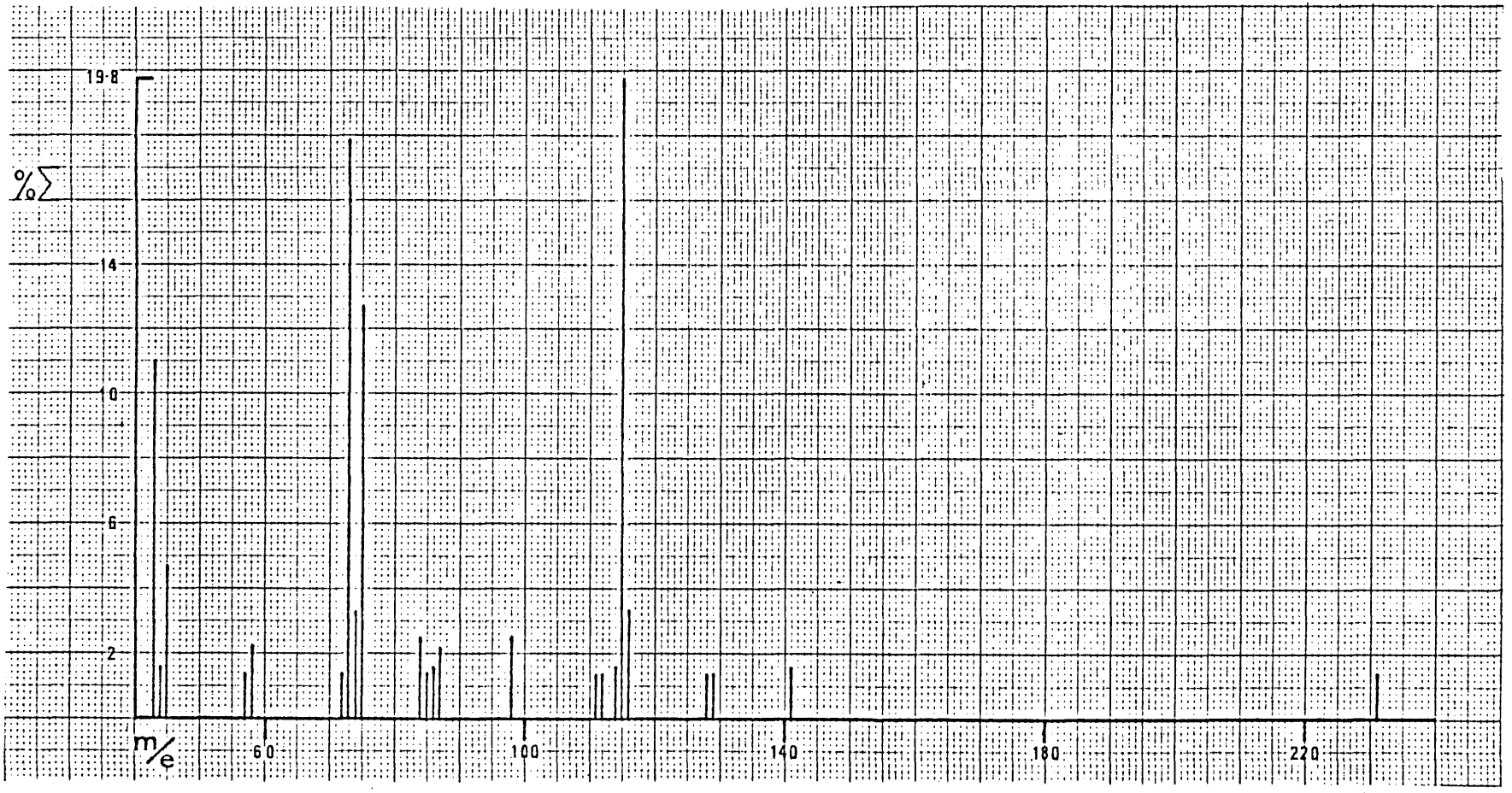
1-DEOXY-D-TALITOL BISBENZENEBORONATE (105°)



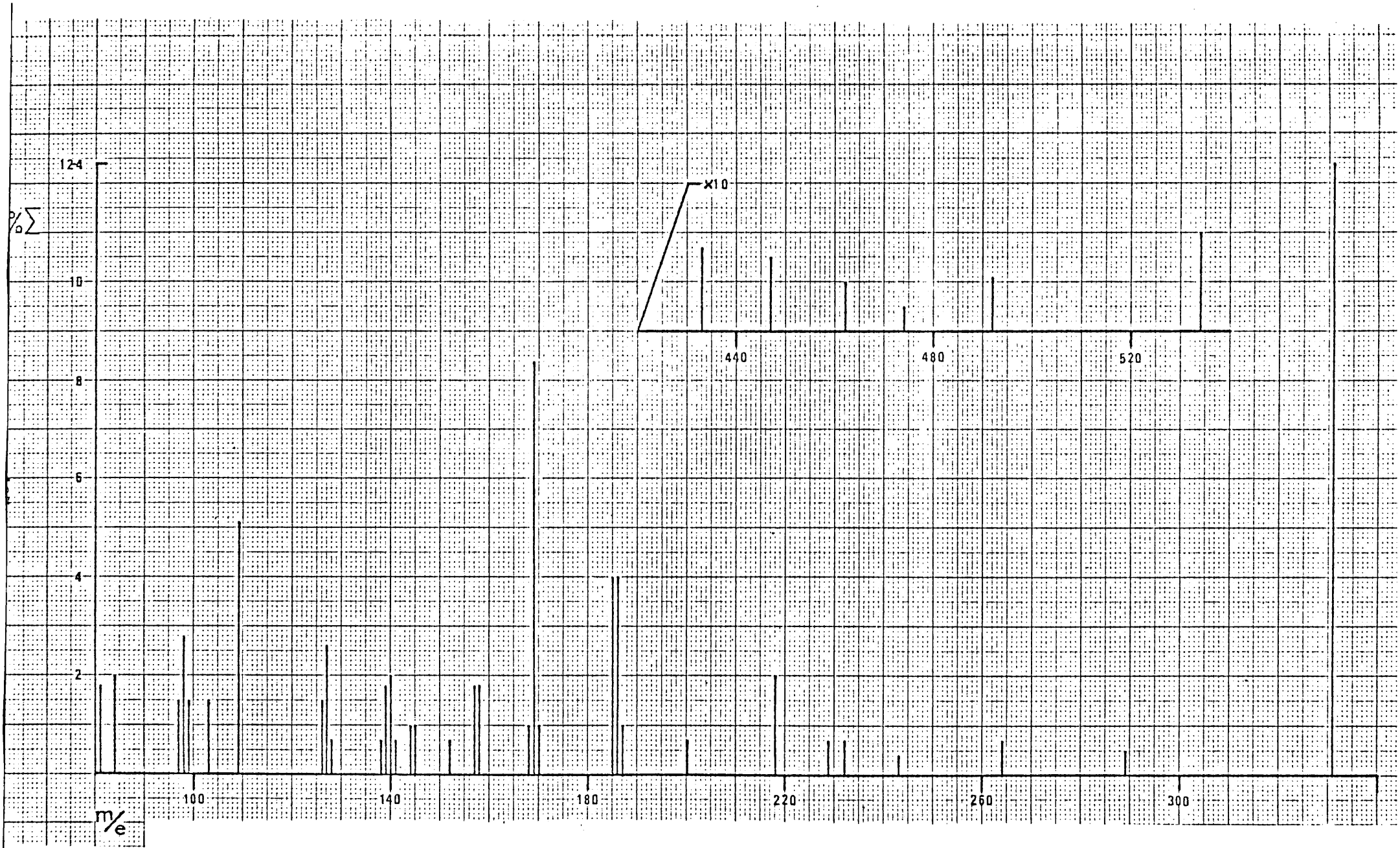
6-DEOXY-D-GLUCITOL BISBENZENEBORONATE (160°)



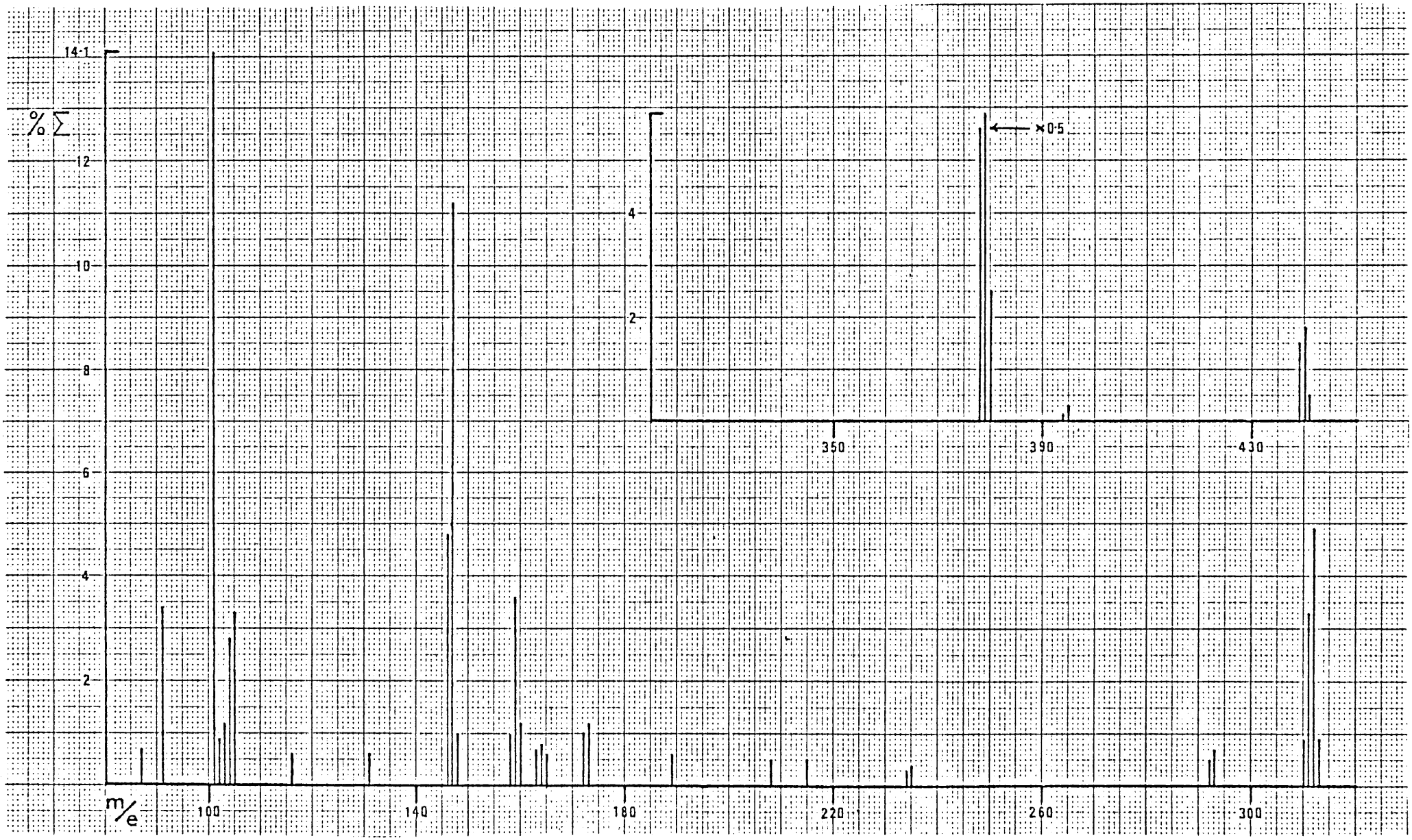
METHYL 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE 4,6-BENZENEBORONATE (200°)



METHYL 2-ACETAMIDO-2-DEOXY-4,6,Di-O-ACETYL-3-O-METHYL-α-D-GLUCOPYRANOSIDE



METHYL 2-ACETAMIDO-2-DEOXY-3-O-(2,3,4,6-TETRA-O-ACETYL-β - D-GALACTOPYRANOSYL)-α - D-GLUCOPYRANOSIDE (200°)



KETOLYSIS PRODUCT OF D-GLUCITOL 1,3 ; 2,4 ; 5,6-TRISBENZENEBORONATE (200°)

B. PRECISE MASS MEASUREMENTS

Measured mass (m/e)	Possible formulae	Calculated mass (m/e)	Multiplet intensity ratio
	Ribitol bisbenzeneboronate		
324.1354	$C_{17}H_{18}B_2O_5$	324.1340	
293.1160	$C_{16}H_{15}B_2O_4$	293.1156	
177.0720	$C_9H_{10}BO_3$	177.0723	
160.0691	$C_9H_9BO_2$	160.0695	
159.0621	$C_9H_8BO_2$	159.0617	
147.0617	$C_8H_8BO_2$	147.0617	
105.0519	C_6H_6BO	105.0512	5/
105.0703	C_8H_9	105.0704	1
104.0437	C_6H_5BO	104.0433	
	Xylitol bisbenzeneboronate		
324.1328	$C_{17}H_{18}B_2O_5$	324.1340	
323.1347	$C_{17}H_{18}{}^{10}BBO_5$	323.1377	
177.0707	$C_9H_{10}BO_3$	177.0723	
176.0693	$C_9H_{10}{}^{10}BO_3$	176.0760	
160.0677	$C_9H_9BO_2$	160.0695	
159.0627	$C_9H_8BO_2$	159.0618	
147.0605	$C_8H_8BO_2$	147.0617	
105.0503	C_6H_6BO	105.0511	
104.0532	$C_6H_6{}^{10}BO$	104.0548	
91.0555	C_7H_7	91.0548	
	D-Arabinitol bisbenzeneboronate		
324.1328	$C_{17}H_{18}B_2O_5$	324.1340	
177.0735	$C_9H_{10}BO_3$	177.0723	
160.0733	$C_9H_9BO_2$	160.0696	
159.0627	$C_9H_8BO_2$	159.0618	
147.0606	$C_8H_8BO_2$	147.0617	
105.0539	C_6H_6BO	105.0512	
92.0640	C_7H_8	92.0626	
91.0554	C_7H_7	91.0548	

Measured mass (m/e)	Possible formulae	Calculated mass (m/e)	Multiplet intensity ratio
	1-Deoxy-L-mannitol bisbenzeneboronate		
338.1498	$C_{18}H_{20}B_2O_5$	338.1498	
337.1534	$C_{18}H_{20}{}^{10}BBO_5$	337.1534	
159.0605	$C_9H_8BO_2$	159.0617	
147.0617	$C_8H_8BO_2$	147.0617	
105.0496	C_6H_6BO	105.0511	
92.0673	C_7H_8	92.0626	
91.0597	C_7H_7	91.0548	
	1-Deoxy-D-glucitol bisbenzeneboronate		
92.0616	C_7H_8	92.0626	
91.0548	C_7H_7	91.0548	
	1-Deoxy-D-talitol bisbenzeneboronate		
338.1504	$C_{18}H_{20}B_2O_5$	338.1498	
263.1062	$C_{15}H_{13}B_2O_3$	263.1050	
177.0723	$C_9H_{10}BO_3$	177.0723	
174.0826	$C_{10}H_{11}BO_2$	174.0852	
173.0774	$C_{10}H_{10}BO_2$	173.0774	
161.0771	$C_9H_{10}BO_2$	161.0774	
159.0662	$C_9H_8BO_2$	159.0617	
147.0611	$C_8H_8BO_2$	147.0617	
105.0339	C_7H_5O	105.0340	1/
105.0512	C_6H_6BO	105.0512	1
104.0549	$C_6H_6{}^{10}BO$	104.0548	1
104.0433	C_6H_5BO	104.0433	/1
91.0548	C_7H_7	91.0548	
	1-Deoxy-L-gulitol bisbenzeneboronate		
159.0597	$C_9H_8BO_2$	159.0617	
147.0592	$C_8H_8BO_2$	147.0617	
105.0497	C_6H_6BO	105.0511	
104.0617	C_8H_8	104.0628	1
104.0397	C_6H_5BO	104.0433	/6

Measured mass (m/e)	Possible Formulae	Calculated mass (m/e)	Multiplet intensity ratio
	1-Deoxy-D-arabinitol bisbenzeneboronate		
174.0815	$C_{10}H_{11}BO_2$	174.0852	
173.0773	$C_{10}H_{10}BO_2$	173.0774	
160.0805	$C_9H_{10}{}^{10}BO_2$	160.0810	4/5
160.0700	$C_9H_9BO_2$	160.0696	
159.0729	$C_9H_9{}^{10}BO_2$	159.0732	4/5
159.0626	$C_9H_9BO_2$	159.0618	
105.0706	C_8H_9	105.0705	1/10
105.0510	C_6H_6BO	105.0511	
104.0549	$C_6H_6{}^{10}BO$	104.0549	2/3
104.0434	C_6H_5BO	104.0434	
91.0544	C_7H_7	91.0547	
	1-Deoxy-D-ribitol bisbenzeneboronate		
174.0845	$C_{10}H_{11}BO_2$	174.0852	
173.0773	$C_{10}H_{10}BO_2$	173.0774	
160.0700	$C_9H_9BO_2$	160.0696	
159.0729	$C_9H_9{}^{10}BO_2$	159.0732	4/5
159.0626	$C_9H_8BO_2$	159.0618	
105.0510	C_6H_6BO	105.0511	5/1
105.0706	C_8H_9	105.0705	
104.0435	C_6H_5BO	104.0434	2/1
104.0549	$C_6H_6{}^{10}BO$	104.0549	
91.0544	C_7H_7	91.0547	
	1-Deoxy-D-lyxitol bisbenzeneboronate		
308.1390	$C_{17}H_{18}B_2O_4$	308.1391	
307.1431	$C_{17}H_{18}B^{10}BO_4$	307.1428	
186.0635	$C_9H_8B_2O_3$	186.0659	
160.0681	$C_9H_9BO_2$	160.0695	
159.0654	$C_9H_8BO_2$	159.0618	
105.0525	C_6H_6BO	105.0512	
104.0455	$C_6H_5{}^{10}BO$	104.0434	
91.0551	C_7H_7	91.0548	

Measured mass (m/e)	Possible Formulae	Calculated mass (m/e)	Multiplet intensity ratio
1,6-Dideoxy-L-mannitol bisbenzeneboronate			
322.1550	$C_{18}H_{20}B_2O_4$	322.1547	
278.1299	$C_{16}H_{16}B_2O_3$	278.1285	
263.1052	$C_{15}H_{13}B_2O_3$	263.1050	
174.0850	$C_{10}H_{11}BO_2$	174.0852	
159.0625	$C_9H_8BO_2$	159.0617	
105.0709	C_8H_8	105.0704	
104.0546	$C_6H_6{}^{10}BO$	104.0548	
1,6-Dideoxy-galactitol bisbenzeneboronate			
322.1541	$C_{18}H_{20}B_2O_4$	322.1547	
307.1310	$C_{17}H_{17}B_2O_4$	307.1312	
278.1293	$C_{16}H_{16}B_2O_3$	278.1285	
263.1054	$C_{15}H_{13}B_2O_3$	263.1050	
174.0850	$C_{10}H_{11}BO_2$	174.0852	
161.0771	$C_9H_{10}BO_2$	161.0774	
146.0530	$C_8H_7BO_2$	146.0539	
105.0512	C_6H_6BO	105.0512	5/
105.0705	C_8H_9	105.0704	1
104.0549	$C_6H_6{}^{10}BO$	104.0548	1/
104.0438	C_6H_5BO	104.0433	1
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate			
322.1462	$C_{15}H_{21}BNO_6$	322.1461	
290.1195	$C_{14}H_{17}BNO_5$	290.1199	
289.1132	$C_{14}H_{16}BNO_5$	289.1121	
272.1092	$C_{14}H_{15}BNO_4$	272.1094	
261.1162	$C_{13}H_{16}BNO_4$	261.1154	
243.1069	$C_{13}H_{14}BNO_3$	243.1067	
201.0942	$C_8H_{14}BO_5$	201.0934	
200.0898	$C_{11}H_{11}BNO_2$	200.0883	
159.0626	$C_9H_8BO_2$	159.0617	
101.0478	$C_4H_7NO_2$	101.0477	
91.0636	$C_3H_9NO_2$	91.0633	
59.0373	C_2H_5NO	59.0371	

Measured mass (m/e)	Possible Formulae	Calculated mass (m/e)	Multiplet intensity ratio
Methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside			
534.1822	$C_{22}H_{32}NO_4$	534.1823	
331.1022	$C_{14}H_{19}O_9$	331.1029	
186.0760	$C_8H_{12}NO_4$	186.0766	8/1
186.0527	$C_8H_{10}O_5$	186.0528	
169.0498	$C_8H_9O_4$	169.0501	
109.0280	$C_6H_5O_2$	109.0290	
Ketolysis reaction mixture of D-glucitol 1,3; 2,4; 5,6-trisbenzeneboronate			
394.1766	$C_{21}H_{24}B_3O_6$	394.1758	
379.1516	$C_{20}H_{21}B_2O_6$	379.1523	
159.0610	$C_9H_8BO_2$	159.0617	
147.0592	$C_8H_8BO_2$	147.0617	
101.0599	C_5H_9O	101.0602	

C. METASTABLE IONS

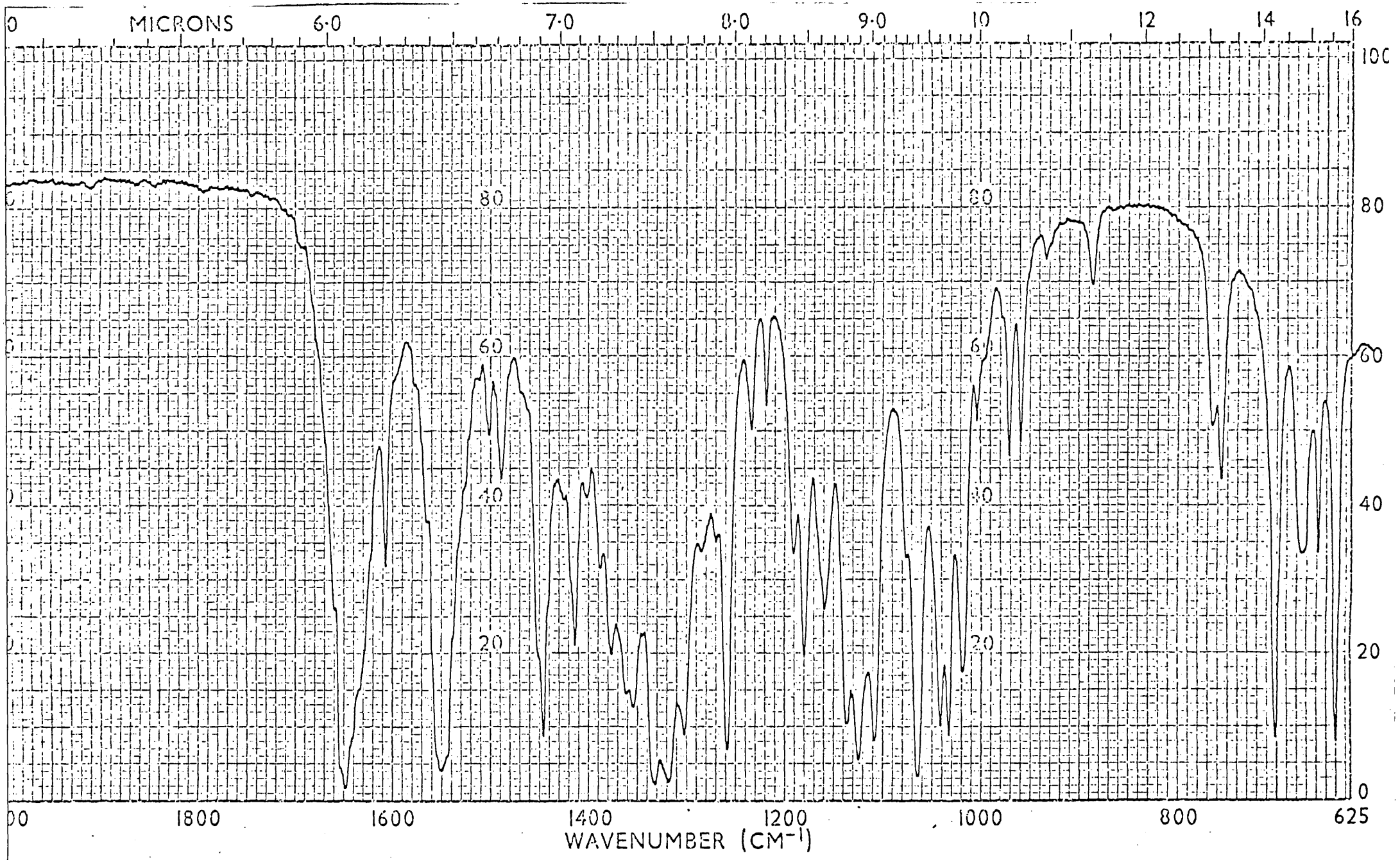
Metastable ions (m/e)		Metastable assignment	
observed	calculated	parent ion	daughter ion
	Xylitol bisbenzeneboronate		
289.0	289.0	324	306
122.8	122.9	294	190
122.1	122.1	177	146
121.1	121.1	176	146
96.7	96.9	324	177
79.0	79.0	324	160
78.0	78.0	324	159
66.7	66.8	324	147
56.3	56.4	147	91
	D-Arabinitol bisbenzeneboronate		
289.0	289.0	324	306
265.0	265.0	324	293
133.1	133.2	190	159
122.8	122.9	294	190
122.1	122.0	177	147
121.1	121.0	176	146
96.7	96.6	324	177
82.6	82.5	306	159
79.0	79.0	324	160
78.0	78.0	324	159
68.0	67.9	159	104
66.7	66.6	324	147
56.3	56.3	147	91
	1-Deoxy-L-mannitol bisbenzeneboronate		
288.0	288.1	323	305
145.3	145.2	174	159
133.1	133.0	190	159
76.7	76.8	338	161
74.8	75.0	338	159
68.5	68.5	161	105

Metastable ions (m/e)		Metastable assignment	
observed	calculated	parent ion	daughter ion
	1-Deoxy-L-gulitol bisbenzeneboronate		
277.8	277.8	338	307
155.7	155.7	190	172
133.0	133.0	190	159
122.8	122.8	294	190
76.7	76.7	338	161
74.8	74.8	338	159
68.5	68.5	161	105
	Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 4,6-benzeneboronate		
260.2	260.2	321	289
226.2	226.2	261	243
212.2	212.0	321	261
166.3	166.3	243	201
164.6	164.6	243	200
126.4	126.4	200	159
125.8	125.8	201	159
99.1	99.3	261	160
39.1	39.1	261	101
35.3	35.3	289	101
34.5	34.5	101	59
	Methyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside		
504.7	504.7	565	534
221.9	222.0	311	271
193.9	193.6	565	331
193.5	193.5	271	229
135.4	135.4	211	169
134.5	134.5	331	211
124.7	124.7	229	169
70.3	70.3	169	109
64.8	65.0	534	186

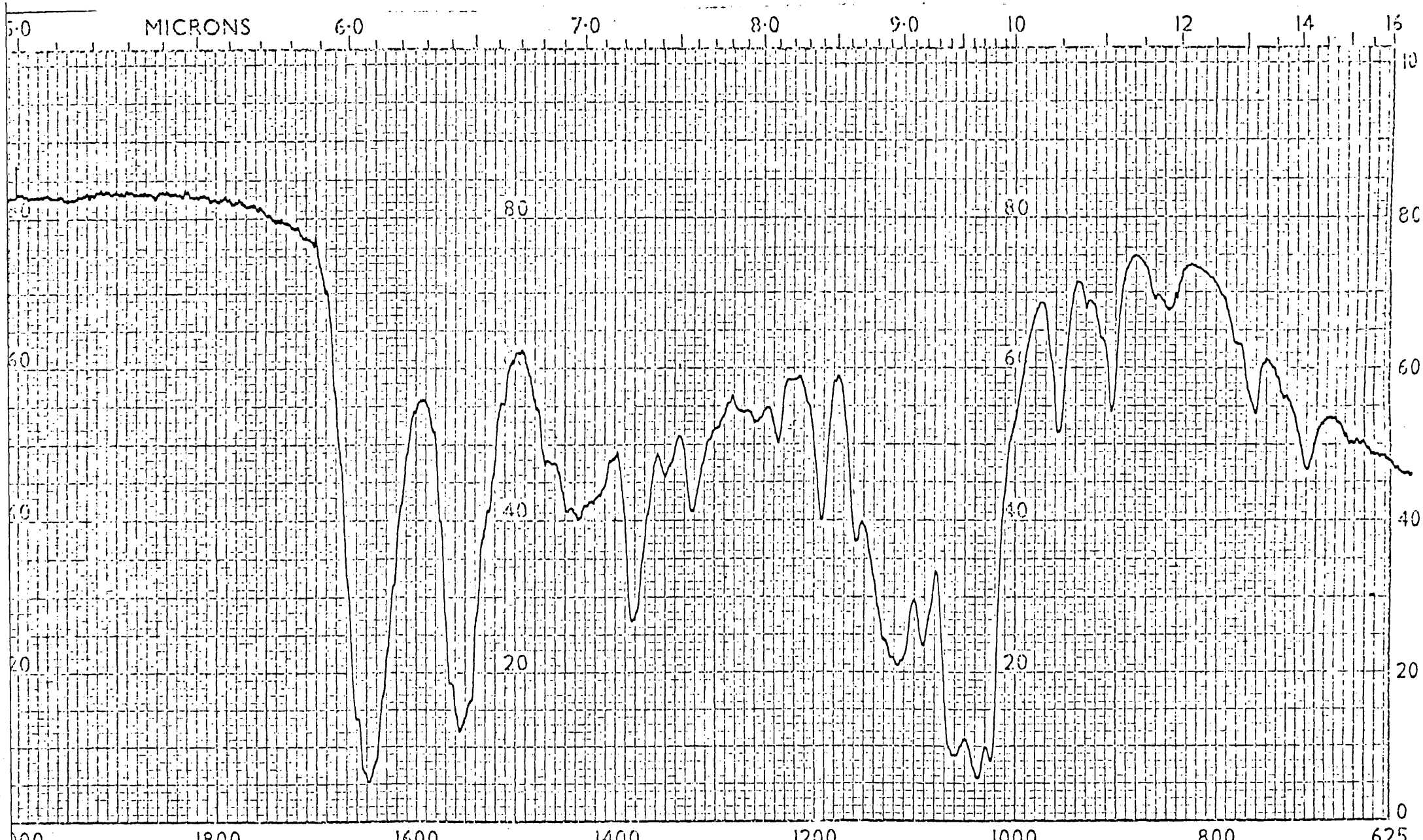
APPENDIX II

INFRARED SPECTROSCOPY

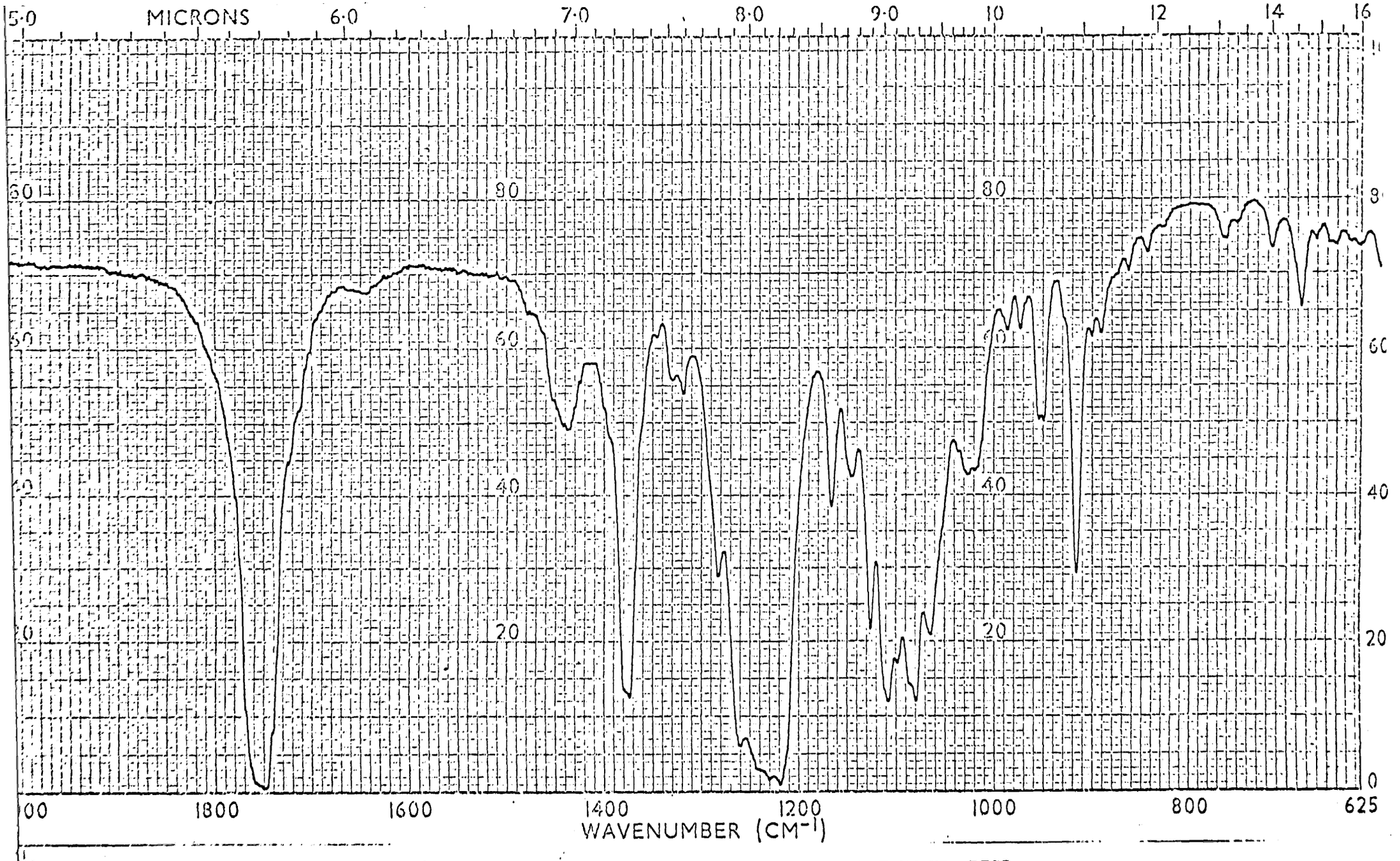
The following infrared spectra show the region: 2000 cm^{-1} - 625 cm^{-1}



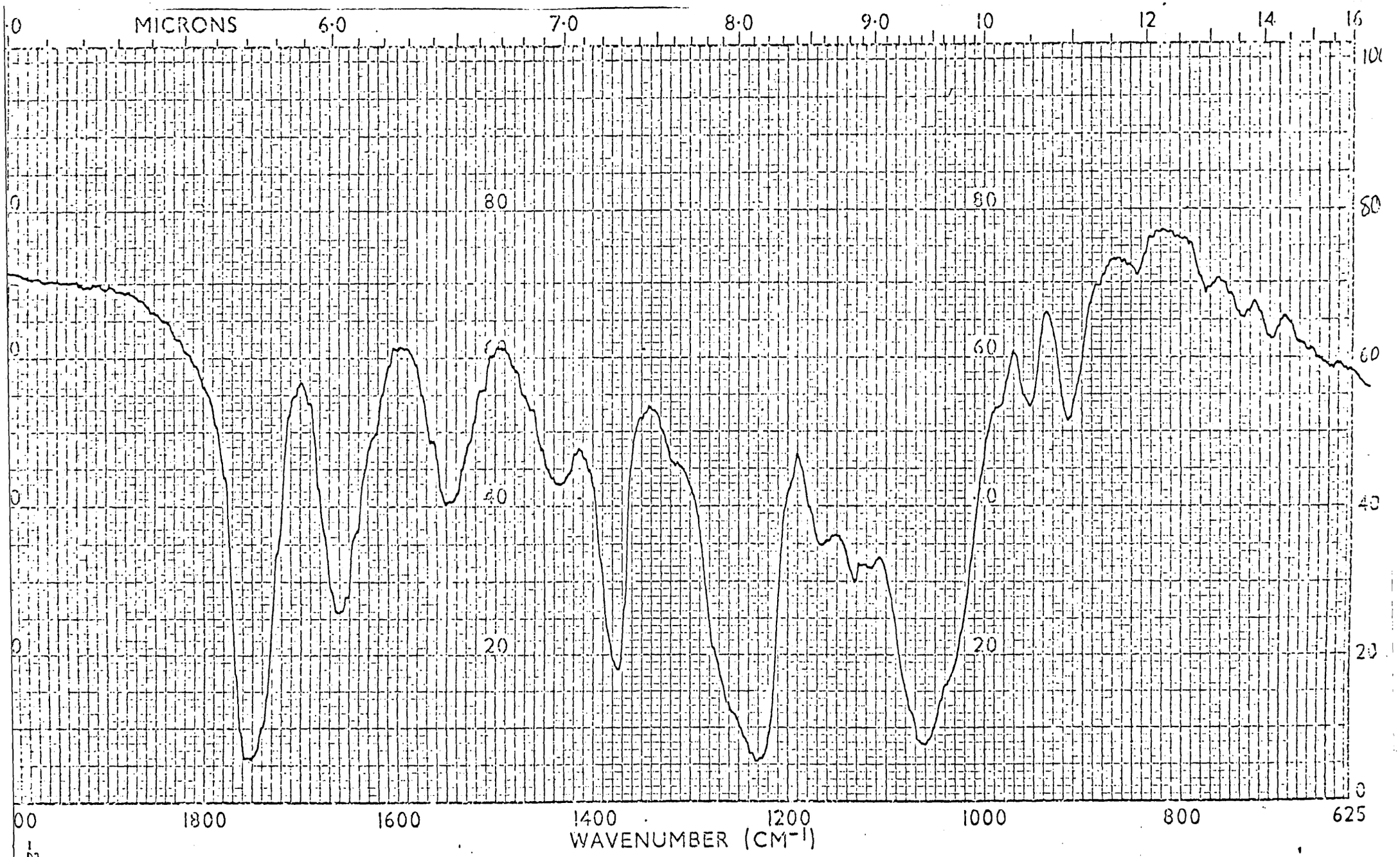
METHYL 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE 4,6-BENZENEBORONATE IN KBr DISC



METHYL 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE IN KBr DISC



2,3,4,6-TETRA-O-ACETYL-α-D-GALACTOPYRANOSYL BROMIDE IN KBr DISC



METHYL 2-ACETAMIDO-2-DEOXY-3-O-(2,3,4,6-TETRA-O-ACETYL-β-D-GALACTOPYRANOSYL)-α-D-GLUCOPYRANOSIDE IN KBr DISC

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