THE APPLICATION OF GAS-PHASE METHODS TO THE ANALYSIS OF NATURALLY OCCURRING COMPOUNDS

A Thesis submitted to the University of Glasgow in part fulfilment of the requirements for the degree of Ph.D.

by

William James Reid

Department of Chemistry

May 1975

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

My sincere thanks are due to Dr. C.J.W. Brooks, for his patient guidance and assistance throughout the last three years, and to Professor R.A. Raphael and Professor G.W. Kirby, for providing me with the opportunity of carrying out this research.

I would also like to thank the gardeners with whom I was associated, namely Mr. Colledge of Logan Gardens, who provided the original samples of the Ligularia and a large sample of Ligularia Tussilaginea, and Mr. Tuck, who looked after the plants for me in the gardens of the Botany Department at Garscube.

Finally, I should like to thank the technical staff of the Chemistry Department, and in particular Mrs. J. Borthwick and Mr. D. Walker, for whose invaluable assistance I am deeply indebted; and my colleagues in the Laboratory, particularly Dr. J.D. Gilbert, for their advice and assistance.

-for my family-

C O N T E N T S

CONVENTIONS

In the text, an Arabic number in brackets, e.g. (127) refers to a reference, while a Roman number, e.g. (CXVII) refers to a diagram or drawing.

In the drawings of chemical compounds, a wedge shaped line represents a bond coming out of the plane of the paper, while a broken line is a bond going below the plane of the paper. A dotted line running between three carbon atoms implies a double bond, the position of which is ambiguous.

In the discussion, whenever both the methyl and phenyl boronates are illustrated, a common diagram will be used for both, with R- denoting the substituents. The illustration will be numbered with a Roman numeral, and the suffix, a, will be used to denote a phenyl ester, the suffix, b, to denote a methyl ester. For example, for the ion XXX the phenyl- form is labelled XXXa, and the methyl- form XXXb.

ABBREVIATIONS

Gas liquid chromatography	GLC
Combined gas liquid chromatography and mass spectrometry	GC–MS
Trimethylsilyl	TMSi
bis(trimethylsilyl) trifluoroacetamide	BSTFA
trimethylchlorosilane	TMCS
hexamethyldisilazane	HMDS
hydrochloric acid	HCl
Electron-Impact	EI
mass to charge ratio	m/e
molecular ion	M
dimethylformamide	DMF
retention index	I
molecular weight	M.W.
Nuclear Magnetic Resonance	NMR
thin layer chromatography	TLC

1. GENERAL INTRODUCTION AND SUMMARY OF THE THESIS

In the last few years, increasing interest has been shown in the related techniques of gas liquid chromatography (GLC), and combined gas chromatography and mass spectrometry (GC-MS), and in particular in the application of these techniques to the analysis of small amounts of material, such as those which may be found in natural systems. This thesis investigates the use of these techniques in two distinct fields; the first is the examination of boronic acids as reagents for the formation of derivatives of sugars suitable for GC and GC-MS analysis, and the second is the application of the techniques to the investigation of extracts from plant tissue for the purpose of locating and identifying any compounds of a particular type which may be present in the plant.

In 1968, Brooks and Watson introduced the concept of boronate esters as suitable derivatives, for GLC and GC-MS, of compounds possessing a cis bifunctionality, such as cis diols. They suggested the applicability of the derivative to carbohydrate analysis, and also showed that pregnanetriol boronate could easily be trimethylsilyated without disturbing the cyclic ester group. Brooks and Harvey also showed, in a later paper, that triple derivatives of a compound could be made, for example, the methyl oxime of a cyclic boronate trimethylsilyl ether. In this same paper the authors demonstrated that acyclic boronates formed from isolated hydroxyl groups could be displaced by trimethylsilyation without affecting a cyclic boronate also present in the molecule. This last observation was extremely important, as it showed that excess boronic acid could be used to form a derivative of a compound and any acyclic boronates formed could be removed in this way. This was ideal for the study of compounds like sugars, where there are a number of hydroxyl groups present. Excess boronic acid could be used to form the cyclic boronates, and the compound would then be trimethylsilylated to eliminate any undesired acyclic

boronates formed. The trimethylsilylation would also result in a less polar, more volatile compound which would have better GLC properties.

Shortly after the inception of the present research, there appeared the first of a number of papers on this topic; these demonstrated that it was possible to use the boronates in such a way, and that where the reaction resulted in one or more hydroxyls remaining underivatised, it was possible to convert them to the trimethylsilyl ethers, without destroying the boronate already present, producing a mixed derivative that was suitable for GLC and GC-MS.

To investigate these derivatives further, a comparison has been made of the GLC properties of the methyl-, n-butyl-, and phenylboronates and boronate TMSi ethers of a number of sugars, each of which was known to be, initially, in a cyclic form, and hence to have a preferred conformation which would influence the course of the boronation reaction in which the boronic acids react only with cis-bifunctional systems. The GC-MS properties of the methyl- and phenyl- derivatives have also been studied, the difference of 62 mass units between the methyl and phenyl groups being an aid to the identification of boron-containing peaks. From the gas chromatographic data and mass spectra, and in some instances the nuclear magnetic resonance data, an attempt has been made to determine the structure of the boronates and boronate TMSi ethers formed by each sugar.

The boronates, and the boronate TMSi ethers, have been found to be good derivatives for GLC, giving sharp, non-tailing peaks on both 1% SE-30 and 1% QF-1, and though the tendency of the sugars to form multiple derivatives would make this method difficult for the routine analysis of a large number of compounds, it does simplify the identification of individual sugars by increasing the number of useful parameters characteristic of each sugar.

The determination of the structures of the sugar derivatives was not found to be possible in all cases simply by consideration of the boron-containing ions, as these can rearrange under electron impact. Thus, for example, ions containing a five-membered boronate ring could be derived from an ester which contained only a six-membered ring, but by combining the information obtained from such ions with that obtained from the rest of the spectrum, it was possible to arrive at the structures in question.

The formation of boronate esters by these sugars is determined by the availability of suitable diol systems, which in turn is governed by the number, position and conformation of the free hydroxyls in the parent sugar. Four hydroxyls may result in either a diboronate, or a number of monoboronates which leave two of the groups unreacted, while three free hydroxyls result in two possible monoboronates, the favoured one being determined by the parent structure.

The mass spectra can also be used to distinguish between very similar compounds, such as: methyl D-mannopyranoside, methyl D-glucopyranoside, and methyl D-galactopyranoside, and to some extent between the anomers of a sugar, for example between the α- and β- anomers of the last two of these compounds.

Thus the boronates and boronate TMSi ethers are suitable derivatives for GC and GC-MS, giving as much information as, and in some cases more than the TMSi ethers by themselves.

The second part of the thesis consists of an examination of the applicability of GLC and GC-MS to the analysis of plant material, and in particular, to the location of sesquiterpenoid compounds in the plants examined.

The classical method of examining plants has been to extract a large amount of the plant material in a heated solvent, which was then removed by evaporation; and then to chromatograph or distil the residue, collecting any fractions which might be pure compounds and

determining their structure, usually by spectroscopic methods. This method has the disadvantages of requiring the collection and processing of a large amount of plant material, and of exposing the extracts obtained to heat or to a chromatographic material which might result in the decomposition or isomerisation of any unstable compounds present.

As an alternative to this, the use of GLC and GC-MS in this type of investigation has been investigated. The macerated plant material is extracted at room temperature in a suitable solvent, which is then removed in a Büchi rotary evaporator, again at room temperature, and the soluble portion of the residue is dissolved in a mixture of benzene and isopropanol in the ratio of 3 : 1. After filtration to remove the insoluble residue this solution is passed through a liquid-gel column acting as a molecular filter, to remove the high molecular weight compounds, which would not be amenable to GLC, but would remain as contaminants at the beginning of the GLC column. The eluate is then examined by GLC and GC-MS, with the aim of locating interesting compounds which can be distinguished by their behaviour on a GC column, and under electron impact.

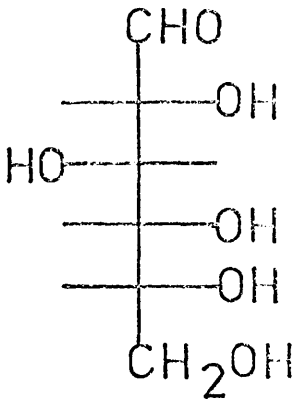
Standardisation of the method was effected while investigations were being carried out on the plant Degeneria vitiensis and a preliminary survey of this plant has been made. Two species of Petasites have been examined and compared, with particular regard to the sesquiterpenoids of the petasin type present in each.

The major part of this investigation has been concerned with the examination of six plants of the genus Ligularia. Plants of this type have been examined by various groups of workers and have been shown to contain a wide range of sesquiterpenoid compounds. This study was made of six plants which were growing in the Logan Botanic Gardens in Wigtownshire, though the plants themselves have an eastern origin, being native to Eastern Asia.

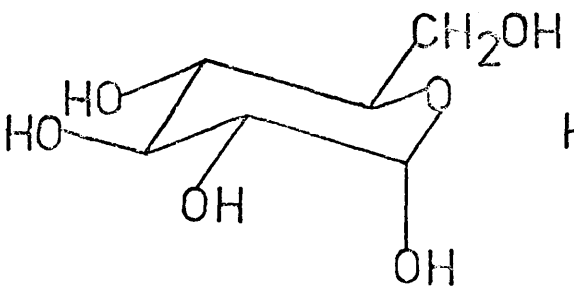
The examination showed in particular the close relation these plants bear to the genus Petasites, since petasin itself was found in two of them, while one other Ligularia tussilaginea, contained a number of compounds similar to furanopetasin. Many of the Petasites are divided into two sub-species, one of which contains petasin type, the other, furanopetasin type esters, and the two classes of compounds are not known to co-occur in the same plant. This may also be true of the Ligularias and would, therefore, explain why none of the plants examined showed the presence of both classes of compound. The examination of Ligularia tussilaginea also pointed out the usefulness of the analysis technique when dealing with unstable compounds, since an important compound was detected which had not been reported in a previous study. This compound was very unstable, being susceptible both to air oxidation and to addition reactions, so it would almost certainly not have been analysable by traditional methods.

Structural identification of the compounds found was not always possible simply from the GLC and GC-MS data. It was found to be necessary to isolate as large a sample of each compound as possible, and to examine this by spectroscopic methods, such as infra-red (IR) nuclear magnetic resonance (NMR) and ultra-violet (UV) spectroscopy. In those cases where this was found to be impossible, however, tentative identifications of the structures concerned were made from the mass spectra.

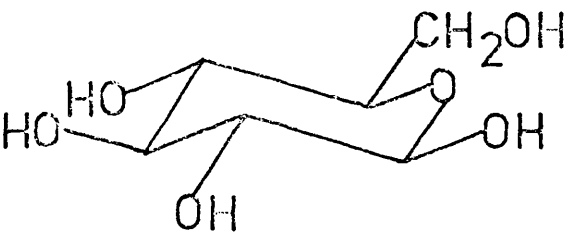
Thus the method is a useful tool for the analysis of compounds occurring in plants, but it is most efficient as part of a system of analysis which involves the use of a complementary range of methods, in which system it operates best as a screening process to discover the presence of any interesting compounds which might make further work worthwhile.



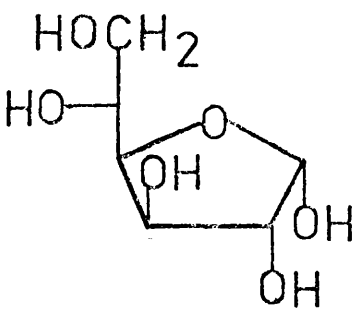
I



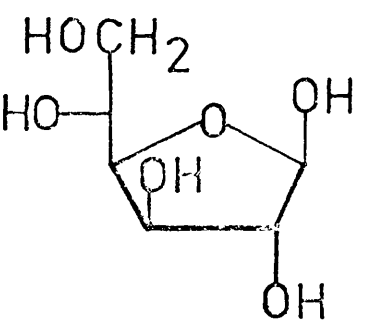
II



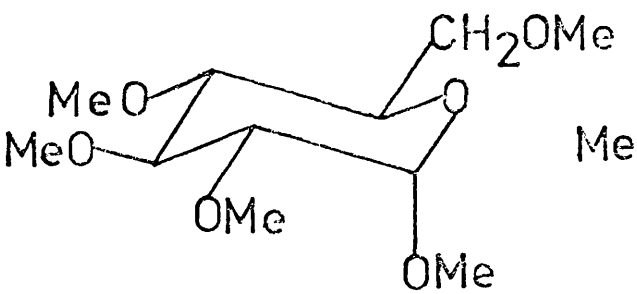
III



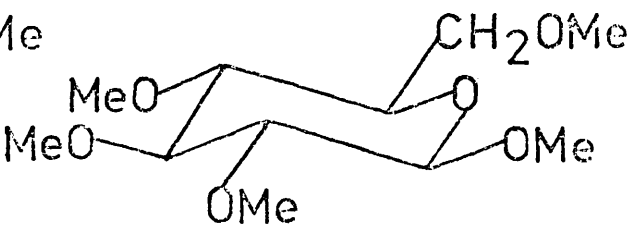
IV



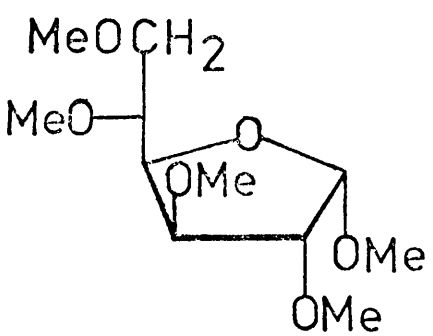
V



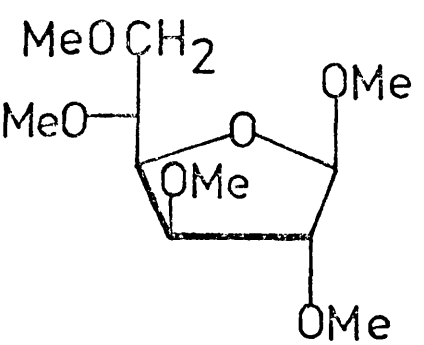
VI



VII



VIII



IX

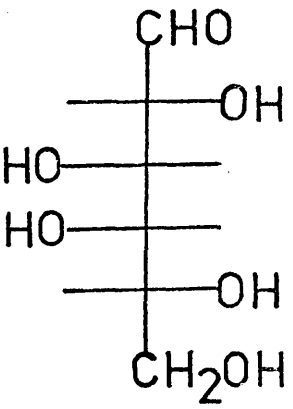
2. AN EXAMINATION OF THE BORONATE ESTERS OF SOME SUGARS

2 - 1 Introduction and Historical Background

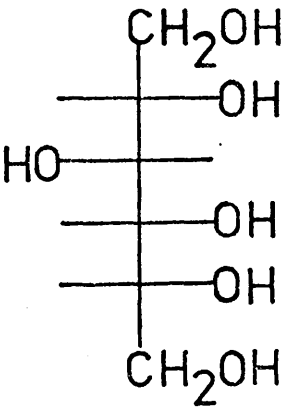
The analysis of monosaccharides can be regarded as a problem in the separation and characterisation of a mixture of isomers, particularly when examining naturally occurring compounds. When gas liquid chromatography, (GLC), and combined gas liquid chromatography and mass spectrometry, (GC-MS), were introduced, interest soon arose in the application of these techniques, which coupled speed with a small sample requirement, to this field.

The free sugars themselves proved to be unsuitable for GLC as they possess several polar hydroxyl groups, which lead to badly trailing peaks and very long retention times, even with the least polar stationary phases such as SE-30. It was found to be necessary to alter the properties of the sugars in some way; ideally some derivative of the hydroxyls was required. This should be easy to form, giving no by-products which would appear on the GLC trace, volatile, and stable under GLC conditions. It should also retain the stereochemical properties of the parent molecule so that derivatives of different compounds should be different enough to be resolvable on GLC, and have distinctive breakdown patterns in the mass spectrometer.

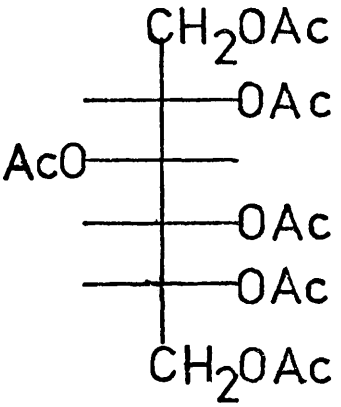
Both methyl ethers (1-9) and acetates (9-19) have been used as derivatives for GLC in this way, but in each case the derivative formation has resulted in multiple GLC peaks due to the formation of isomers during the reaction (16). A free sugar, e.g. glucose, can exist in five forms, the acyclic isomer (I), and the α- and β- anomers of the pyranose (II, III) and furanose (IV, V) rings. Formation of a derivative, e.g. the methyl ether, results in four isomers, the α- and β- anomers of each cyclic form (VI-IX). Each of these compounds



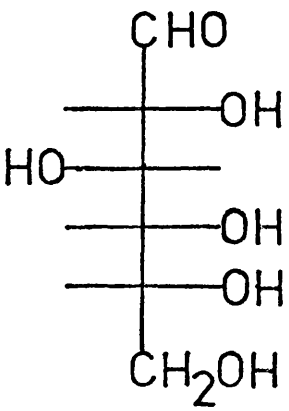
X



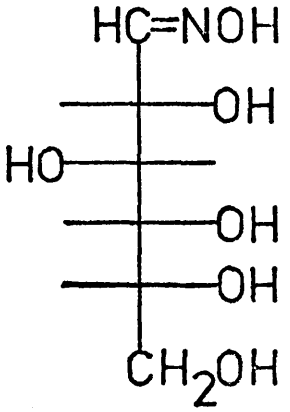
XI



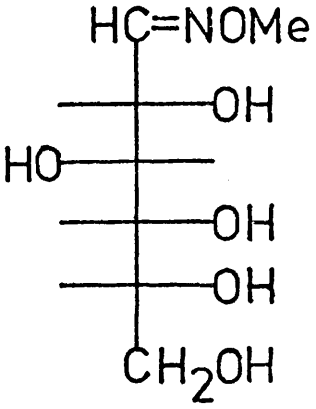
XII



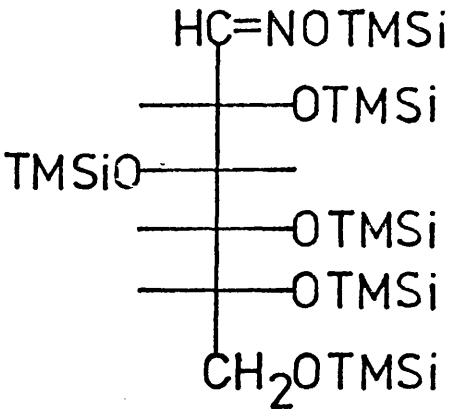
I



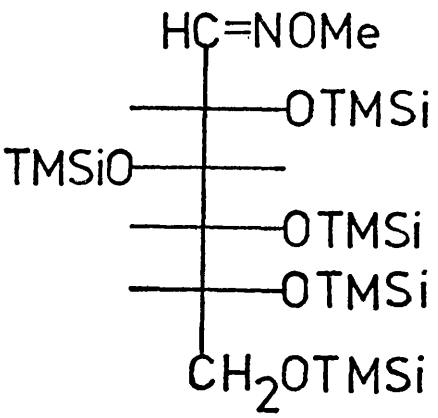
XIII



XIV



XV



XVI

gives a peak on the GLC trace, increasing the difficulty of analysing a mixture of sugars. Many different ways of overcoming this difficulty have been attempted; for example the analysis of aldose sugars, such as glucose (I) and galactose (X) has been investigated by J.S. Sawardeker et al. (16). They first reduced the sugar to the alditol with sodium borohydride, then reacted it with acetic anhydride to form the acetate. Glucose (I) has, for example been reduced to glucitol (XI) which was then converted into the acetate (XII). Another approach has been the use of mixed derivatives, for example, the trimethylsilyl (TMSi) ethers of sugar oximes (20–22). The sugar e.g. glucose (I) is converted to the oxime (XIII) or the O-methyloxime (XIV) and then treated with trimethylsilylating reagents, for example, bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS). The free hydroxyls are converted into the TMSi ethers, the oximes into O-trimethylsilyloximes (XV) and the O-methyloxime (XVI) is unaffected (22). The mass spectral fragmentation patterns of such O-methyloximes have been studied (23), as have those of methyl ethers and acetates.

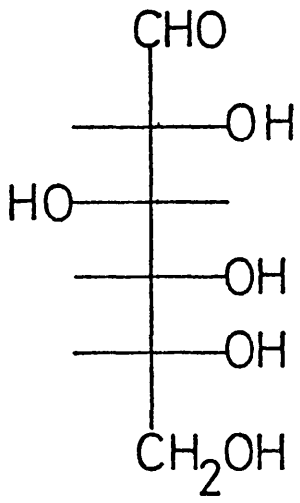
In the case of the acetates the pyranose and furanose forms can be differentiated (24–26), though rearrangement of the ions from the six-membered ring to the five-membered ring is possible (26) and care should be taken when analysing the mass spectrum of an unknown compound. Again, to simplify the analysis, the alditol acetates have been made and their spectra examined (16, 27, 28). The methyl ethers give characteristic peaks in the high-mass end of the spectrum, but, since these are of low intensity, they call for careful evaluation during interpretation (29–34).

The most widely used, and most successful derivative to date, however, is the TMSi ether. The preparation of carbohydrate TMSi ethers was first described by Hedgley and Overend (35), and later standardised for GLC by Sweeley et al., who also gave retention data for over a hundred compounds (36). The ethers were prepared by treating the sugar with a 2 : 1 mixture of hexamethyldisilazane (HMDS) and TMCS in pyridine. Excess of the reagents was used to ensure complete

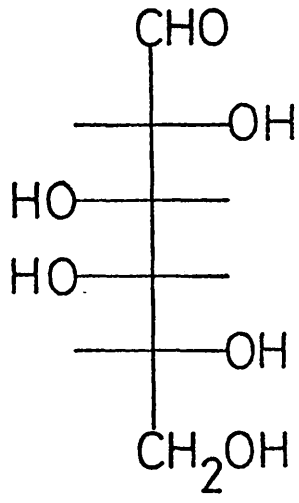
derivatisation, and to eliminate any traces of water in the reaction mixture. An aliquot was then drawn off and injected straight on to the GLC column, or, to avoid the very large solvent peak caused by the pyridine, the derivatives were partitioned between water and chloroform (or petroleum ether) and recovered from the organic solvent, in which they could be injected (36). Pyridine could also be removed by evaporation under a stream of nitrogen, then, with or without the removal of the insoluble pyridine hydrochlorides, re-dissolved in a more volatile solvent (38). Injection of the inorganic precipitate onto the GLC column does not appear to do any damage, even over prolonged periods of use (37).

The derivatives formed in this way fit closely to the ideal. They are easy to form, convenient to handle, stable at the temperatures used in GLC and they are also readily hydrolysable back to the free sugar, a distinct advantage when they are used for preparative GLC (36). In only a few cases the reaction has not proceeded satisfactorily, generally when using compounds containing acid groups, for example, uronic acids (39), or ascorbic acid (40). It is thought that hydrochloric acid (HCl) is formed during the reaction, and that this inhibits the ether formation (17). Modification of the acid group or use of a different silylating reagent (39) were the methods used to overcome this difficulty. The reaction is quantitative (36), and there is good agreement between the results obtained by quantitative gas and paper chromatography of sugars (41, 42). The derivatives have good retention characteristics, giving good peak shapes with little tailing, even on fairly polar columns, and retention times (adjusted to give good resolution) of between five and thirty minutes (36, 43-64).

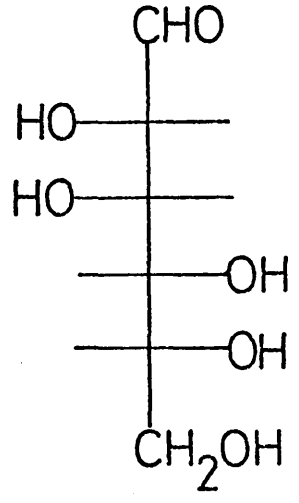
The major disadvantage of the TMSi ethers is the formation of isomer peaks. Each sugar TMSi ether gives two to four peaks, corresponding to the α- and β- pyranosides and the α- and β- furanosides, (17, 36, 45, 64, 65). This occurs especially when water is present in the reaction mixture, catalysing the equilibration of the mixture of



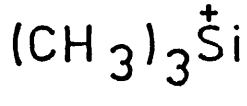
I



X



XVII



XVIII

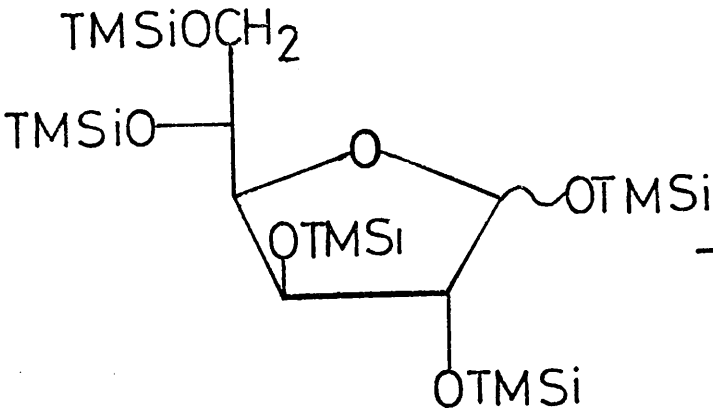
isomers (17). Thus for mixtures of TMSi ethers of sugar stereoisomers, for example, glucose (I), galactose (X) and mannose (XVII), the resolution of anomers and ring isomers gives many coincident and overlapping peaks (17).

Under given conditions of derivative formation, the number of peaks and their relative proportions are found to be constant for each sugar. It is thus possible to calculate the total amounts of each sugar present from any one single, completely resolved and identified peak (36, 67, 68, 69). Tables of these proportions are available (17).

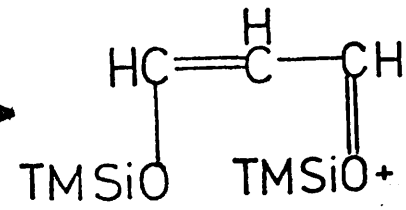
Considerable variation is found, however, between sugars analysed from pyridine and aqueous equilibrium mixtures, and between sets of values based on similar preparative procedures but different solvents. In pyridine, trimethylsilylation is faster than mutarotation (36), therefore, it is possible to determine naturally occurring ratios of sugar anomers if analysis is made soon after derivative formation, and if the reaction has been carried out in scrupulously dry pyridine (19). Recently, also, equilibria of this type have been studied by using the trifluoroacetates as derivatives of the sugars (70).

Identification of individual peaks does, however, present a problem. Such a peak may be known to be related to a specific compound, but the absolute identification of it as one isomer or anomer can be difficult. GLC can provide only relative information from standards, not direct structural or chemical data. It is possible to separate each compound by preparative GLC and determine its structure by classical methods, but this is cumbersome, and the time required would cancel the advantage of using GLC. Such a procedure is impracticable, if the sample under consideration is too small. Hence the use of GC-MS.

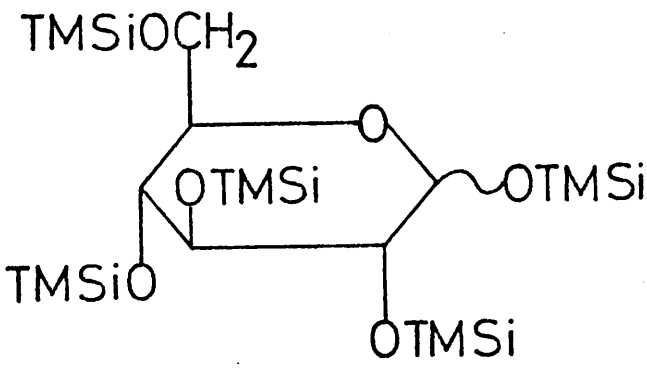
In 1957, Sharkey et al, reported mass spectral data of silylated, long chain, aliphatic alcohols, and most of the observations and ideas of these authors are still tenable for other classes of compounds (72). The electron-impact (EI) mass spectra were characterised by the fragment of mass to charge ratio (m/e) 73. (XVIII), which is present in the



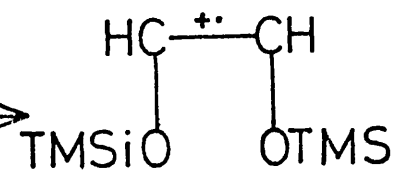
XIX



XX



XXII

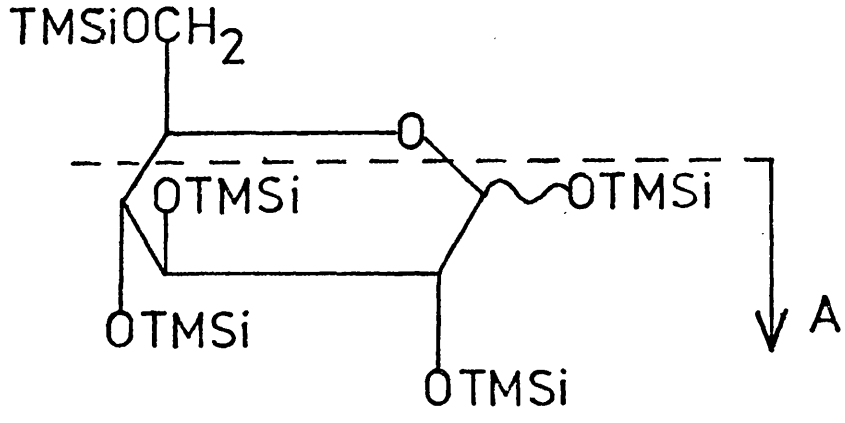


XXI

highest abundance. The highest m/e value is generally that of the molecular ion $(M)^+$ minus 15 due to the loss of methyl from one of the TMSi groups. In a recent paper, (73) Richter and Hunneman have compared the spectra of the TMSi ethers and dimethylsilyl (DMSi) ethers of primary alcohols, and have shown that, for the latter derivative, the relative intensity of the M-15 ion is severely reduced with respect to that of the corresponding TMSi ethers. A number of rearrangements have been reported as occurring during the breakdown of a TMSi ether; for example, the loss of a methyl group with subsequent replacement by a hydrogen atom (74), intramolecular migration of complete TMSi groups (75, 76) and McLafferty-type rearrangements (77, 78).

Turning back to the problem of peak identification, it has been shown that structural and geometrical isomerism influence mass spectral fragmentation patterns (79, 80). For example, from the consideration of quantitative mass spectra of twenty TMSi ethers of aldohexoses and partially methylated derivatives, Petersson and Samuelson (81) developed a method of determining the number and position of methoxyl substituents in monosaccharides. DeJongh et al. (71) established detailed fragmentation patterns of D-glucose, Me D-glucopyranoside, and ethyl D-galactopyranoside by the use of labelling techniques and high resolution mass spectrometry. In agreement with other work (66, 81-84) they showed differences between the pyranose and furanose ring isomers, e.g. for a furanose TMSi ether such as that of glucose (XIX) the intensity of the fragment m/e = 217 (XX) is significantly larger than that due to m/e = 204 (XXI), while for the corresponding pyranose (XXII) compound the reverse is true. These differences have been used to identify minor peaks in the GLC of silylated aldoses (71), and in those of fructose where the open-chain derivative of the compound could be distinguished (66).

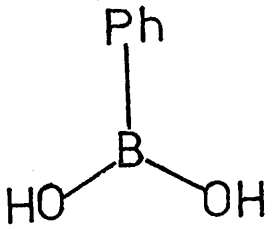
The mass spectra of α- and β- anomers have been examined with varying results; Reed et al. originally showed differences between the spectra



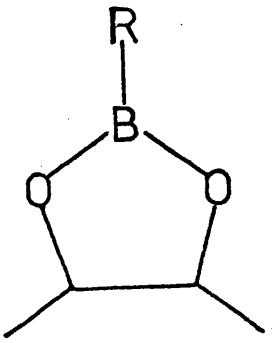
XXIII

of Me α-D-glucopyranoside and Me β-D-glucopyranoside (85, 86) but DeJongh et al. claimed that the spectra of anomeric forms of penta-O-trimethylsilylated glucopyranose were identical as were the anomers of the corresponding galactopyranose (71). Similarly, no differences were noted in the spectra of the corresponding pertrimethylsilylated methyl glycosides. These experiments were repeated using a lower ionisation voltage in the mass spectrometer in an attempt to limit secondary fragmentation processes, and so accentuate stereochemical differences (87). The spectra of the trimethylsilylated anomers run at 17eV do show minor intensity differences, particularly in the ratio of the two peaks of m/e = 435 and m/e = 393. The former is due to the loss of a methyl group from the parent molecule, the latter to fragment A (XXIII), minus a methyl (71). The ratio 435/393 is larger in the spectrum of the β-anomer than in that of the α-anomer. Similar differences were found in galactopyranose and mannopyranose, and in the methyl glycosides of all three sugars. There the ratio considered was that of m/e = 377 caused by loss of $-CH_3$ and trimethylsilanol (TMSiOH) from the molecule, to m/e = 361, caused by loss of OCH_3 and TMSiOH (89). These ratio differences are useful for analysis but they have two main drawbacks. The ratios vary if the ion source temperature of the mass spectrometer is altered, which may cause difficulty in reproducing them, particularly from machine to machine, and secondly a very large body of data would have to be built up before the technique could be used for routine analysis.

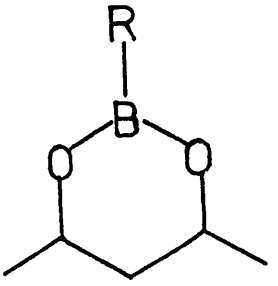
Scheuer and co-workers (89) examined, in glycol TMSi ethers, the m/e = 147 fragment common to many dihydroxy compounds (76, 91) in which the hydroxyls need not be adjacent to each other (76). In the glycol derivatives this fragment arises from a cyclic elimination leading to $(TMSiO = Si(CH_3)_2)^+$. They argued that the intensity of this ion could be related to the ease of formation with which the transition state could occur, and predicted the order of intensities that should occur in the α- and β-anomers of glucose, galactose and mannose. These were a close match to the experimental values, and so the basic postulate



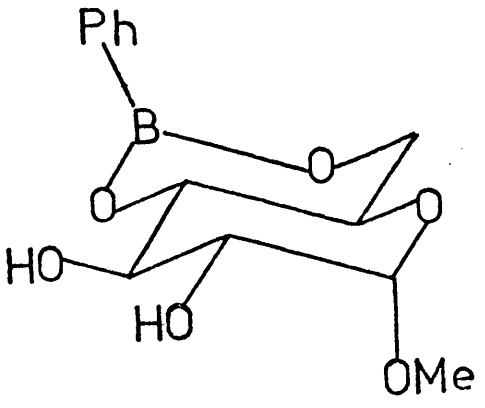
XXIV



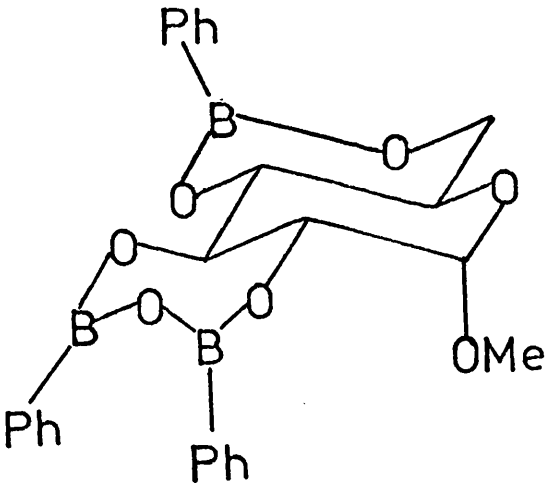
XXV



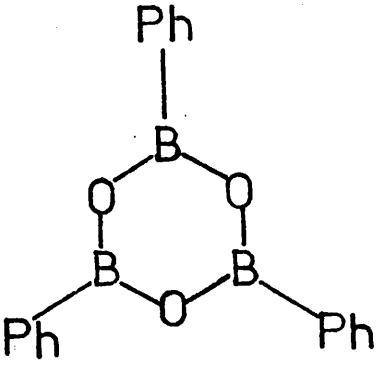
XXVI



XXVIIa



XXIXa

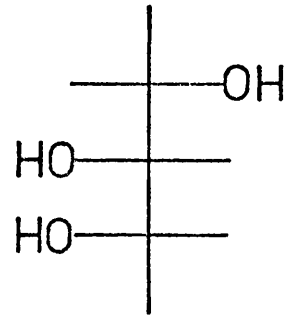


XXVIIIa

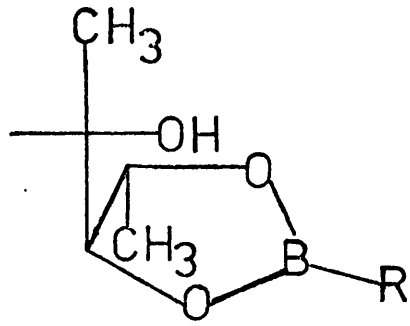
may hold and may in time be applied to structure determination.

Applications of the TMSi ether to sugar chemistry are many and various. Zinbo and Sherman have studied the fragmentation of some sugar phosphates (75), as have Harvey, Horning and Vouros (92, 93), Petersson has examined the TMSi ethers of acyclic alditols (94) and of the lactones from the corresponding aldonic acids (95) and he has also used the methyloxime-TMSi ethers in a very general method for the analysis of sugars, sugar acids and lactones (96). TMSi ethers have been used by deWilt (98) in the study of the oxidation of sorbose to 2-keto-L-gulonic acid, and by Kamerling, Vliegenthart, Vink and de Ridder for the analysis of oligo-saccharides and disaccharides (99, 101), and in the study of partially methylated sugars occurring in the sphingoglycolipid found in oyster mantle (102).

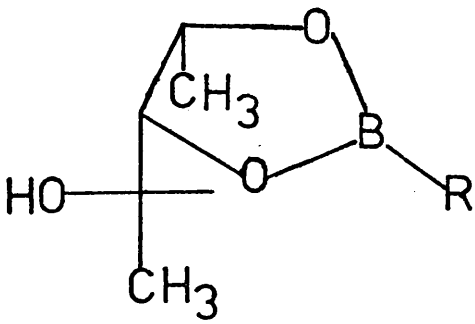
Recently, a new derivative has been used for the GLC and GC-MS analysis of sugars, viz, the cyclic boronate ester. This is formed by the reaction of, (for example) a 1,2- or 1,3- diol system with a boronic acid, e.g. phenylboronic acid (XXIV), resulting in a five-membered (XXV) or six-membered ring (XXVI) boronate respectively. The first sugar boronates were made by Wolfrom and Solms (103), who fused a mixture of phenylboronic acid and the sugar and extracted the products with petroleum ether to give the ester. Later workers, however, used different reaction conditions since the realisation that a rapid equilibration was taking place (104) suggested the use of anhydrous solvents and the removal of water from the reaction to promote the formation of the ester (105). The 4,6- cyclic ester of Me α-D-glucopyranoside (XXVIIa) was prepared by treatment of the sugar with phenylboronic acid in boiling benzene, removal of the water being effected with a Dean and Stark apparatus, as was the per(phenylboronate) (XXIXa) (106, 114). Treatment of the sugar with the acid anhydride (XXVIIIa) (107) under similar conditions was also shown to give the ester (108-110), as was the use of 2-methoxyethanol (113), Me-cellosolve (115), or a methanol and water mixture (111,112) as a solvent for the sugar and anhydride reaction.



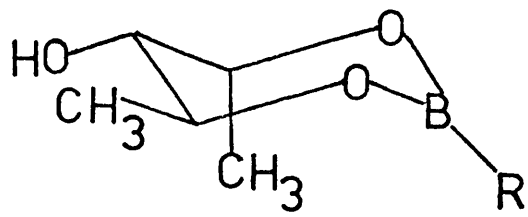
XL



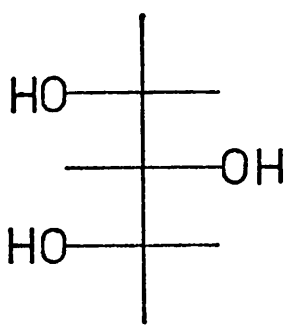
XLI



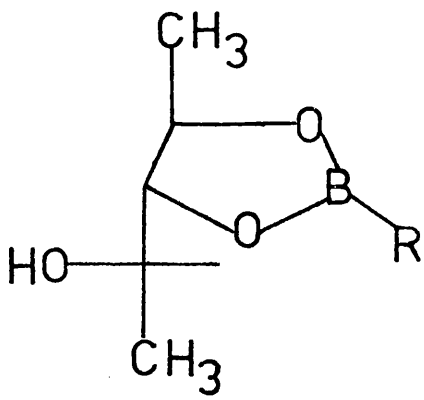
XLII



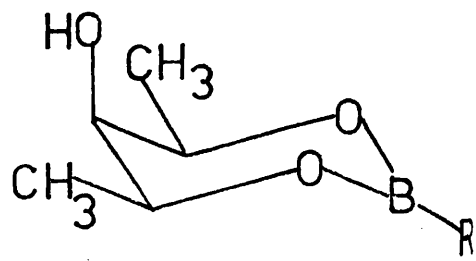
XLIII



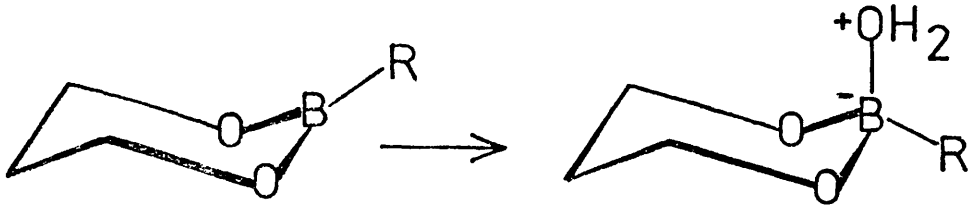
XLIV



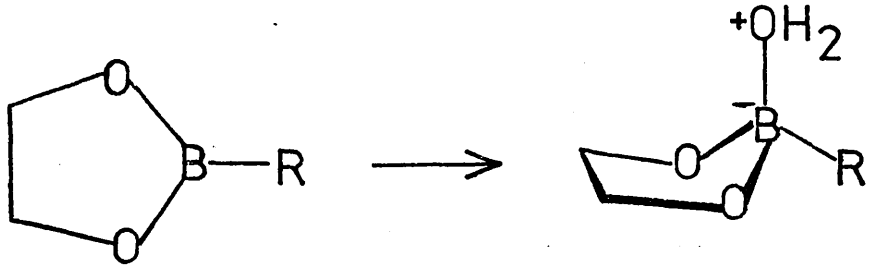
XLV



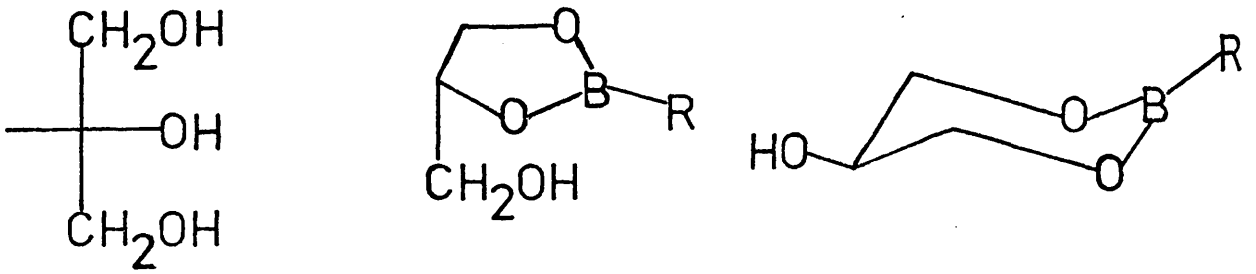
XLVI



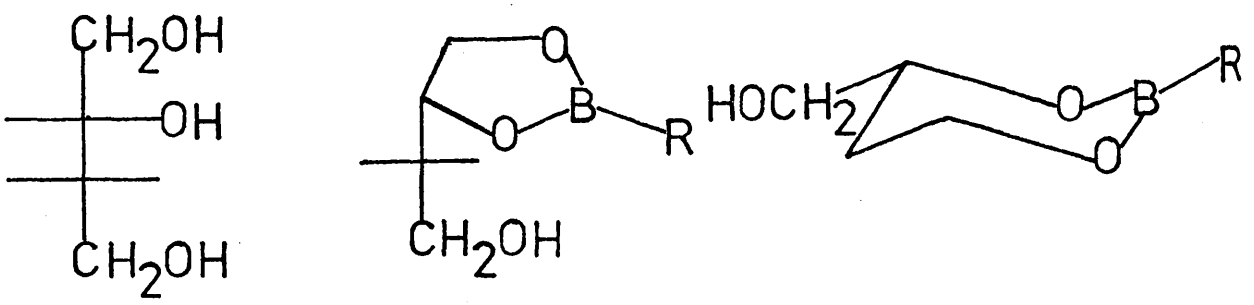
XXX XXXII



XXXI XXXIII



XXXIV XXXV XXXVI

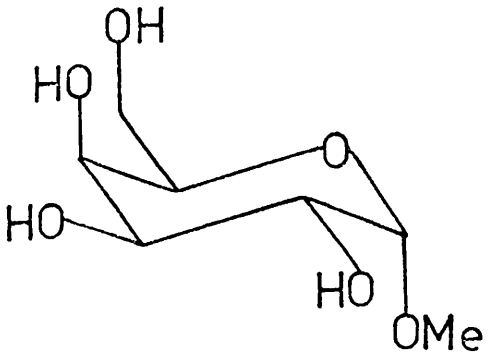


XXXVII XXXVIII XXXIX

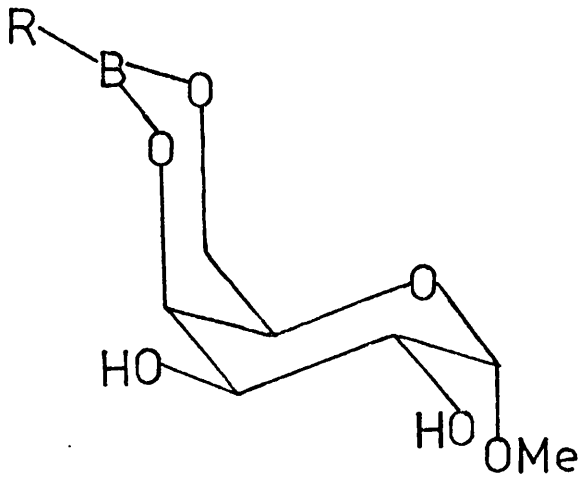
The relative stabilities of the various sizes of the ring that can be formed as boronate esters have been investigated in several ways. Bowie and Musgrave have shown (116) that the stability of the rings towards hydrolysis depends on two factors, the size of the ring and the number of alkyl substituents. The seven-membered ring was the most easily hydrolysed of the 5-, 6- and 7-membered rings examined, while the six-membered ring was the most stable. As the number of alkyl substituents on the ring increases, the stability of each of the types of ester to hydrolysis increases. The hydrolysis is thought to proceed by an attack of water on the boron atom (XXX, XXXI), resulting in a tetrahedral configuration round this atom (XXXII, XXXVII). The greater strain in the seven- and five-membered rings make the next step, cleavage of one of the boron-oxygen bonds and so breakdown of the ring, more favourable in these esters than in the six-membered derivative, where there is very little ring strain. The presence of alkyl substituents on the ring results in a steric hindrance to the formation of the tetrahedral configuration, and so to the hydrolysis.

The same authors also comment on the effect upon the stabilities of the esters, of different groups attached to the boron atom. A comparison was made of the phenylboronate and the methylboronate six-membered esters and it was shown that the phenylboronate was less stable to hydrolysis.

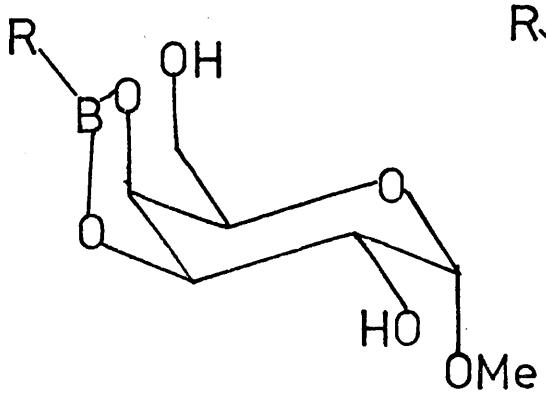
A later paper by McKinley and Weigel (117) investigated the formation of cyclic boronates by reaction of the boronic acid with acyclic triols, allowing the possibility of the formation of either the five-membered or six-membered boronates, and the authors produced three general rules governing the situation. Considering the four examples shown, with each of their possible derivatives, glycerol (XXXIV), DL-butane 2,3,4-triol (XXXVII), L-arabino-pentane 2,3,4-triol (XL), and xylo-pentane 2,3,4-triol (XLIV), if the non-hydrogen substituents of a six-membered ring ester are equatorially disposed (XXXVI, XXXIX) this isomer will be the major product; thus the 1,3-ester of glycerol (XXXVI) and the



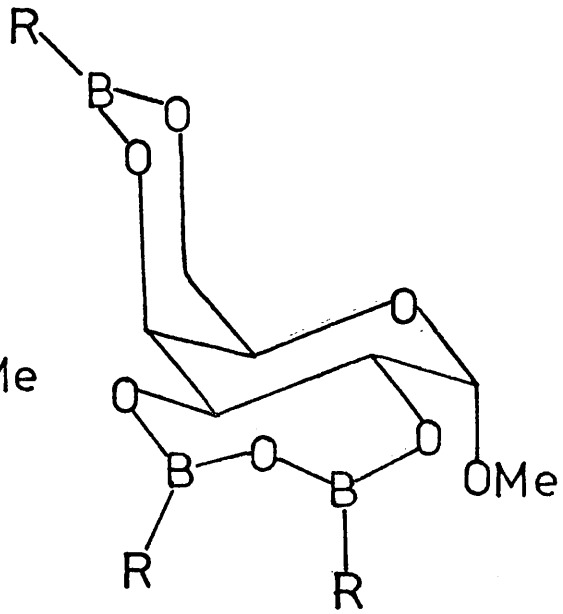
XLVII



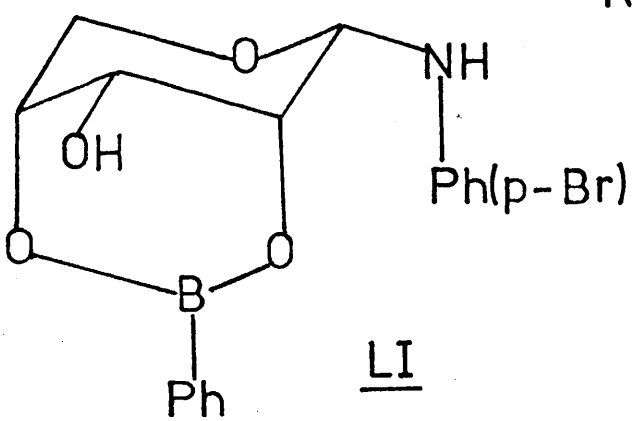
XLVIII



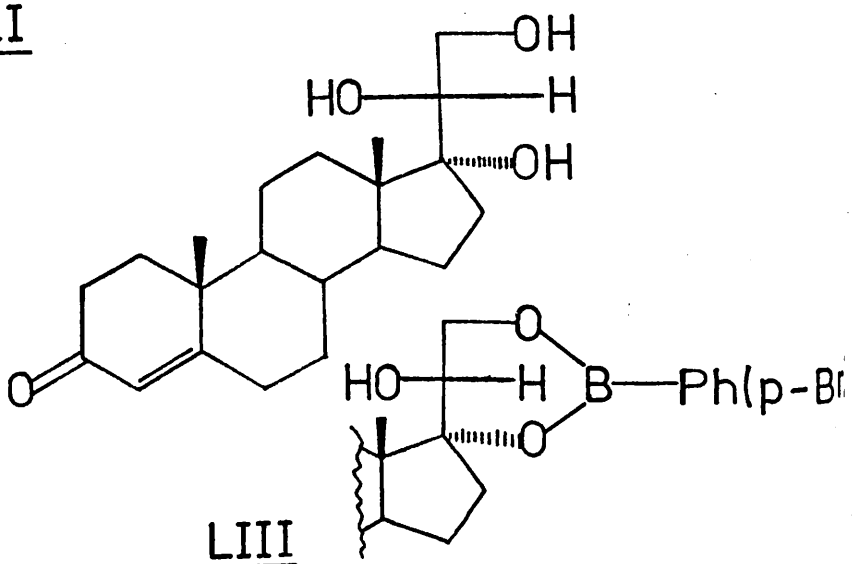
XLIX



L



LI



LII

LIII

2,4-ester of DL-butane 1,2,4-triol (XXXIX) are the major products of their respective reactions. If the six-membered ring would possess axial substituents (XLIII, XLVI) the five-membered ring isomers are the exclusive products (XLII, XLV). Lastly, five-membered rings with trans-substituents (XLI) are formed more easily than those with cis-substituents (XLII).

These rules do not seem to apply to all the cyclic boronates. For example, in Me α-D-galactopyranoside (XLVII), where it is possible to form either the 4,6- (XLVIII) or the 3,4-esters (XLIX), the six-membered ester would possess axial substituents and so the five-membered ester should be formed exclusively. The reaction is, however, known to produce either the six-membered cyclic ester (XLVIII) or the per (phenylboronate) ester (L) in which the six-membered ring is present (L). Similarly, the absolute structure of N-(p-bromophenyl)- α-D-ribopyranosylamine 2,4-phenyl boronate (Ll) established by X-Ray diffraction analysis of the crystals, shows that this compound is in the six-membered form, while the rules would suggest that it should have the five-membered form which is also available to it (118).

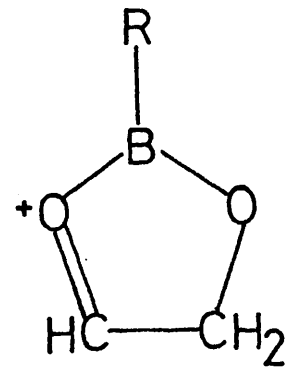
The boronates of some corticosteroid triols are also known to form the six-membered ring (129, 131) although the configuration of the hydroxyl groups is that of a 1,2,3-triol system, for example: 17, 20, 21-trihydroxy-4-pregnen-3-one (LII) which has been shown, by an X-ray crystallographic structure determination, to form the six-membered ester (LIII) (119).

Thus it would seem that while these rules may hold true for the simpler acyclic systems they are less applicable to cyclic systems. In these cases the formation of the boronate ester would more probably be governed by the configuration of the substrate molecule, which would result in a preferred conformation of the hydroxyls participating in the esterification.

LIV R = n-Bu,

LVa Ph,

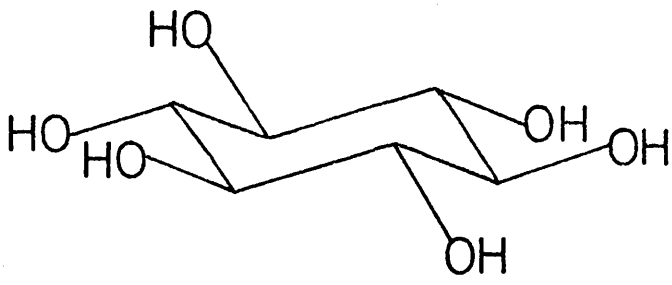
LVb Me.



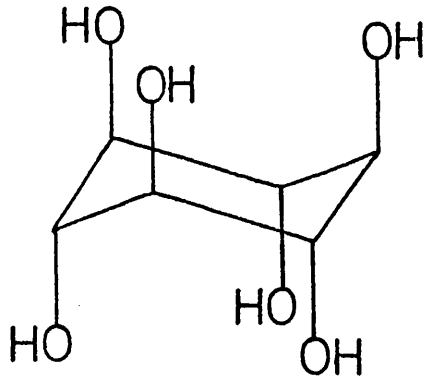
The boronates have been shown to be stable to esterification conditions (106, 108, 114), to trimethylsilylation (122, 123), but not to methylation with methyl iodide (106, 108, 114). They are readily hydrolysed under neutral conditions with an aqueous solvent (106) or by transesterification with propane-1,3-diol (106, 108), thus they can be used to protect certain groups of the correct configuration, in a complex molecule during a sequence of reactions. This ability to protect selectively, groups in a molecule has been used in synthetic work (113-121).

The application of boronate esters in general to GLC and GC-MS was begun by Brooks and co-workers in 1967 (122-131). They showed that the esters could be readily formed at room temperature by mixing equimolar amounts of the acid and a suitable diol, at room temperature, in a solvent which can form azeotropes with, and so remove, the water evolved, i.e. acetone (122, 123) or pyridine (123). The GLC properties of the boronates were better than those of the free diols, and in general the mass spectra showed significant ions, due to the presence of the boronate group, which were an aid to the characterisation of the diol. In particular, the presence was noted of some ions containing the cyclic boronate structure, for example, the ion m/e = 127 for the n-butyl boronate (LIV) and = 147 for the phenylboronate (LVa) (125). The presence of such ions, and those due to the 1,3-diol system might be used as a diagnostic test for distinguishing between these two types of diol.

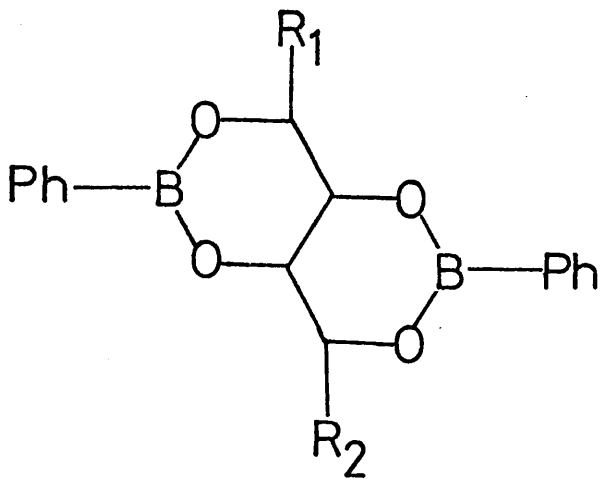
GLC of sugar boronates as the n-butyl ester was first reported in 1971 by Wood and Siddiqui (132) who carried out the reaction in pyridine, at room temperature. By storing the reaction mixture and taking out samples, at intervals, to be trimethylsilylated (37) and run on GLC, they showed that the best results were obtained by leaving the reaction to stand for at least four hours. By similar methods they also demonstrated that the best ratio of acid to sugar, for the aldoses that they were using, was 2 : 1, otherwise multiple peak formation was



LVIa



LVIb



LVII R = H,

LVIII CH_2OH.

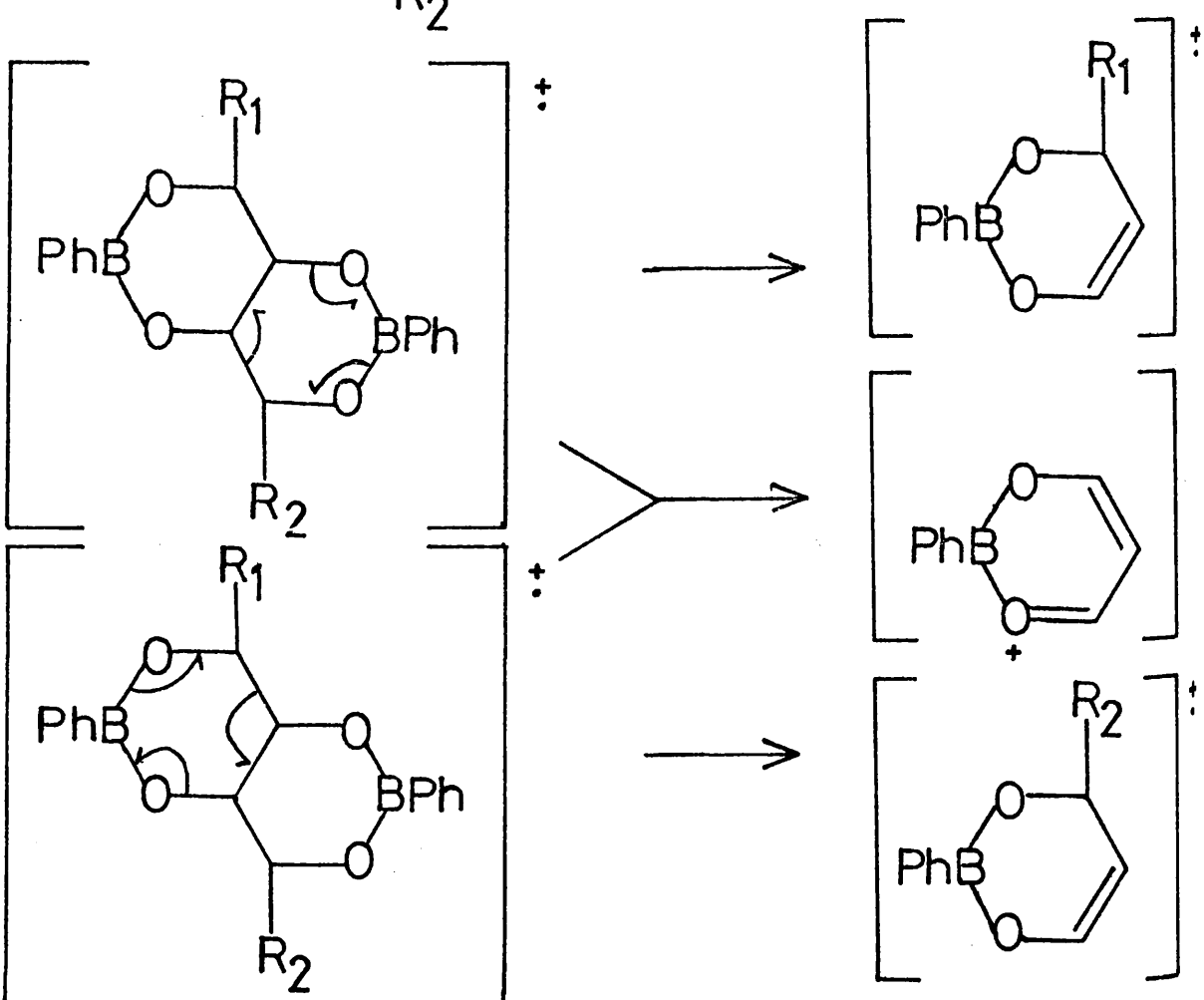
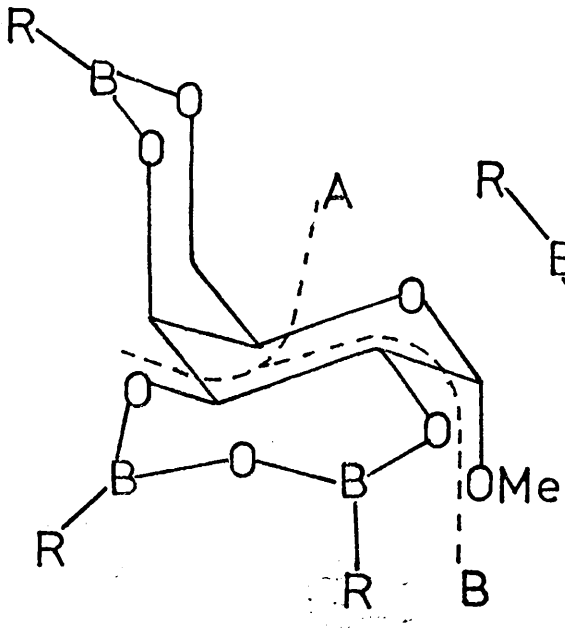


Fig. I

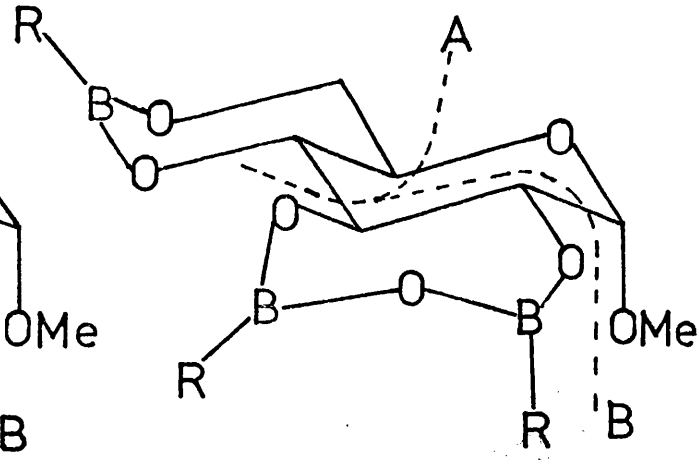
noticed. They obtained single peaks for a single anomer if it was freshly dissolved, but if they allowed it to stand in aqueous solution before doing the reaction, minor peaks were obtained on the chromatogram. These may be caused by the anomer, or by by-products of the reaction due to the traces of water present (132).

Eisenberg (133) reported the retention times of a series of sugars and polyols as the butylboronates, which he injected straight on to the column from the pyridine solution. He collected a pure sample of myo-inositol butylboronate from the effluent gas of the gas chromatograph and recorded its mass spectrum. This showed the molecular ion at m/e = 378, a fragment at m/e = 321 indicating the loss of a butyl group, and the main fragment at m/e = 139 implying the structure $C_3H_3O_2BC_4H_9$ derived by rupture of the ring and loss of two boronate residues. These results support the identification of the derivative as myo -inositol tris(dibutylboronate), $C_6H_6O_6B_3(C_4H_9)_3$.

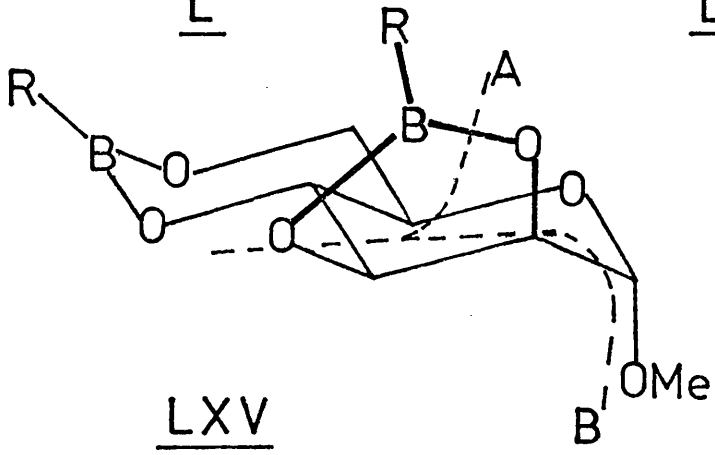
In a later paper (134) Eisenberg went into more detail about GLC of the esters. The best derivatives that he found were those of compounds containing an even number of free hydroxyls, e.g. pentoses and hexitols, while molecules containing odd numbers of free hydroxyls, such as hexoses and pentitols, behaved badly on GLC, due to the incomplete derivatisation of the polar hydroxyl groups. Number of available sites for the reaction is not, however, the only criterion for the formation of good derivatives; the orientation of the hydroxyls is also important. Thus scyllitol (LVI) is said to be inert to reaction with the boronic acids, because in the preferred conformation (LVIa) all the hydroxyls are equatorial and therefore trans- to each other, inhibiting the reaction which requires that the hydroxyls involved be cis-; only in the conformation where all the hydroxyls are axial (LVIb) could there be any reaction, for here there are 1,3- hydroxyl pairs cis- to one another, but this conformation is sterically hindered because of the close proximity of each set of three hydroxyls on either side of the molecule, making it energetically unfavourable. Any



L

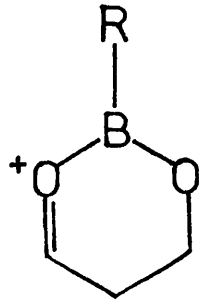


LXIV

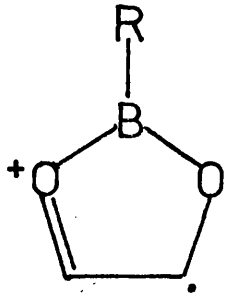


LXV

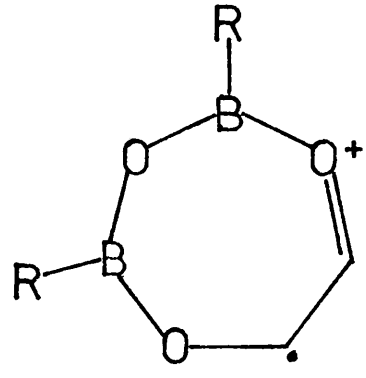
α≡ R=Ph



LXVI



LXVII



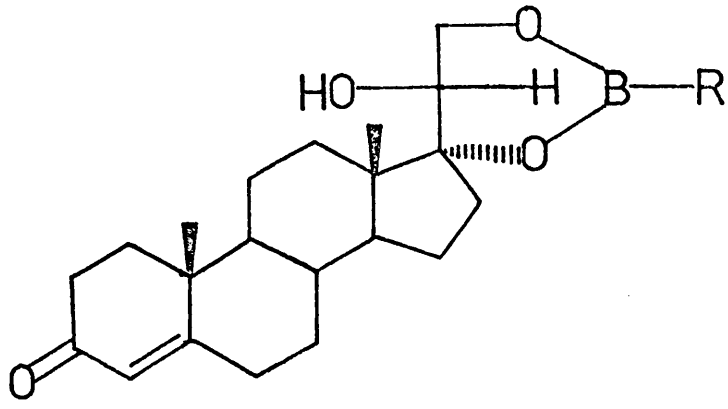
LXVIII

reaction of the scyllitol with a boronic acid would, therefore, probably result in a series of polymeric compounds which would be involatile, and thus indetectable by GLC.

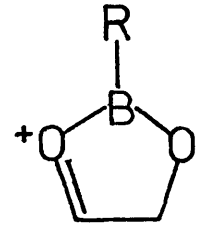
Mass spectrometry of a set of 5-, 6- and 7-membered cyclic phenylboronates showed a pattern of fragmentation modes, one of them exclusive to the 6-membered ring (135). This fragmentation pattern gives rise to diagnostic boron-containing ions in the spectra of some polyol bisphenylboronates, e.g. those of erythritol (LVII) and ribitol (LVIII), which break down by the general process (Fig. 1) to give the ions shown. The presence of such ions in the spectra of the polyol derivatives confirms their structures as the 6-membered ring esters.

The phenyl- and n-butylboronates of arabinose and xylose have also recently been prepared and examined by nuclear magnetic resonance spectrometry (NMR) and MS, and have been shown to be the 1,2,3,4- and 1,2,3,5- diboronates respectively (136). Comparison of the mass spectra of the butyl- and phenyl esters of each compound showed that similar boron-containing ions occurred in each with a difference of 20 mass units. The spectra did not show any prominent fragment exclusive to either of the sugars, but a major, distinguishing feature in the boronates is the relatively greater intensity of the peak at m/e = 127 (147) for the xylose derivative. This intensity difference is constant and occurs in both types of ester, making it of diagnostic value.

A recent paper by Robinson et al., (120) describes the formation of the per(phenylboronates) of Me α-D-galactopyranoside (La), Me α-D-glucopyranoside (LXIVa), and Me α-D-mannopyranoside (LXVa) and the mass spectra of each of these compounds which was obtained by direct insertion of the esters, on the probe, into an AEI 902 mass spectrometer. Two major fragmentations were observed. The first was that shown as A, leading to the splitting off of the six-membered boronate ring to give the ion of m/e = 161 (LXVIa), while the second, B, resulted, in the last of the esters, in the ion at m/e = 146 (LXVIIa) and in the first two esters, in the seven-membered ring ion of m/e = 250 (LXVIIIa) which



LIII



LVb R = Me

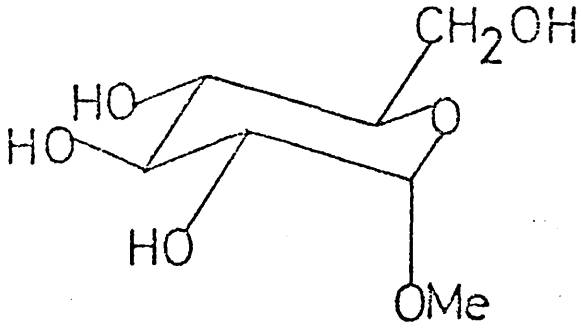
XXIX R = n-Bu

breaks down to give the m/e = 146 ion (LXVIIa). This last ion is the most abundant ion in each of the mass spectra. Because of this rearrangement from the seven-membered ring ion to the five-membered, the authors state that the presence of the m/e = 146 ion cannot be taken as proof of the presence of the five-membered ester in the parent compound.

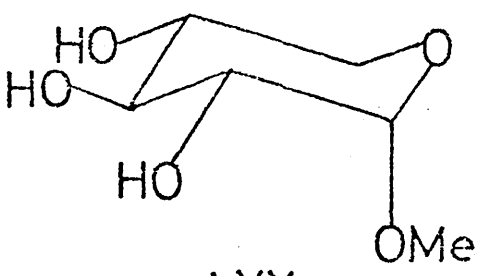
A similar rearrangement of a six-membered cyclic boronate ester resulting in a five-membered ion is postulated by Brooks, Middleditch and Harvey (131), in the mass spectrum of some corticosteroid 17α, 20, 21-triol boronates, for example, the methyl and n-butyl boronates of 17α , 20α , 21-trihydroxy-pregn-4-en-3-one (LIII). The mass spectrum of each of these esters shows peaks that correspond to the five-membered ions (LVb), at m/e = 85 and XXIX at m/e = 127, respectively.

Therefore, the basic boronate ions alone do not seem to afford an unambiguous way of assigning the structure of a boronate ester from the mass spectrum, and attention must be paid to the other ions in the spectrum.

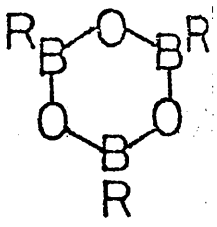
The object of this section of the research, which was begun in 1971, just before the publication of the first paper on the GLC and GC-MS of sugar boronates (132), has been the examination of the GC-MS properties of the boronate esters and boronate TMSi ethers of a set of sugars.



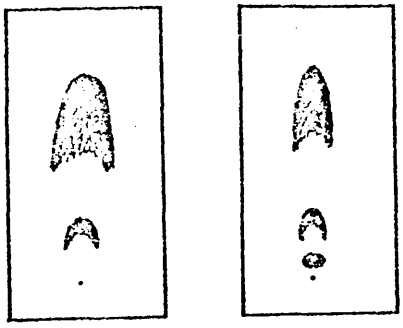
LXIX



LXX

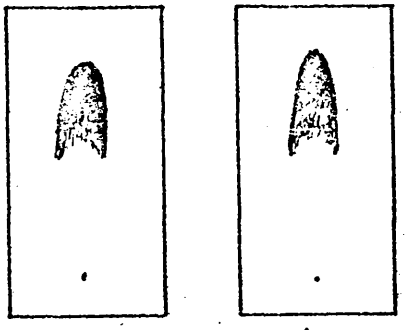


XXVIII



	Rf
Ester	0.73
Acid	0.36
Sugar	0.09

a Fig II b



a Fig III b

a - Diphenylcarbazone spray only.

b - Diphenylcarbazone & ceric sulphate sprays.

a Me α-D-mannopyranoside di(phenylboronate). 10ft., 1% SE-30,

b Phenylboronic acid anhydride. 150°C, N_2 - 35ml/min

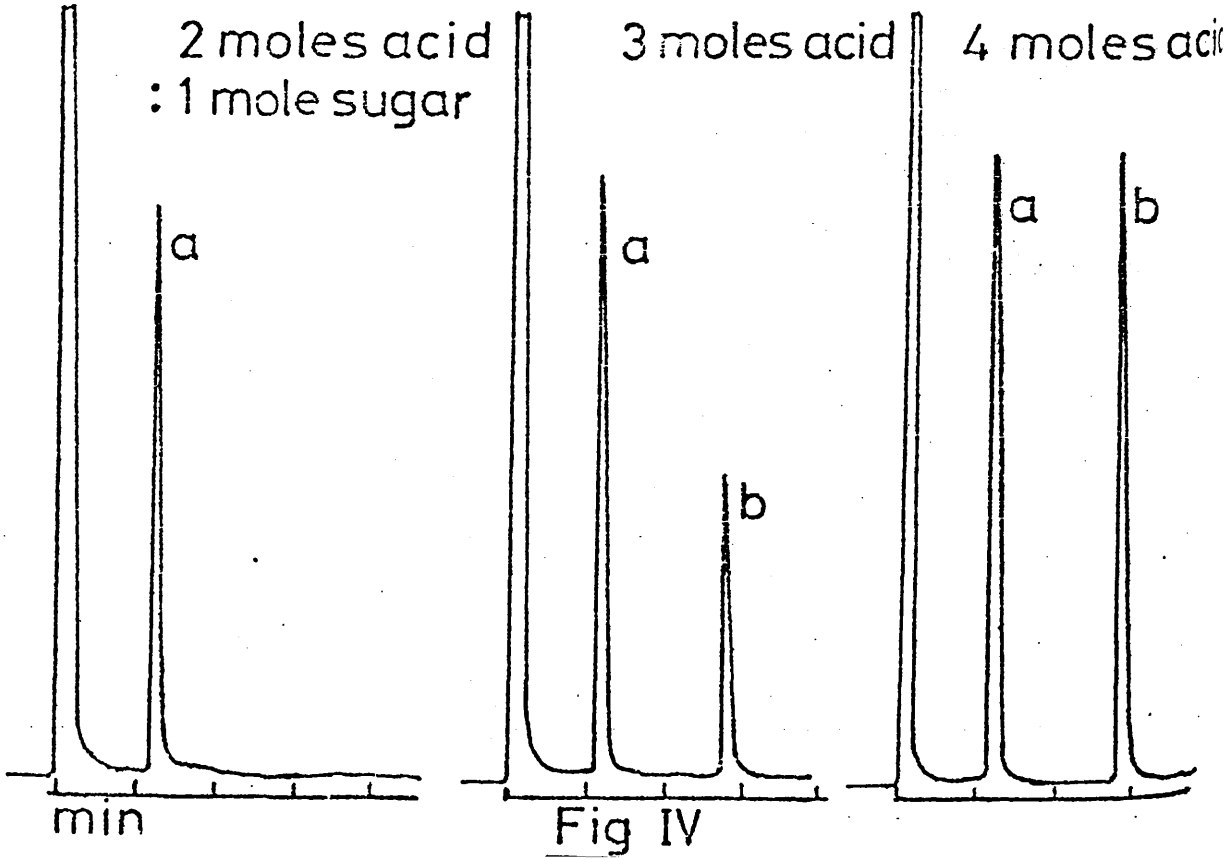


Fig IV

2 - 2 Results and Discussion

2 - 2.1 Formation and GLC Properties of the Boronates

The sugars chosen for examination as the boronate TMSi ethers were all initially in the pyranose or furanose forms, and, where possible, they were specifically one anomer, as for example, the methyl glycosides Me[1] α-D-glucopyranoside (LXIX) or Me α-D-xylopyranoside (LXX). Thus, in each case, the hydroxyl groups in the sugars are held in a preferred conformation which will affect the availability of each for reaction with the boronic acids (113).

Pyridine and dimethylformamide (DMF) were examined as possible solvents for the esterification, the reaction being followed by thin layer chromatography (TLC) using selective spray reagents to visualise the different components; the boron-containing compounds gave a deep pink colour when sprayed with a saturated solution of diphenylcarbazone in ethanol, while the sugars were located by further spraying with ceric sulphate solution and heating the TLC plate. In both cases, using impure reagent produced the same result, a partial conversion of the sugar into the ester (Fig. II). After drying the reagents, however, it was found that in both cases the reaction went to completion (Fig. III). The reaction was carried out by mixing the sugar and the acid, in the ratio of 1 mole to 2 moles, in dry solvent and heating at 100°C for fifteen minutes (132). The excess acid seems to be necessary to force the equilibrium of the reaction towards the formation of the ester and to allow for any side reactions, for example, with water, which is bound to be present no matter how scrupulous the drying conditions, but too great an excess can lead to the formation of by-products, for example, the acid anhydride (XXVIII), which has GLC properties similar to those of the boronates, and so can interfere with them (Fig. IV). In the TLC plates sketched in Fig. III the excess boronic acid is probably in the anhydride form and so runs with the same R_f value as the ester, giving only one spot.

[1] "Me" is here used as an abbreviation of methyl

Fig V

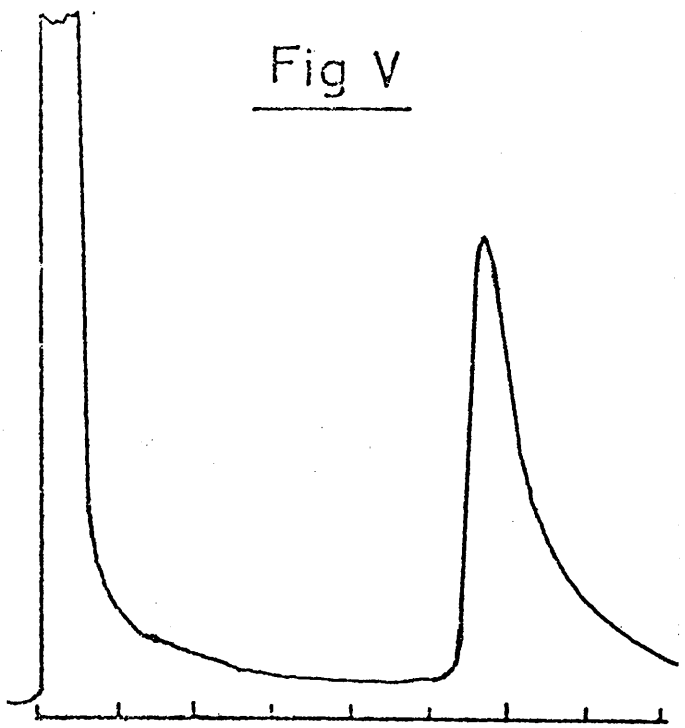
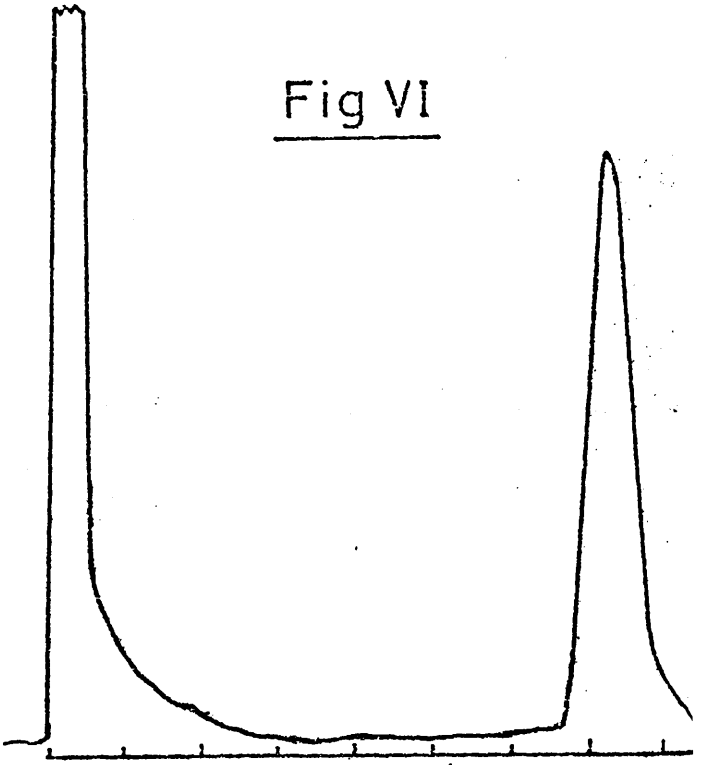


Fig VI



min

1 ft., 1% SE-30, 175°C, N_2 - 35ml/min.

Me α-D-glucopyranoside phenylboronate.

Me D-galactopyranoside phenylboronate.

Fig VII

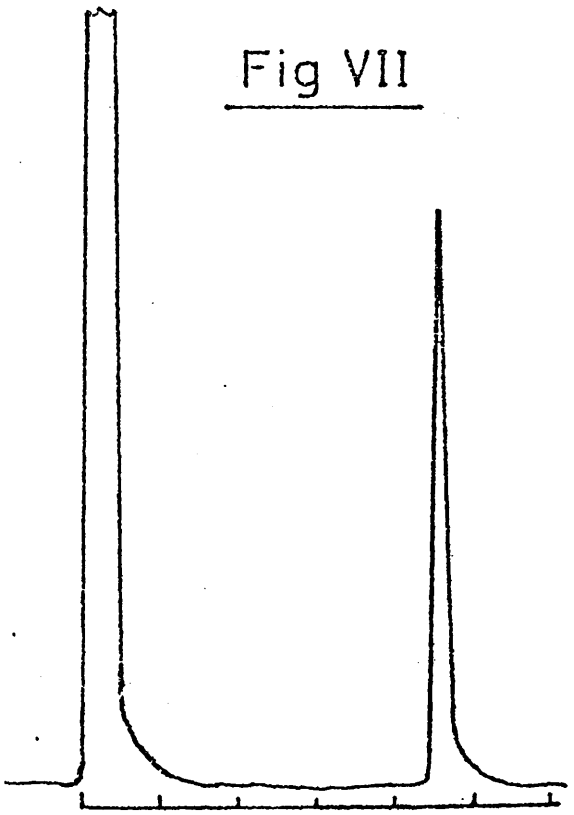
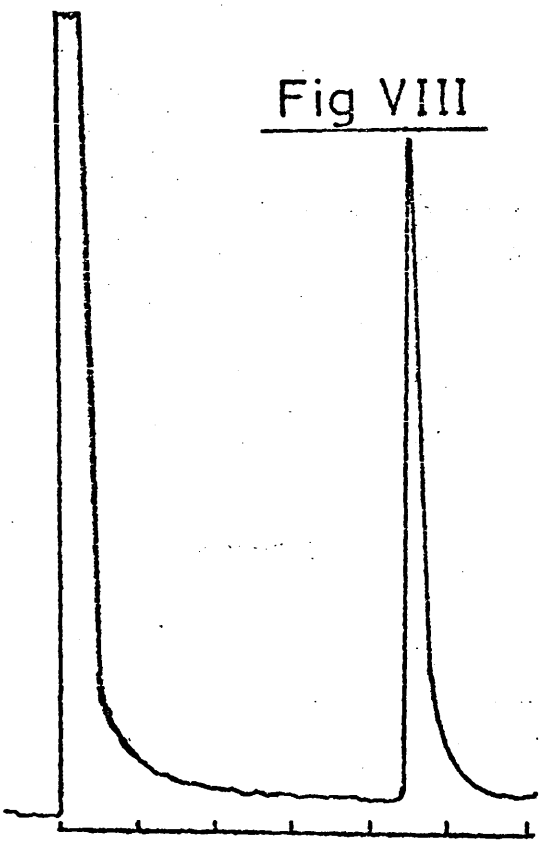


Fig VIII



min

10ft., 1% SE-30, 125°C, N_2 - 35ml/min.

Me α-D-mannopyranoside di(methylboronate).

Me 2,3-di-O-Me-α-D-glucopyranoside methylboronate.

The methyl, n-butyl and phenyl boronates of the standard sugars were made by this method, and their GLC properties were examined by injecting them onto a 10ft 1% SE-30 column straight from the reaction mixture. The boronates were each found to give one of two distinct types of behaviour; Table 1 gives the retention indices of those which exhibited the first type.

In this case the peak shapes are broad and show distinct tailing, presumably due to unreacted hydroxyls which are increasing the polarity of the compound, e.g. Me α-D-glucopyranoside phenyl boronate which gives the trace shown in Fig. V, and the mixture of α- and β - anomers of the Me D-galactopyranoside phenyl boronate which give the trace shown in Fig. VI. The differences in retention indices between the boronates of a single sugar are fairly consistent, and are of the order of 300 retention index units, which corresponds to the difference of three carbon atoms between the methyl and the n-butyl esters, and the n-butyl and phenyl boronate esters. The retention indices of similar compounds are also fairly similar, for example, those of the glucoside and galactoside derivatives.

In the second case the boronates show sharp, almost non-tailing peaks, such as that of Me α-D-mannopyranoside (Fig. VII), and, apart from the esters of Me 2,3-di-O-Me α-D-glucopyranoside, the difference between the various boronates of a single sugar is of the order of 600 retention index units implying the presence of two ester groups in each of the derivatives. Me α-D-mannopyranoside has already been reported as forming the 2,3 ; 4,6-diester (LXV) (106, 120), and presumably both 6-deoxy-D-glucose and 6-deoxy-D-galactose can also form diboronates (see discussion of the GC-MS properties). Me 2,3 -di-O-Me- α-D-glucopyranoside (LXXI) has only two hydroxyls available for boronation, the others having been methylated, and so, after formation of the monoboronate, the molecule has no free polar groups, and gives good results on GLC, as seen in Fig. VIII which shows the methyl boronate. The retention indices of these compounds are summarised in Table 2.

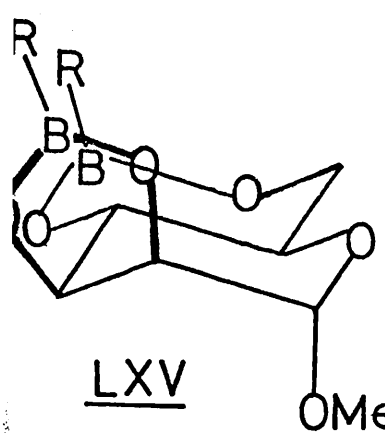
TABLE 1

Compound	Retention Index (10ft. 1% SE-30) Me-boronate		Bu-boronate		Ph-boronate	
D-digitoxose	1475	b	1760	c	2055	d
Me β-L-arabinopyranoside	1180	a	1425	b	1710	c
Me α-L-rhamnopyranoside	1305	b	1640	c	1950	d
1,2 -isopropylidene-D-glucofuranose	1450	b	1760	c	2075	d
2-deoxy-D-ribose	1180	a	1465	b	1750	c
2-deoxy-D-galactose	1310 1345	b	1650 1670	c	2070 2085	d
Me α-D-xylopyranoside	1120	a	1475	b	1850	d
Me β-D-xylopyranoside	1235	b	1570	c	1880	d
Me α-D-glucopyranoside	1555	b	1900	c	2225	d
Me β-D-glucopyranoside	1555	b	1900	c	2225	d
Me α-D-galactopyranoside	1550	b	1910	c	2240	d
Me β-D-galactopyranoside	1550	b	1910	c	2240	d

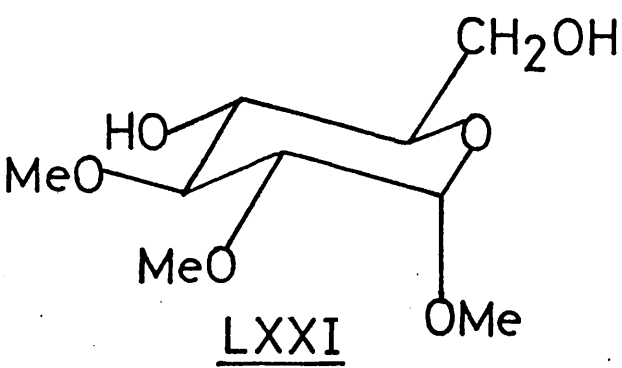
TABLE 2

Compound	Retention Index (10ft. 1% SE-30) Me-boronate		Bu-boronate		Ph-boronate	
Me 2,3 -di-O-Me-α-D-glucopyranoside	1475	b	1760	c	2055	d
Me α-D-mannopyranoside	1430	b	1990	c	2410	d
6-deoxy-D-glucose	1235	a	1855	c	2430	d
6-deoxy-D-galactose	1240	a	1775	c	2310	d

a - 100°C; b - 125°C; c - 150°C; d - 175°C

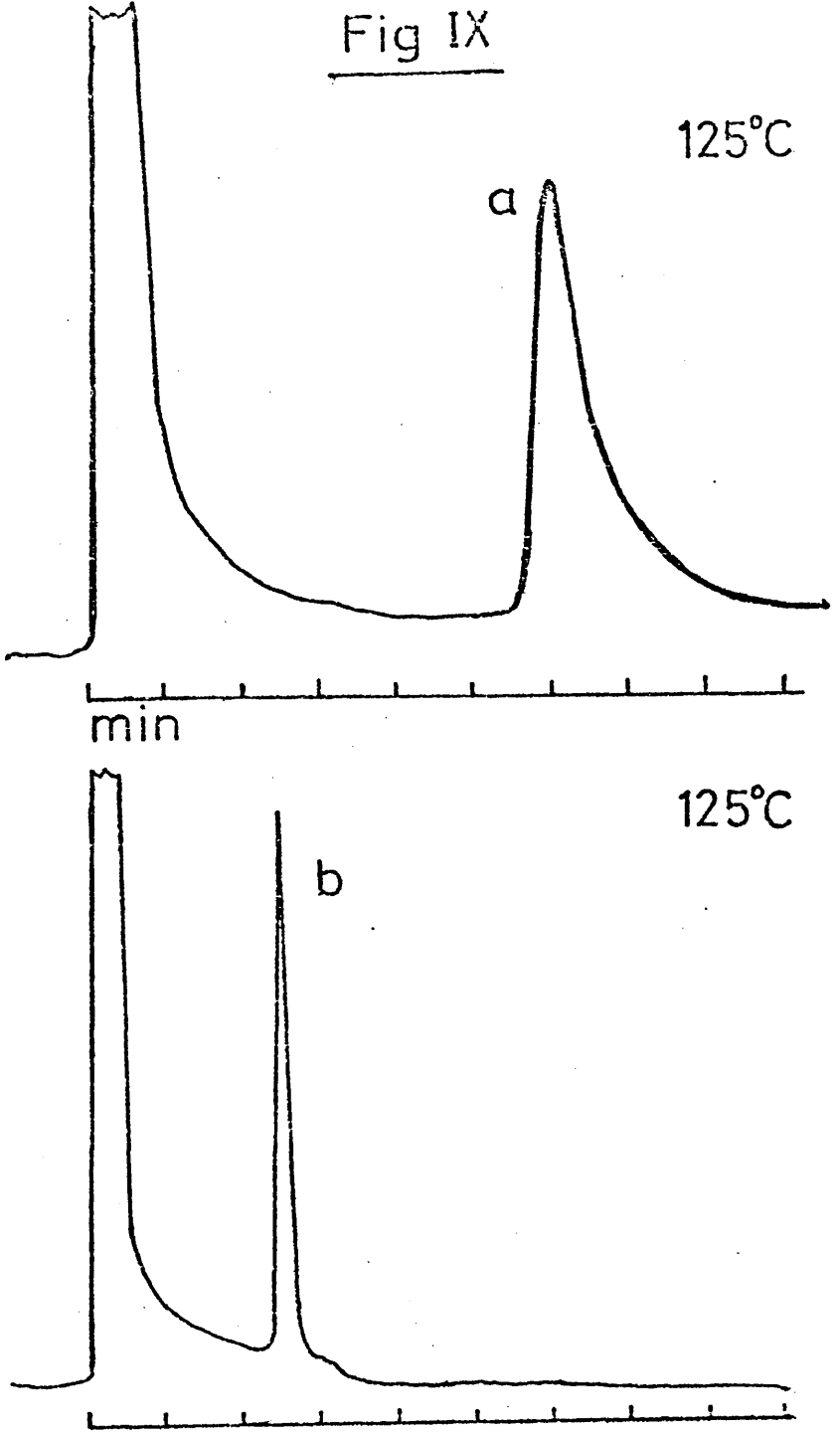


LXV



LXXI

Fig IX



Me β-D-glucopyranoside.

a – Phenylboronate.

b – Phenylboronate TMSi ether.

c – TMSi ether.

10ft., 1% QF-1,

N_2 – 35ml/min.

2 - 2.2 Formation and GLC Properties of the Boronate TMSi Ethers

The method used to trimethylsilylate the unreacted hydroxyl groups, in the sugars of the first group, was the standard one of treating the boronate in dry pyridine with HMDS and TMCS in the ratio of 2:1, and heating at 100°C for fifteen minutes (37). The sugar boronate esters have already been shown to be stable to this treatment (132) and in all cases this was found to hold, the boronate TMSi ether being formed. Since the boronation reaction was also done in pyridine, the total reaction sequence consisted of treatment of the sugar with the boronic acid in dry pyridine followed by the addition of the silylating reagents. The product could be examined on GLC by injecting straight from the reaction mixture (37), but to avoid a large solvent peak due to the pyridine, the solvent was blown off at 50°C under a stream of nitrogen, the product dissolved in ethyl acetate and the insoluble precipitate filtered off. The last was done, not to preserve the GLC column which it is reported not to harm (37), but to minimise the background when the sample was applied to the GC-MS. The pertrimethylsilyl ethers were also formed by reacting the free sugars, in the same way, with the silylating reagents.

The boronate TMSi ethers were examined by GLC, using both QF-1 and SE-30 (both 10ft. 1%), and the results are summarised in Tables 3 and 4. Generally the peak shapes are improved and the retention times shortened: for example Fig. IX shows the phenylboronate (a) and the phenylboronate TMSi ether (b) of Me β-D-glucopyranoside run under the same conditions. The latter has a much sharper peak shape with almost no tailing, while the former is broad and tails markedly. The TMSi ether peak is also shown, run at a lower temperature (Fig. IXc). The retention indices of the derivatives show some of the same characteristics as those of the boronates. The difference between the three boronate TMSi ethers of each compound is fairly constant, being of the order of 275 retention index units for SE-30 and 350 units for QF-1, again corresponding to the three-carbon difference between the isomers. The

TABLE 3

Compound	Retention Index (1% SE-30 10ft)							
	Me-boronate TMSi Ether		Bu-boronate TMSi Ether		Ph-boronate TMSi Ether		TMSi Ether	
D-digitoxose	1255 1285 1310	a	1560 1595 1600	b	1850 1890 1910	c	1525	b
Me β-L-arabinopyranoside	1285	a	1610	b	1915	c	1565	b
Me α-L-rhamnopyranoside	1385	a	1640	b	1935	c	1605	b
1,2-isopropylidene-D-glucofuranose	1575	b	1870	c	2150	d	1835	c
2-deoxy-D-ribose	1255	a	1525 1545	b	1835 1855	c	1510	b
2-deoxy-D-galactose	1325 1350 1390	a	1645 1665 1700	b	1930 1940 1975	c	1790	c
Me α-D-xylopyranoside	1415	a	1635	b	1980	c	1660	b
Me β-D-xylopyranoside	1350	a	1595	b	2005	c	1670	c
Me α-D-glucopyranoside	1690	c	1960	c	2280	d	1910	c
Me β-D-glucopyranoside	1715	c	2005	c	2310	d	1940	c
Me α-D-galactopyranoside	1635 1655	b	1875 1890	c	2130 2165	d	1865	c
Me β-D-galactopyranoside	1615 1655	b	1880	c	2125 2165	d	1890	c

TABLE 4

Compound	Retention Index (1% QF-1, 10ft) Me-boronate TMSi Ether		Bu-boronate TMSi Ether		Ph-boronate TMSi Ether		TMSi Ether	
D-digitoxose	1405 1410 1530	e	1760 1765 1820	a	2175 2185 2305	a	1730	a
Me β-L-arabinopyranoside	1435	e	1775	a	2105	a	1800	a
Me α-L-rhamnopyranoside	1430	e	1780	a	2100	a	1805	a
1,2-isopropylidene-D-glucofuranose	1725	a	2105	a	2510	b	2050	a
2-deoxy-D-ribose	1400 1510	e	1755 1880	a	2120 2240	a	1710	e
2-deoxy-D-galactose	1555 1620 1670	e	1900 2005 2075	a	2260 2405 2490	b	1925	a
Me α-D-xylopyranoside	1700	e	2040	a	2380	b	1800	a
Me β-D-xylopyranoside	1470	e	1850	a	2225	b	1825	a
Me α-D-glucopyranoside	1585	e	2035	a	2460	b	1930	a
Me β-D-glucopyranoside	1605	e	2060	a	2500	b	2040	a
Me α-D-galactopyranoside	1555 1610	e	1950 2045	a	2330 2470	b	1950	a
Me β-D-galactopyranoside	1585 1630	e	1980 2075	a	2345 2510	b	2025	a

Temperature Code:- e = 75°C a = 100°C b = 125°C
c = 150°C d = 175°C

retention indices of the Me β-L-arabinopyranoside and Me α-L-rhamnopyranoside derivatives are more similar than those of the boronates themselves on both columns, but those of the glucosides and the galactosides are more distinct making identification more certain in a mixture; for example Fig. X shows the phenylboronate and the phenylboronate TMSi ether of a mixture of α- and β- Me-D-galactopyranosides. In a number of cases, more than one peak is observed; this is due to the possibility of forming more than one cyclic boronate, inherent in the free sugars involved, and will be discussed in more detail later in the thesis, when the mass spectra of the derivatives are considered. In common with the TMSi ethers (46, 48-50) the α-sugars generally have lower retention indices than those of the β- sugars, except in the case of the xylopyranosides where the position is reversed. This also is discussed later.

The second group of sugar boronates was treated in the same way to see if the diboronates were also stable towards the silylating reagents. As can be seen from Tables 5 and 6, all the boronates proved to be so, except that of Me α-D-mannopyranoside which gave rise to two more boronate TMSi ethers, presumably the 2,3-boronate and the 4,6-boronate TMSi ethers. This too will be discussed later with consideration of the mass spectra.

TABLE 5

Compound	Retention Index (1% SE-30 10ft.) Me-boronate		Bu-boronate		Ph-boronate		TMSi	
	+ silylating reagents							
Me 2,3, di-O-methyl α-D-glucopyranoside	1475	a	1760	b	2055	c	1655	b
Me α-D-mannopyranoside	1655	b	1860 1970	c	2145 2220	d	1815	c
6-deoxy-D-glucose	1235	a	1855	c	2430	d	1760	c
6-deoxy-D-galactose	1240	a	1775	b	2310	d	1715	b

TABLE 6

Compound	Retention Index (1% QF-1 10ft) Me-boronate		Bu-boronate		Ph-boronate		TMSi	
	+ silylating reagents							
Me, 2,3, di-O-methyl α-D-glucopyranoside	1540	e	1935	a	2360	b	1845	a
Me α-D-mannopyranoside	1540	e	1910 2005	a	2290 2410	b	1900	a
6-deoxy-D-glucose	1430	e	2120	a	2780	c	1920	a
6-deoxy-D-galactose	1420	c	2100	a	2750	c	1850	a

Temperature Code:- e = 75°C a = 100°C b = 125°C c = 150°C
d = 175°C

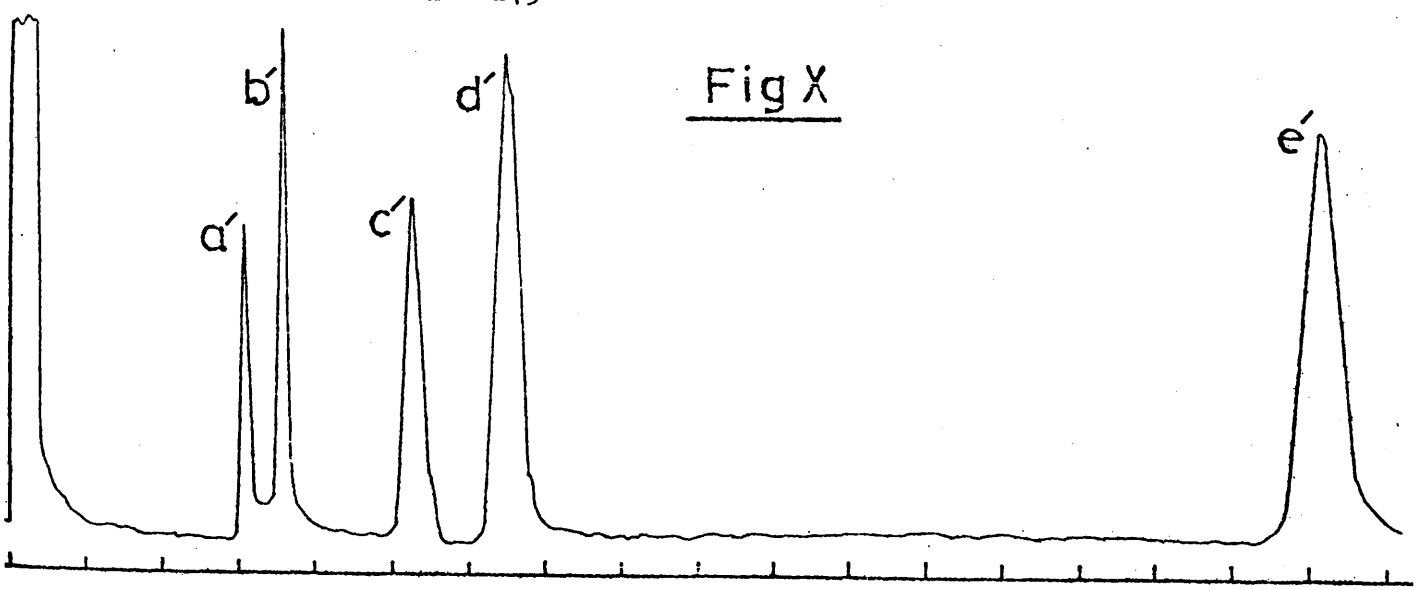
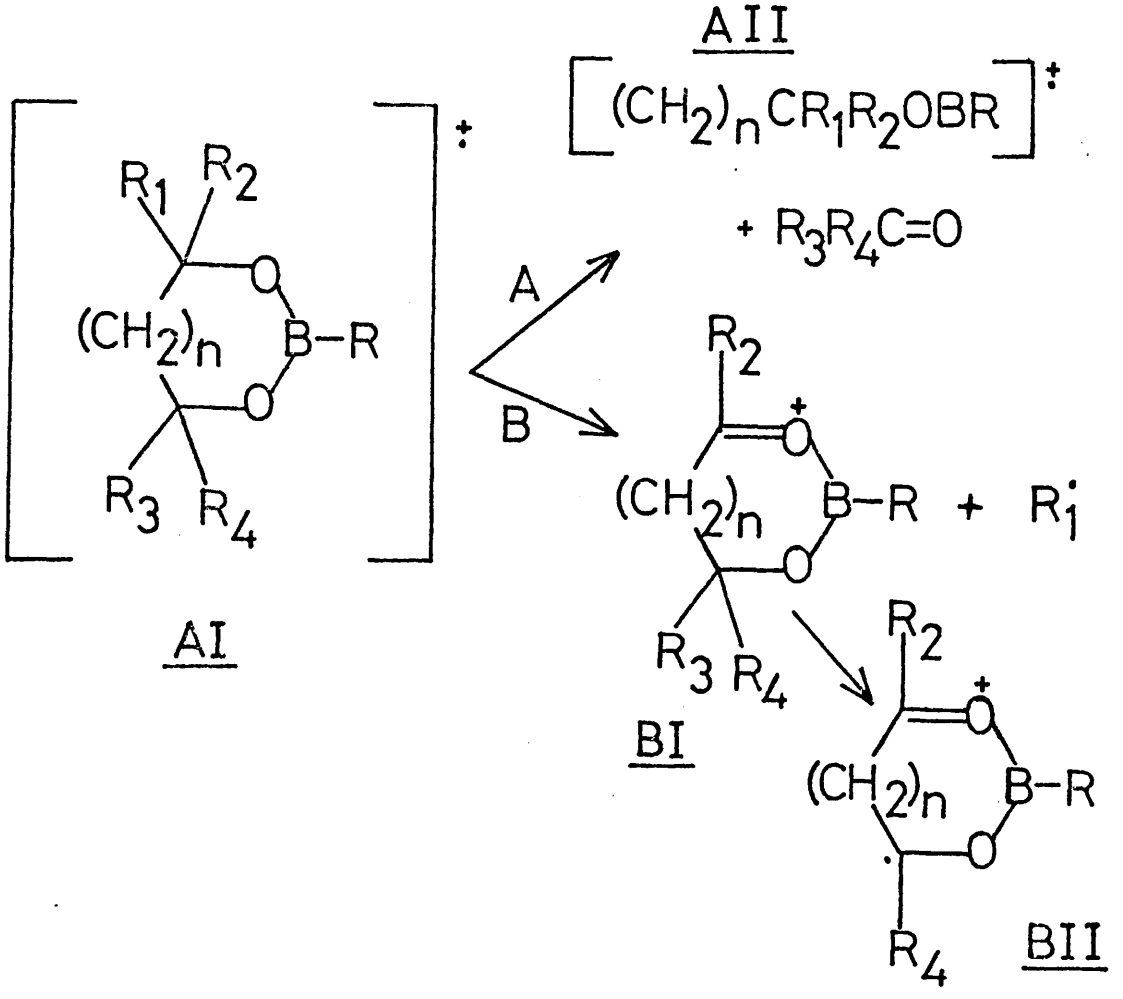
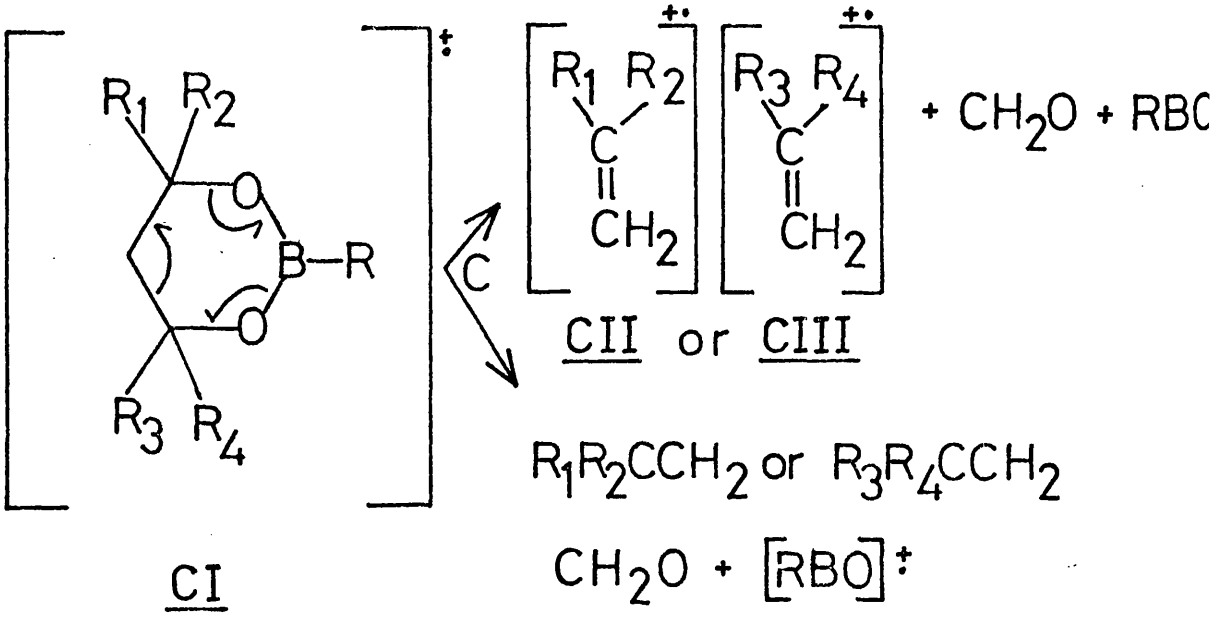


Fig X

a´, c´ - Me α-D-galactopyranoside phenylboronate TMSi ether.
b´, d´ - Me β-D-galactopyranoside phenylboronate TMSi ether.
e´ - Me D-galactopyranoside phenylboronate.

10ft., 1% QF-1, 125°C, N_2-35ml/min


AII
$[(CH_2)_nCR_1R_2OBR]^{\ddagger}$
$+ R_3R_4C=O$
A
B
AI
BI
BII
$+ R_1^{\cdot}$

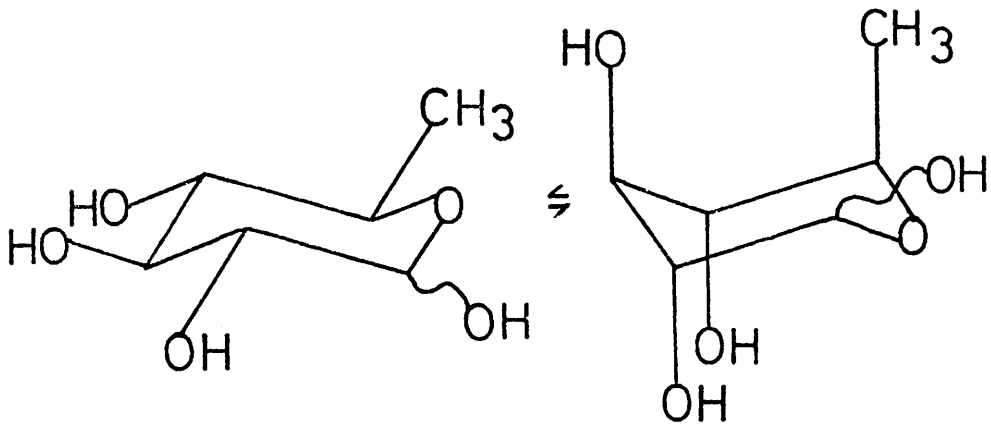

C
CII or CIII
$+ CH_2O + RBO$
$R_1R_2CCH_2$ or $R_3R_4CCH_2$
$CH_2O + [RBO]^{\ddagger}$
CI

2 - 2.3 Examination by GC-MS of the Boronate TMSi Ethers

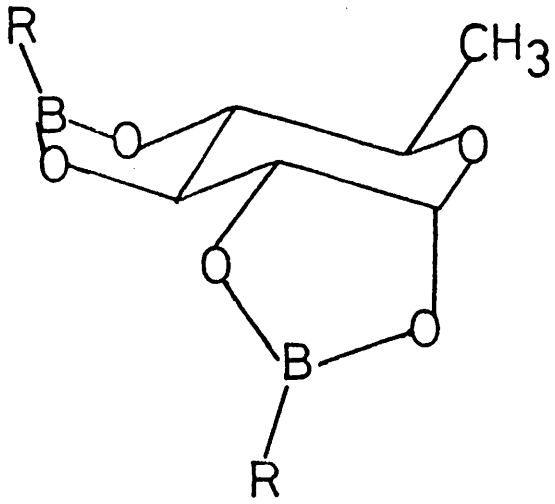
The fragmentation of the boronate ester groups in the mass spectrometer seems generally to follow the four routes outlined by McKinley and Weigel (136). Elimination of an oxo-compound from the molecular ion (AI) results in ion AII (sequence A), which seems to break down further to give the ion $RB\overset{+}{O}H$, of m/e = 105 when R = phenyl, and 43 when R = methyl or the ion $RB\overset{+\cdot}{O}$, of m/e = 104 and 42 which was initially described by Brooks and Watson (122). Cleavage of one of the exocyclic C-C bonds in the ion, i.e. α-cleavage of the sugar ring, produces the oxonium ion (sequence B), which can undergo further bond-fission outside the boronate ring to give the ion BII. A third fragmentation (sequence C) is shown only by the six-membered cyclic boronates (CI), which break down to give either the ions CII or CIII, or the ion $RB\overset{+}{O}$, as shown. These three fragmentation modes all produce boron-containing ions, which are recognisable by the ^{10}B isotope ion which occurs, with about a 20% abundance, at an m/e value of one less than the ion in question, for example, at m/e = 103 accompanying the ion $RB\overset{+}{O}$, at m/e = 104, where R is equivalent to phenyl. Comparison of the spectra of the methyl and phenyl boronates of a sugar would also show the boron-containing ions, since they would present in the former with an m/e value of 62 less than in the latter, and so are readily identifiable.

The last fragmentation (sequence D) would result in ions which did not contain any of the boronate group, since it consists of a breakdown and rearrangement of the sugar ring, and of the TMSi ether groups that are attached to it, resulting in the formation of various TMSi ether-containing ions as described by De Jongh et al. (71). These are more easily described as they arise, since there seem to be no general rules covering their formation.

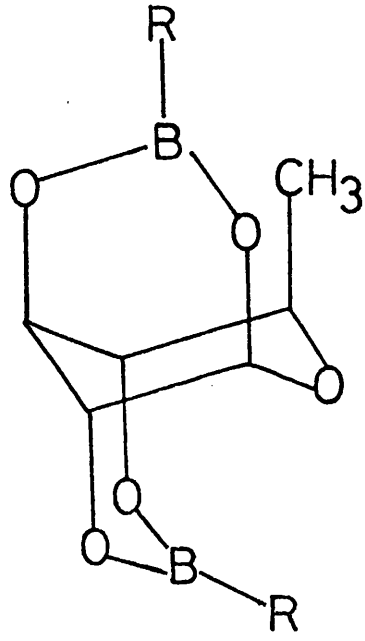
In the following discussion, the line diagrams of the mass spectra referred to can be found in Appendix I.



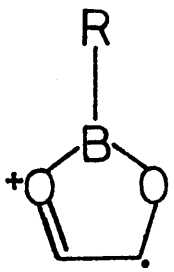
LXXII



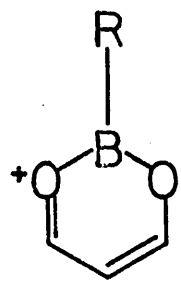
LXXIII



LXXIV



LXVII



LXXV

6-deoxy-D-glucose

The methyl and phenyl diboronates of each of the sugars were run on GC-MS, the injections being made from a solution in ethyl acetate. A summary of the results for 6-deoxy-D-glucose is shown in Table 7. In each case the molecular ion is present, and has the value expected for the diboronates, namely 212 and 336 for the methyl and phenyl groups respectively. The difference between the two molecular weights corresponds to the mass difference between the weight of the two methyl and two phenyl substituents. This difference occurs in the comparison of each boron-containing ion in the pair of esters. The molecular ion of the phenyl boronate is much stronger than that of the methyl, possibly because the phenyl group has a greater stabilising effect.

6-deoxy-D-glucose can be seen to possess four free hydroxyls (LXXII); for the α-anomer the diboronate could only be formed by a distortion of the sugar ring to give the 1,2 ; 3,4-diester (LXXIII), but for the β-anomer the 1,3 ; 2,4-compound (LXXIV) could be formed without any distortion. In the former case we would expect to see a five-membered cyclic boronate ion (LXVII) (120), which would have an m/e value of 146 for the phenyl ester (123), and 84 for the methyl, while in the latter case the six-membered ring (LXXV) would be expected (136) giving a peak at m/e = 159 and 97 for the phenyl and methyl esters. In fact, as can be seen from the results, the latter ions are the most abundant in their respective spectra, implying the formation of the 1,3 - 2,4-diboronate. To check the composition of the m/e = 159 ion a high resolution mass spectrum was determined. The mass was found to be 159.06181, while the theoretical value for $C_9H_8BO_2$ is 159.061731, confirming the structure of the ion, and hence of the ester.

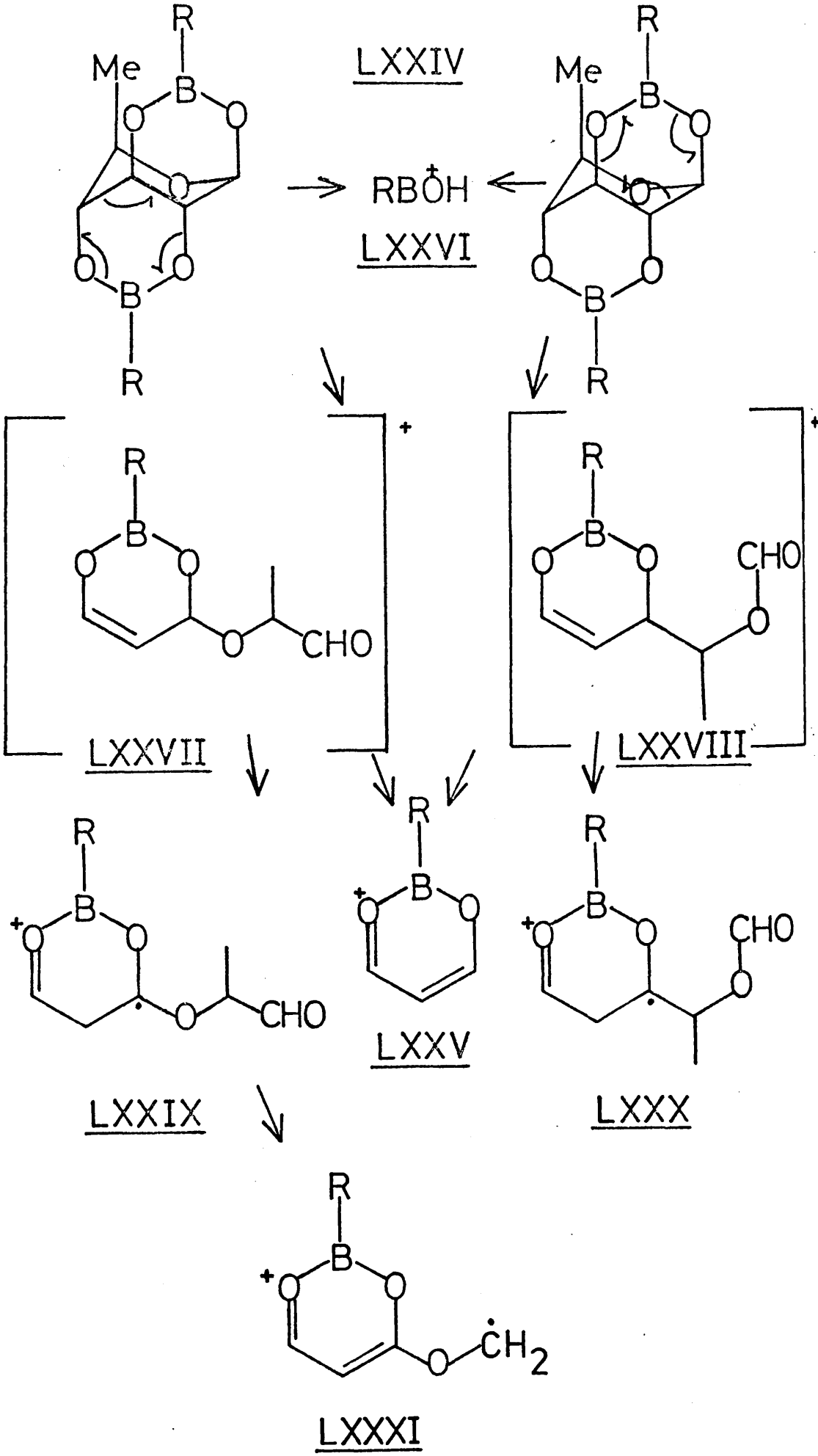
From the structure shown (LXXIV) for the boronate fragmentation ought to proceed in a similar way to that detailed by McKinley and Weigel (136, 45) i.e. by the breakdown of one of the rings, as shown, giving the ions LXXVI, LXXVII and LXXVIII. Of these, only LXXVI is

TABLE 7

6-deoxy-D-glucopyranose diboronate

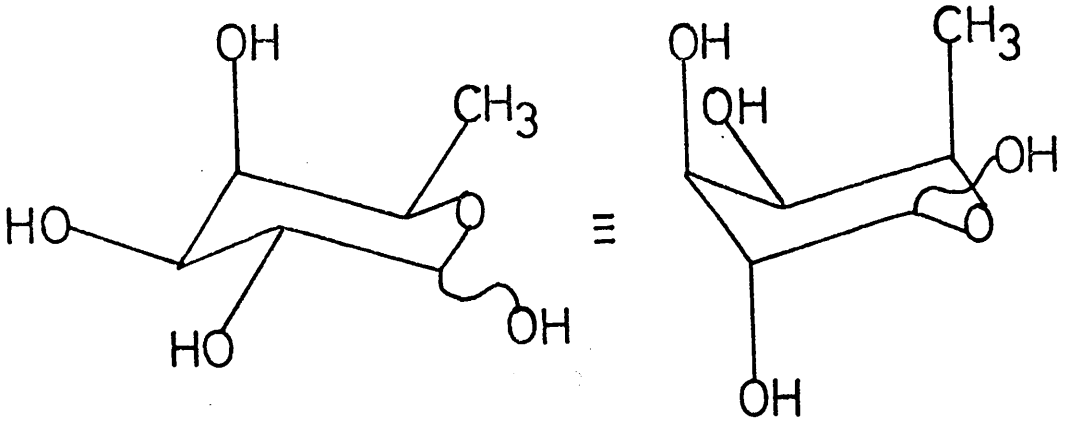
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 1*	1235	212	97	1	168(14)	126(44)	96(25)
					84(20)	43(29)	
Phenyl 2	2340	336	159	17	336(17)	188(29)	158(26)
					146(14)	105(22)	

* Number of mass spectral line diagram

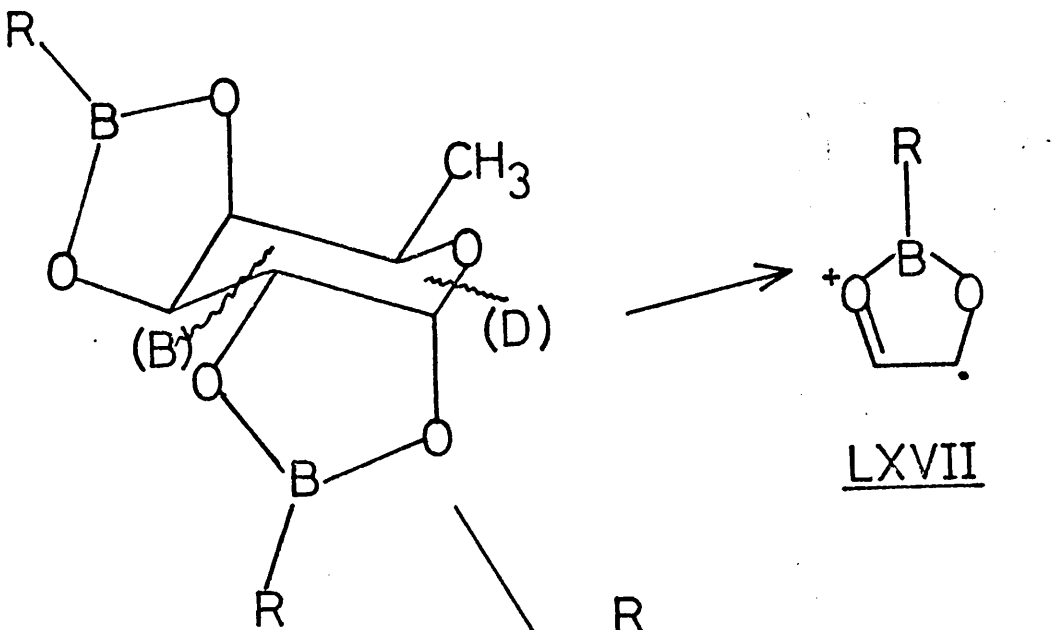

LXXIV
RBOH
LXXVI
LXXVII
LXXVIII
LXXV
LXXIX
LXXX
LXXXI

stable, giving peaks at m/e = 105 and 43 for the phenyl and methyl esters respectively (122, 137). The other ions break down to give the six-membered cyclic boronate ion (LXXV) of m/e = 159 and 97, while in the case of the methyl boronate ester loss of hydrogen from either LXXVII or LXXVIII results in the ion of m/e = 168, which might have the structure LXXIX or LXXX. Alternatively, breakdown could result in the fragment with m/e = 188 and 126 respectively (LXXXI).

the mass spectra of
In both esters there is also present, however, the ion due to the five-membered ring (LXVII). This most probably arose from rearrangement of the boron-containing fragment during the breakdown of the molecule. Here it is present in a fairly low ratio to the six-membered ring ion, but the fact that it is present at all shows that care must be taken when interpreting the mass spectra of the boronates, since the five and six-membered ring ions are not exclusively formed from the respective 1,2- and 1,3-boronate esters. Consideration should also be taken of any other major ions occurring in the spectrum.

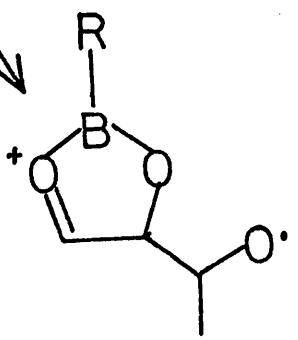


LXXXII

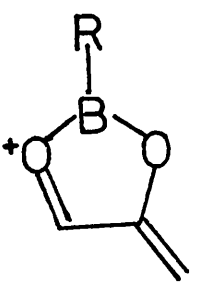


LXXXIII

LXVII



LXXXIV



LXXXV

6-deoxy-D-galactose

The boronates of this compound also have molecular weights corresponding to those of the diboronate esters, namely 336 for the phenyl, and 212 for the methyl isomer. Both of the molecular ions are again present, though in this case the difference between their relative intensities is much smaller than it was for the 6-deoxy-D-glucose esters.

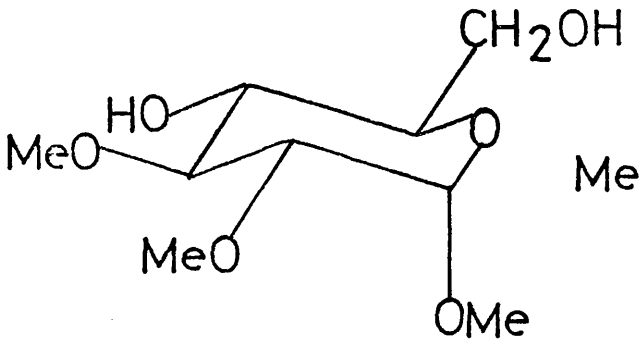
As can be seen from the diagrams (LXXXII), the only possible diboronate is the 1,2 ; 3,4-diester (LXXXIII) of the α-anomer. This contains two five-membered cyclic boronate groups and would, therefore, be expected to show the five-membered ring ion of m/e = 146 and 84 for the phenyl and methyl boronates respectively (LXVII). In both cases this ion is the most abundant in the spectrum; a high resolution mass spectrum of this ion gave an accurate mass of 146.053906, which compares favourably with the theoretical value for $C_8H_7BO_2$ of 146.053982 confirming the structure of the ion and hence of the diester.

Cleavage α- to the ester groups (B), followed by the fragmentation of one of them would account for the $RB\overset{+}{O}H$ ion of m/e = 105 and 43 for the phenyl and methyl boronates (127, 136, 137) while cleavage of the sugar ring as shown (D) would result in the ion (LXXXIV) or the most stable ion (LXVII). The small peaks at m/e = 159 and 97, may be due either to rearrangement to the six-membered ring ion or to the ion LXXXV, pointing up once again the difficulties in the unambiguous assignment of the ions in the spectra of these compounds.

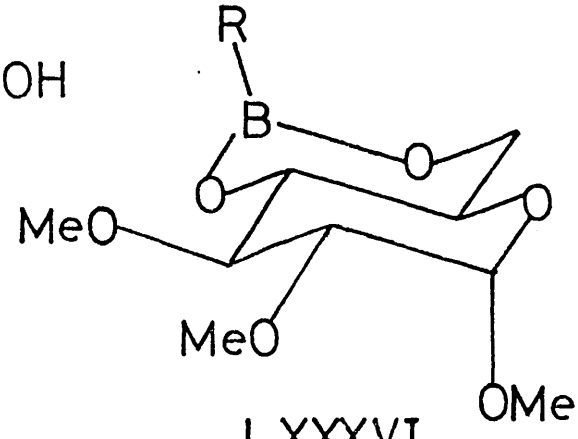
TABLE 8

6-deoxy-D-galactopyranose diboronate

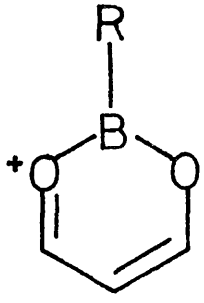
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)			
Methyl 3	1240	212	84	1	128(9)	97(3)	83(24)	43(15)
Phenyl 4	2310	336	146	3	190(9)	159(2)	145(25)	105(15)



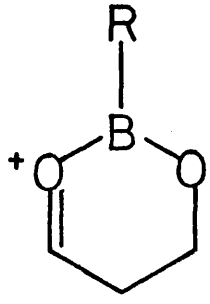
LXXI



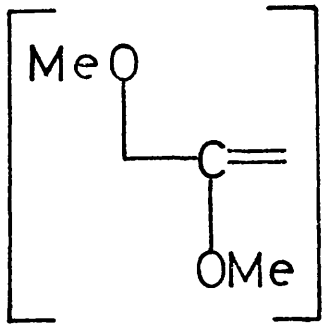
LXXXVI



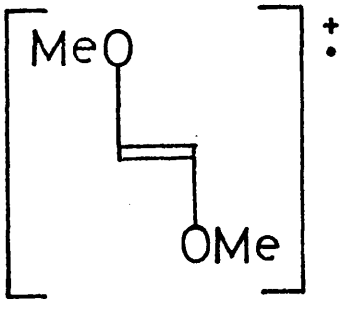
LXXV



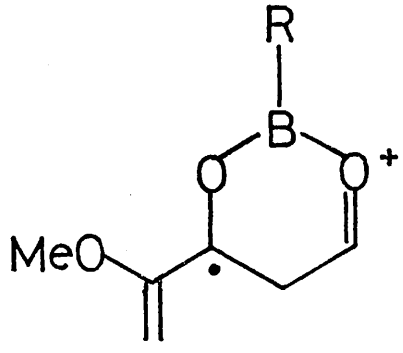
LXVI



LXXXVII



LXXXVIII



LXXXIX

Me 2,3-di-O-Me ∝-D-glucopyranoside

As can be seen from the diagram (LXXI), there are only two free hydroxyls in this molecule available for boronation, so the only possible simple derivative would be the 4,6-boronate (LXXXVI), with a molecular weight of 308 for the phenyl ester, and 246 for the methyl ester. In both cases this molecular ion is present in the spectrum, though in neither case is it very prominent. As expected there are peaks in each spectrum which correspond to the six-membered cyclic boronate ion of m/e = 159 and 97 (LXXV) but there are also ions at m/e = 161 and 99 for the phenyl and methyl boronates presumably having the structures shown (LXVI) (120, 137). The spectra each show ions at m/e = M-31, and M-63, corresponding to loss of first a methoxy group, then methanol respectively, while the ions at m/e = 101 and 88, the latter being the base peak, are both methoxy- containing (LXXXVII, LXXXVIII) arrived at by breakdown of the sugar ring itself, while a similar breakdown is responsible for the largest boron-containing fragment, m/e = 216 and 154 in each ester, which is presumably as shown (LXXXIX).

In this case, too, there seems to be rearrangement to the five-membered cyclic esters (131), for there are peaks in the methyl-boronate spectrum at m/e = 84, and in the phenylboronate spectrum at m/e = 146. This shows that rearrangement from one form of the cyclic ester to the other under electron bombardment is feasible, because the original structure of the ester was of necessity the six-membered form attached to the 1,3-diol system unless a methyl migration occurred during the boronation reaction, which is unlikely.

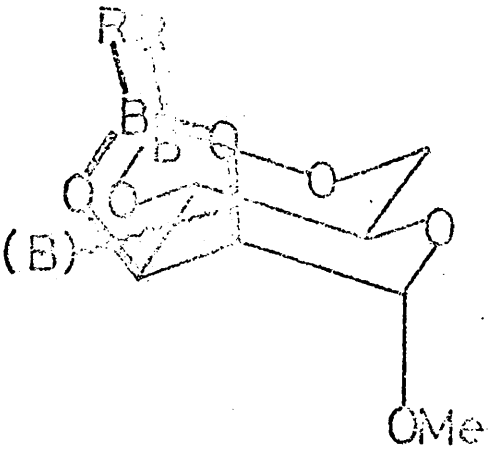
Me ∝-D-mannopyranoside

Since for this sugar both the diboronate and the monoboronate TMSi ethers can be formed, both of them were examined by GC-MS, first the diboronate, then the mixed derivative.

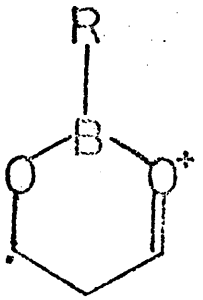
TABLE 9

Me 2,3-di-O-Me- α-D-glucopyranoside boronate

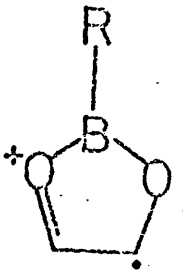
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 5	1475	246	88	.25	215(2)	183(6)	154(6)
					101(6)	99(2)	97(4)
					89(6)		
Phenyl 6	2055	308	88	.1	277(2)	245(3)	216(8)
					161(3)	159(3)	101(6)
					89(6)		



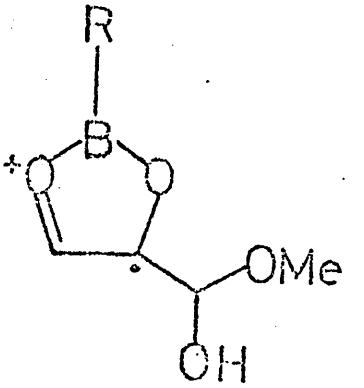
LXV



XC



LXVII



XCI

6ft., 1% SE-30, a - 105°C, b - 150°C, N_2 - 35ml/min.

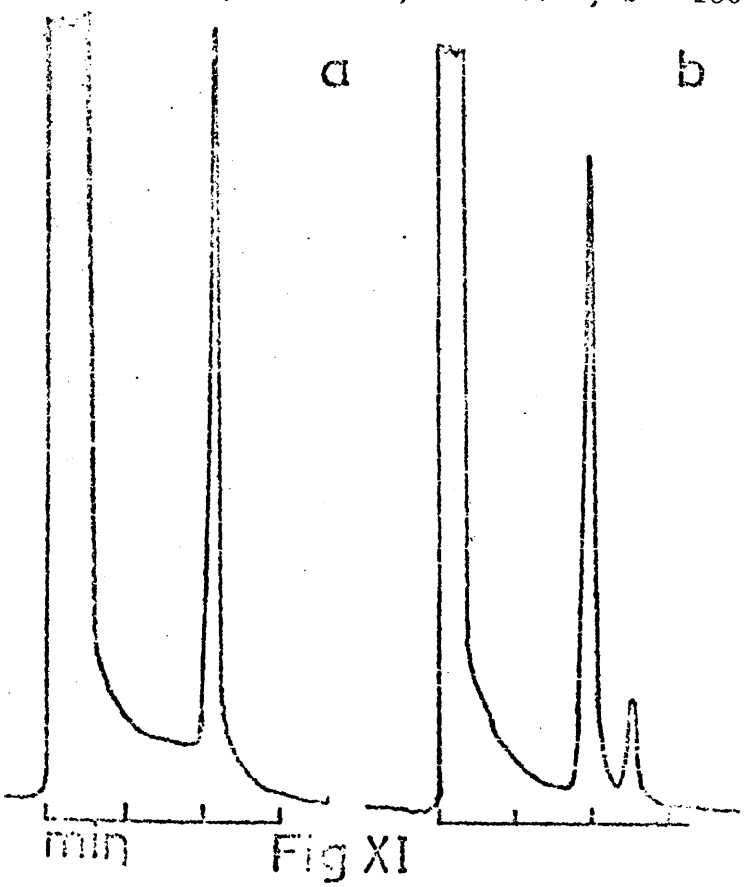


Fig XI

Me α-D-manopyranoside boronate TMSi ether.

a - methyl,
b - phenyl.

2.3-4,6-diboronate

Me α-D-mannopyranoside has previously been shown to form the diester (LXV) (106, 120), and as can be seen from the results in Table 10, though the molecular ion is not present in either of the spectra, the ion of m/e = 331 and 211 equivalent to the loss of a methoxy group from the phenyl- and methyl-boronates is present in each. Also present in the spectra are ions derived from both the five-membered and six-membered boronates; both types are present in sufficient intensity to rule out the possibility of one being the rearrangement product of the other; however, the ratio of their intensities is different in each of the esters. In the methyl-boronate, the base peak is due to the ion of m/e = 98 (XCb), and the five-membered ring ion m/e - 84 (LXVIIb) is present with 32% of its intensity, while for the phenyl-boronate the base peak is due to the five-membered ring ion m/e = 146 (LXVIIa) while the six-membered ring ion m/e = 160 (XLa) has 50% of its intensity. Probably, fragmentation proceeds by cleavage of the bond α- to both of the boronate rings, as shown (B). Subsequent breakdown of one of the boronates to give the $RB\overset{+}{O}H$ ion of m/e = 105 and 43, would result in the formation of the stable ions containing the other ring; thus, cleavage of the five-membered ring would result in the ion (XC), while cleavage of the six-membered ring would give the ions of m/e = 206 and 146 (XCI) and m/e = 146 and 84 (LXVII). The more stable boronate ring would be expected to give rise to the more intense ring ion in the mass spectrum, so in the methylboronate the six-membered ring is the more stable, while in the phenylboronate the reverse is true. This suggests that the phenyl group is either stabilising the five-membered ring, or destabilising the six-membered ring, relative to the methyl group.

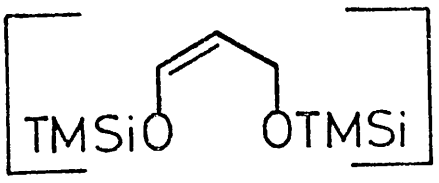
Boronate TMSi ether

After treatment of the diboronate with the trimethylsilylating reagents, the methyl boronate gave one peak, while the phenylboronate gave two peaks, in the approximate ratio of 7 : 1 (Fig. XI).

TABLE 10

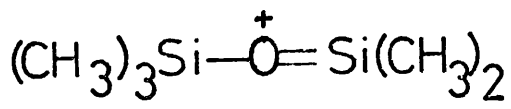
Me α-D-mannopyranoside diboronate

	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 7	1430	242	98	-	144(6)	97(60)	84(32)
					83(10)	61(41)	43(25)
Phenyl 8	2410	366	146	-	206(10)	160(50)	159(43)
					147(12)	145(19)	105(12)



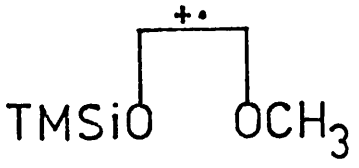
m/e = 218

XCII

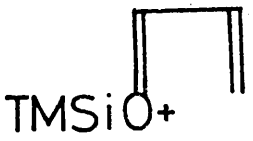


m/e = 147

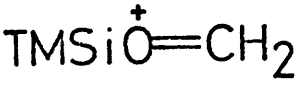
XCIII



XCIV m/e = 146

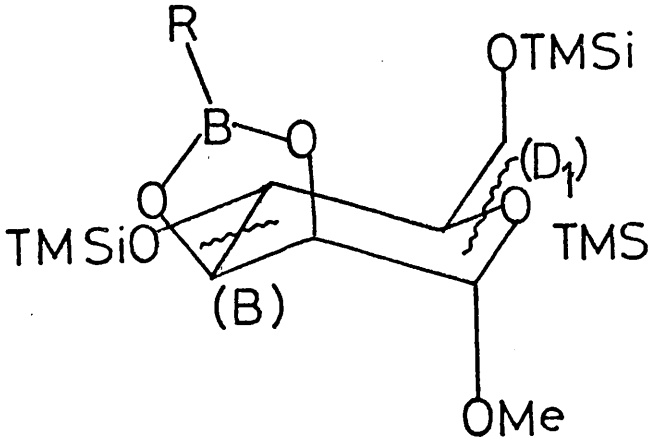


XCV m/e = 129

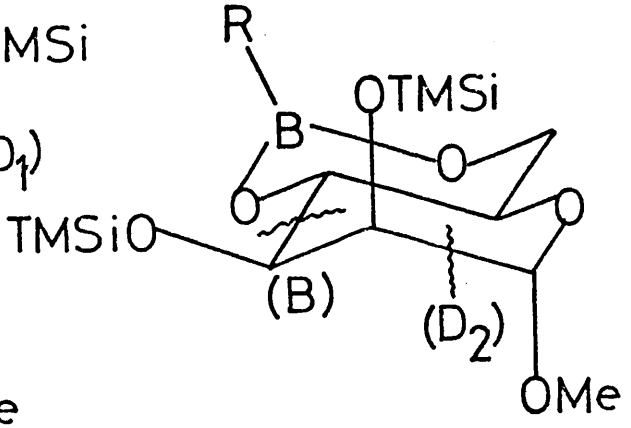


m/e = 103

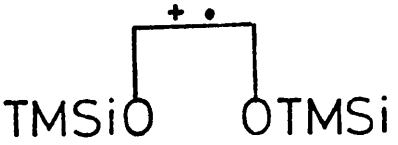
XCVI



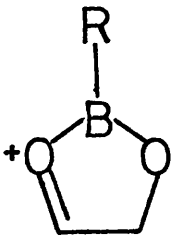
XCVII



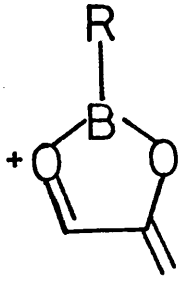
XCVIII



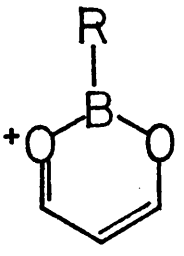
XXI



LV



LXXXV



LXXV

Examination of the methyl- and phenylboronate TMSi ethers by GC-MS gave the results that are summarised in Table 11. The peak at m/e = 73 is neglected, here and in other instances; this is due to the ion $(CH_3)_3 Si^+$ (68), and is almost always the most abundant ion in the 70eV mass spectrum of a TMSi ether. The methylboronate TMSi ether and the first of the phenylboronate TMSi ethers both have a base peak of m/e = 218 (XCII) (71), while that of the second phenylboronate has an m/e of 204 (XXI) (71). These and most of the other ions present in the mass spectrum are well-known ions derived from the fragmentation and rearrangement of the TMSi ether groups (XCIII-XCVI) (71).

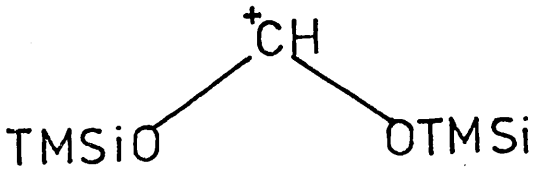
Examination of the two possible structures, the 2,3-boronate TMSi ether (XCVII) and the 4,6-boronate TMSi ether (XCVIII) suggests that the former would be more likely to give the ion of m/e = 218 (XCII) directly, by cleavage from the sugar ring of carbons 4 and 5 as shown (B, D_1) while the latter would be expected to give the m/e = 204 ion (XXI) by a similar cleavage of the sugar ring resulting in the liberation of carbons 2 and 3 (B, D_2). This evidence, coupled with the occurrence, in the spectra of both the methylboronate TMSi ether and the first of the phenylboronate TMSi ethers, of the five-membered boronate ion (LV) of m/e = 85 and 147 (122, 131) respectively, and its absence in the spectrum of the latter phenylboronate TMSi ether suggests that the former pair are the 2,3-boronate TMSi ethers and the other the 4,6-boronate TMSi ether respectively. The peaks at m/e = 159 in the 2,3- derivatives would then probably be due to the ion LXXXV while that in the 4,6- derivative would be due to the six-membered ring ion (LXXV) (136).

The molecular ions are not present in any of the spectra, the ion of highest mass in the methylboronate TMSi ether being that of m/e = 249, equivalent to loss of 73 mass units($(CH_3)_3Si^+$) from the molecule, and in the phenylboronate TMSi ethers that of m/e = 409 due to loss of a CH_3 group, probably from one of the TMSi ether groups.

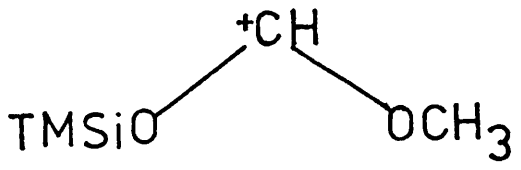
TABLE 11

Me α-D-mannopyranoside boronate TMSi ethers

	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 9	1655	362	218	-	249(1)	147(72)	146(15)
					129(30)	103(35)	97(32)
					85(18)		
Phenyl 10	2145	424	218	-	409(2)	191(10)	159(23)
					147(53)	146(20)	129(24)
					103(24)		
11	2220	424	204	-	409(1)	159(9)	147(38)
					146(11)	133(2)	129(15)
					103(31)		



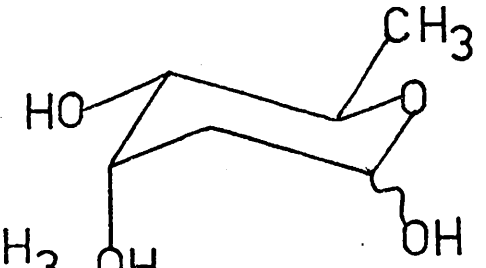
XCIX



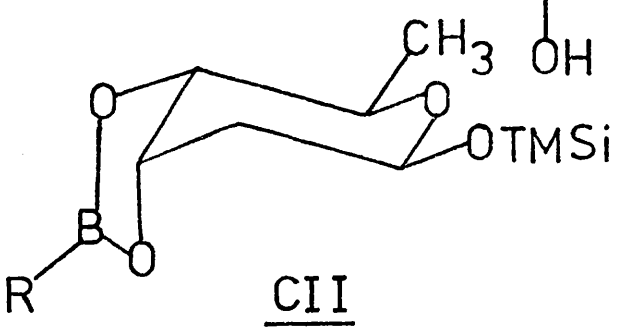
C

For the 2,3- phenylboronate (XCVIIa) the TMSi ions are the same as those which occurred in the methylboronate (XCVIIb) with the addition of a peak at m/e = 191, equivalent to the ion (XCIX) which probably arises from rearrangement of the molecule during fragmentation with the result that both TMSi groups become attached to carbon 6. This ion does not occur to any great extent in the 4,6-boronate TMSi ether, as would be expected, since there is no TMSi ether group on carbon 6, nor any other $-CH_2OTMSi$ group in the molecule. For similar reasons the ion of m/e = 103 (XCVI) occurs to a much larger extent in the 2,3- isomer than in the 4,6-isomer. In this latter the ion at m/e = 133 (C) is present in greater abundance than in the former isomer. Presumably this ion is formed by a rearrangement taking place during the fragmentations of both isomers, with a TMSi ether group becoming attached to carbon 1 which is then eliminated. In the 4,6-phenylboronate TMSi ether (XCVIIIa) the TMSi ether groups are closer to carbon 1 than those in the 2,3-phenylboronate TMSi ether, hence, in the former case the rearrangement proceeds more readily, resulting in the larger abundance found in the mass spectrum.

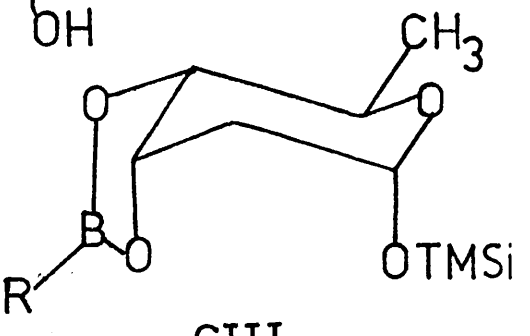
In the original Me α-D-mannopyranoside diboronate, both the five-membered and six-membered cyclic boronate ester groups were present. The reaction with the trimethylsilylating reagents resulted in the preferential formation of the 2,3-boronate TMSi ether from the di(phenylboronate), and its exclusive formation from the di(methylboronate). It would seem that, under the reaction conditions, one of the ester groups is being hydrolysed; since the trimethylsilylation reaction is known to be rapid (37), the TMSi ether groups would probably be formed with whichever hydroxyls were liberated by the hydrolysis, before the monoboronate thus formed had time to rearrange to any other form, i.e. the TMSi ether groups would trap the kinetic product of the hydrolysis. This would imply that the six-membered boronate ring was, in this case, more susceptible to hydrolysis than the five-membered ring.



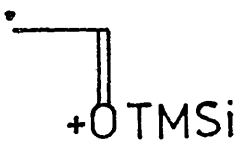
CI



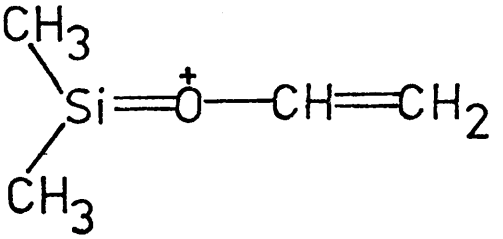
CII



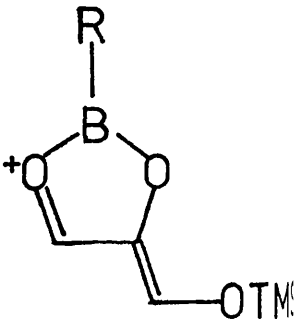
CIII



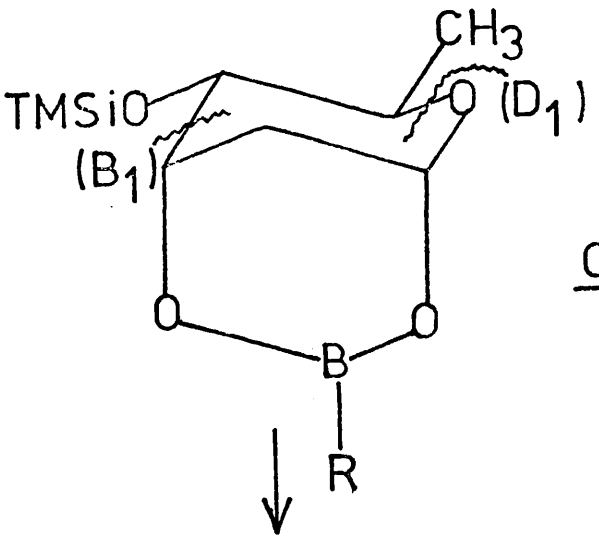
CV



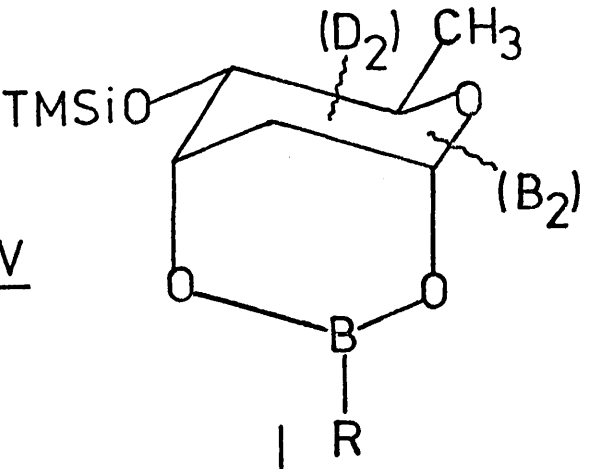
CVI

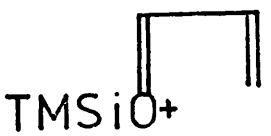


CVII

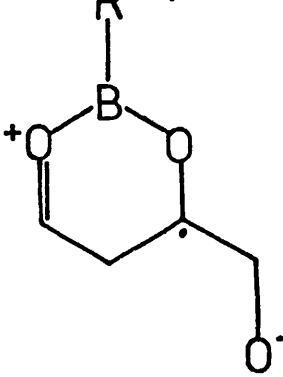


CIV





XCV



CVIII

D-digitoxose

The sample of this sugar used for these experiments was a mixture of the α- and β- anomers (CI), and, as a result there are three possible boronate TMSi ethers that can be formed; the α- and β- TMSi ethers of the 3,4-boronate (CII, CIII), and the TMSi ether of the 1,3-boronate (CIV). After the reaction three products were obtained in both cases, and the GC-MS results are summarised in Table 12. The ratio of the peak heights on GLC was found to be approximately 1 : 1 : 2 for the phenyl- and 2 : 2 : 1 for the methylboronate TMSi ethers.

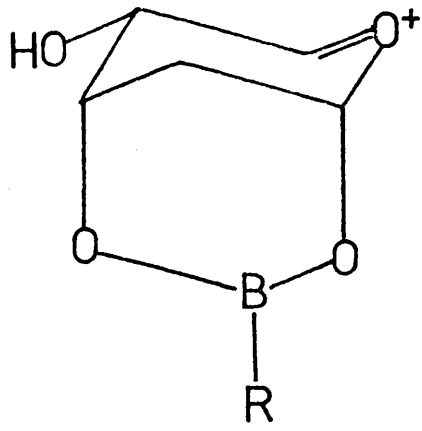
In both the phenyl- and methylboronates the first pair of peaks are very similar to each other and to the corresponding peaks in the other isomer, each of the four having a base peak of m/e = 116 (CV), and peaks corresponding to the five-membered cyclic boronate ion (XLI) i.e. at m/e = 146 and 84. This suggests that these pairs of peaks are due to the 3,4-boronate TMSi ethers (CII, CIII), the base peaks arising by elimination from the sugar ring, of carbons 2 and 1, the latter of which is bonded to the TMSi ether, while the ion of m/e = 101 (CVI) arises by the same process. The ion in the methylboronate of m/e = 185 and its counterpart (m/e = 247) in the phenylboronate ester are formed by a rearrangement process during the fragmentation of the sugar ring to give the ion CVII.

The other pair of esters would then be the 1,3-boronate TMSi ethers (CIV), and the base peak at m/e = 129 (XCV) (67) would arise by cleavage, from the sugar ring, of the fragment containing carbons 4,5 and 6 as shown (B_1, D_1). A breaking of the carbon - oxygen bond α- to the ester ring (B_2), followed by elimination of a CH_3CHO group, by cleavage of the bond between carbons 4 and 5 (D_2), would result in the ion of m/e = 262 and 200 for the phenyl and methylboronate esters respectively (CVIII). The M-87 peak at m/e = 219 and 157 is caused

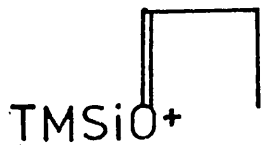
TABLE 12

D-digitoxose boronate TMSi ethers

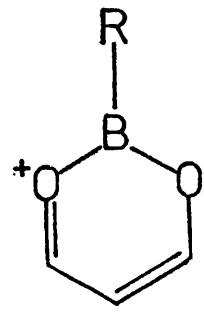
		$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)			
Methyl	12	1255	244	116	-	185(15)	101(53)	84(28)	73(23)
	13	1285	244	116	-	185(13)	101(53)	84(22)	73(23)
	14	1310	244	129	1	200(46)	157(34)	116(51)	101(30)
						73(120)	97(10)	43(40)	
Phenyl	15/16	1850	306	116	-	146(41)	105(17)	101(35)	73(27)
		1890	306	116	-	146(40)	105(14)	101(32)	73(26)
	17	1910	306	129	-	262(53)	219(32)	159(24)	131(55)
						116(53)	105(80)	101(61)	73(190)



CIX



CX

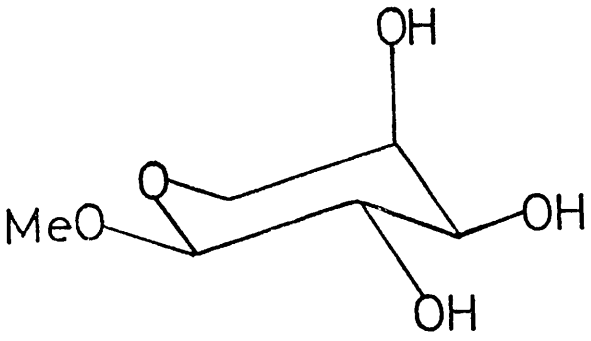


LXXV

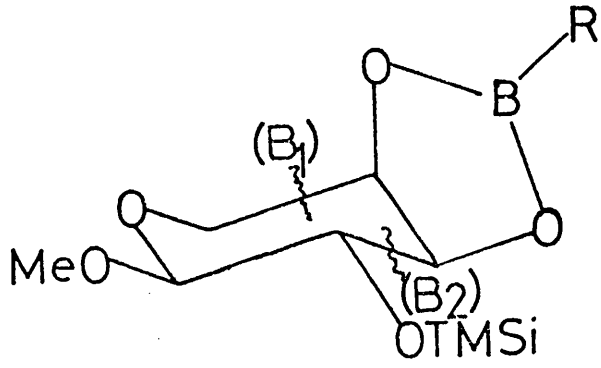
by loss of both the TMSi group and the methyl group giving the ion shown (CIX). The other significant ions in the spectrum are all standard ions observed in the fragmentation of a TMSi ether, i.e. those of m/e = 131 (CX), 116 (CV), and 101 (CVI), except for the boron- containing fragments of m/e = 159 and 97, which are due to the six-membered boronate ring (LXXV), and 105 and 43, due to the $RB\overset{+}{O}H$ ion.

Since trimethylsilylation is a rapid reaction (37), treatment with the reagents may result in the trapping of whatever boronates were present at the time of reaction, in the same way as the TMSi ethers can be used to trap the products of the equilibration of a sugar (87). Thus, in a sugar like D-digitoxose, where the reaction sequence can result in a number of products the form and ratio of these will be the result of a rearrangement during trimethylsilylation or that existing before the reaction. These last could be the result of one or both of two processes, the kinetic products of the boronation, in which the most readily formed boronates predominate, or the equilibrium products, in which the boronates have had time to rearrange to the most stable forms. These topics will be discussed in more detail later in the thesis.

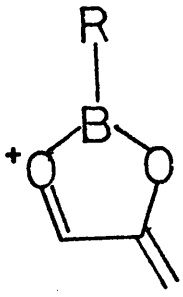
To investigate which of these processes was operating, samples of D-digitoxose were treated separately with methyl- and phenylboronic acid and heated to 100°C. Samples were taken out at intervals, trimethylsilylated, and examined by GLC. It was found that there was no apparent change in composition, so if equilibration is taking place it must do so very quickly, and so the mixture treated with the trimethylsilylating reagents will contain the most stable compounds, which in D-digitoxose prove to be the 3,4-boronate TMSi ethers (CII, CIII) and to a lesser extent the 1,3-boronate TMSi ether (CIV).



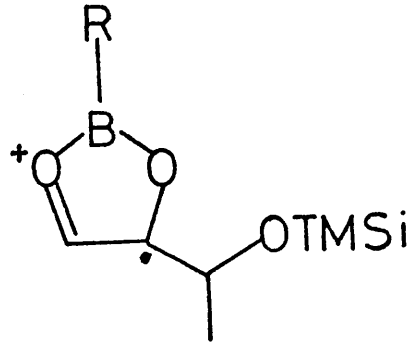
CXI



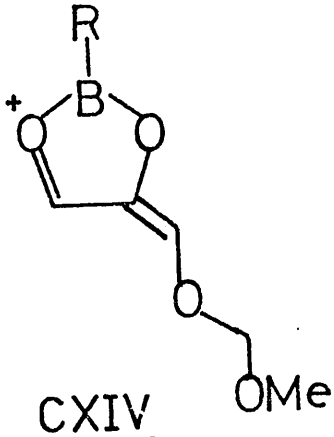
CXII



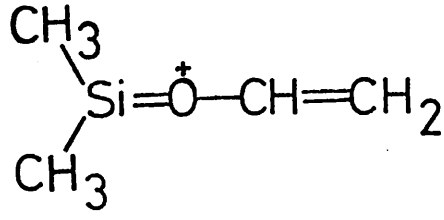
LXXXV



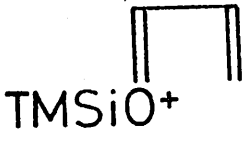
CXIII



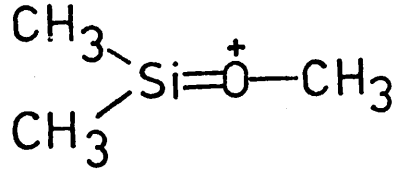
CXIV



CVI



XCV



CXV

Me β-L-arabinopyranoside

This sugar (CXI) has three free hydroxyl groups available for derivatisation, but of these, only two, on carbons 3 and 4 are cis- to each other and so available for boronate formation, giving the 3,4-boronate TMSi ether (CXII) (108) after the normal reaction sequence. From the GC-MS properties summarised in Table 13, it can be seen that while the molecular ions themselves were not obtained in each case the M^{+}-60 ion, m/e = 262 and 200 was seen in the spectrum. The cyclic five-membered boronate ion at m/e = 146 and 84, appears in the spectra, along with an ion at m/e = 159 and 97, which must in this case, as with Me α-D-mannopyranoside 2,3-boronate TMSi ether, be due to the ion (LXXV). The ions of m/e = 262 and 200 (CXIII) would be the result of initial cleavage of the sugar ring, α- to the boronate (B_1) while the M-103 ion, m/e = 219 and 157 (CXIV) would arise from a similar α- cleavage (B_2) with subsequent loss of carbon 4 and the TMSi ether group to which it is bonded. This last fragment would give rise to the ion of m/e = 101 (CVI). The ions of m/e = 129 and 89, each of which occur in both spectra, are derived from the fragmentation and rearrangement of the TMSi ether group and have the structures XCV and CXV respectively.

Me α - L-rhamnopyranoside

After the reaction sequence, this sugar (CXVI) was found to give one derivative of each boronate ester. Only in the case of the phenyl-boronate did the molecular ion, m/e = 336 appear in the mass spectrum, but in both cases the M-15 peaks, m/e = 321 and 259, were seen, confirming the formation of the boronate TMSi ether. As in the last mentioned sugar, there are three free hydroxyls, but only two of them are cis- to each other and hence available for boronation, those on carbon 2 and carbon 3, giving the 2,3-boronate TMSi ether (CXVII).

Apart from the base peak, m/e = 130 (CXVIII), which arises by cleavage

TABLE 13

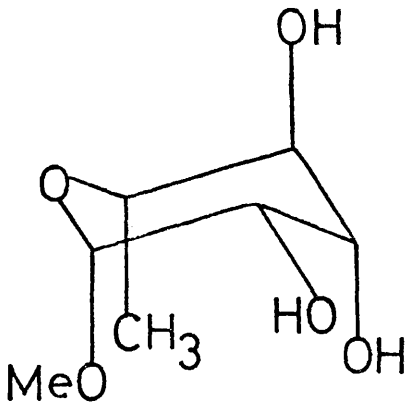
Me α-L-arabinopyranoside boronate TMSi ethers

	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 18	1285	260	116	-	200(15)	157(20)	129(63)
					101(63)	97(23)	89(37)
					84(12)		
phenyl 19	1915	322	116	-	262(11)	219(11)	159(19)
					146(38)	129(45)	101(46)
					89(34)		

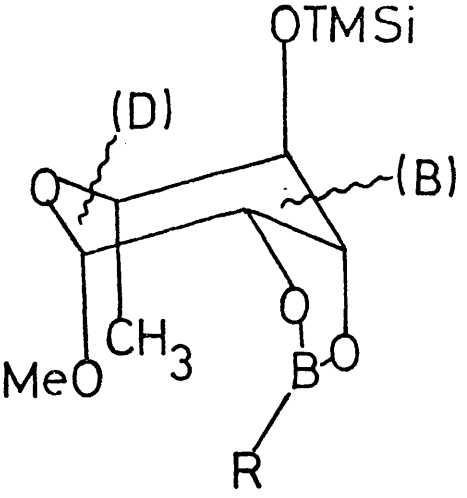
TABLE 14

Me α-L-rhamnopyranoside boronate TMSi ether

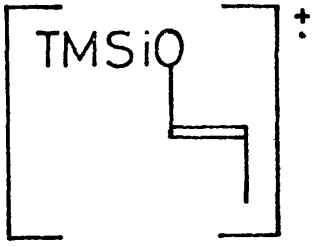
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)			
Methyl [20]	1385	274	130	0	157(13)	146(14)	115(25)	84(5)
Phenyl [21]	1935	336	130	1	219(7)	146(16)	115(15)	



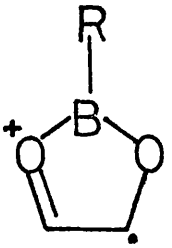
CXVI



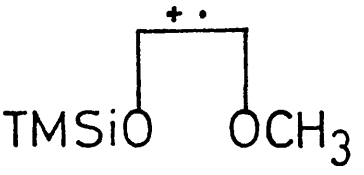
CXVII



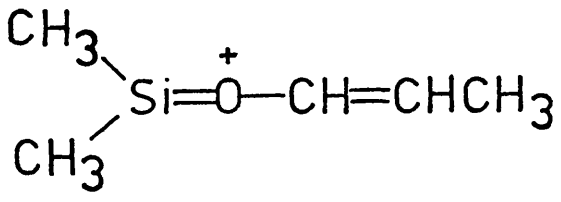
CXVIII



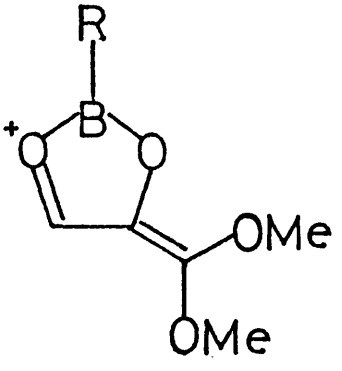
LXVII



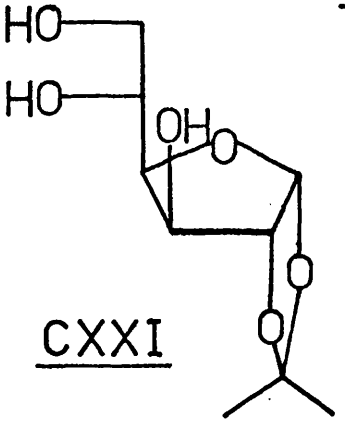
XCIV



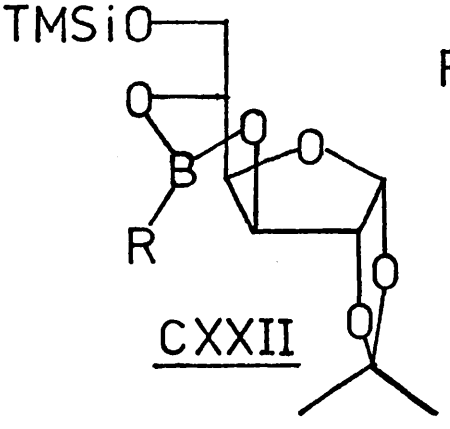
CXIX



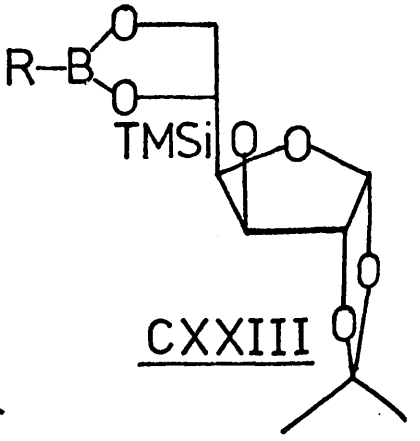
CXX



CXXI



CXXII



CXXIII

of the sugar ring, α- to the boronate ring, between carbons 3 and 4 (B) and between carbon 5 and the oxygen (D) the spectra show very few peaks of any appreciable intensity. The ions derived from the five-membered cyclic ester at m/e = 146 and 84 [LXVII], are present in very low amounts, and the former is masked by the TMSi ether fragment of m/e = 146 (XCIV), which is present in both spectra. Examination of this peak, in the phenylboronate, by high resolution mass spectrometry, shows that it is composed of two separate peaks, of m/e = 146.05368 and 146.26314. The first of these is the boron-containing ion (LXVII) which has a theoretical, accurate mass of 146.053982 while the second is the TMSi ether fragment (XCIV) which has a theoretical accurate mass of 146.262833.

The peak at m/e = 115 seems to be a rearrangement ion formed by the shift of the methyl group to carbon 3, followed by the liberation of this carbon to give either this ion CXIX, or that of m/e = 219 and 157, which has the structure CXX.

1,2-O-isopropylidene- α-D-glucofuranose

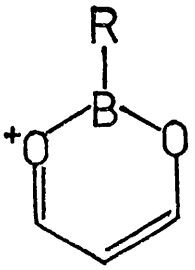
All three of the free hydroxyls in this sugar (CXXI) are available for esterification, giving two possible alternatives, the 3,5-boronate TMSi ether (CXXII) or the 5,6-isomer (CXXIII).

Both observed derivatives show the ion of m/e = 159 and 97 (LXXV) and not the corresponding five-membered ring ion (LXVII) suggesting the presence of the six-membered boronate ring, i.e. the 3,5-boronate TMSi ether (LXXII): this is further borne out by the fact that the 5,6-boronate TMSi ether might be expected to form the five-membered ring ion of m/e = 147 (LV) in the same way as the boronates of some monoglycerides (122), and of corticosteroid 20, 21-diols (131), by cleavage α- to the ester ring. Thus the more likely derivative in this case is the 3,5-boronate TMSi ether (CXXII).

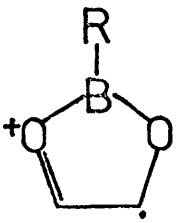
TABLE 15

1,2-isopropylidene α-D-glucofuranose boronate TMSi ether

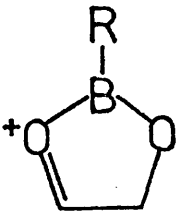
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)			
Methyl 22	1575	316	103	-	301(32)	143(15)	131(16)	117(89)
					100(15)	97(65)	43(20)	
Phenyl 23	2150	378	117	2	363(40)	159(69)	143(17)	131(20)
					105(17)	103(90)	100(14)	



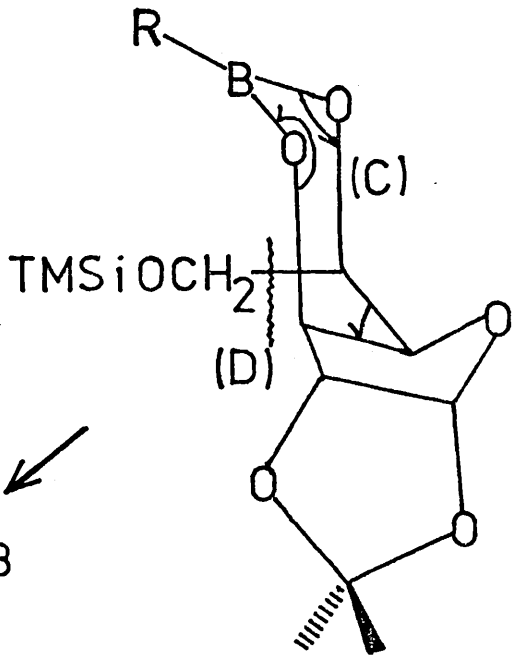
LXXV



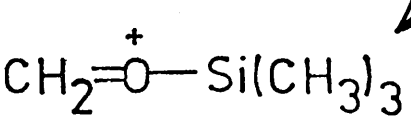
LXVII



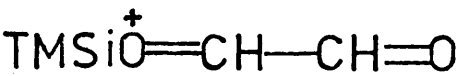
LV



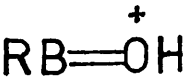
CXXII



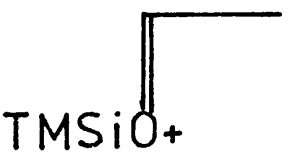
XCVI



CXXIV



LXXVI



CXXV

The mass spectra of the boronates (Table 15) show the molecular ion only for the phenyl derivative, but in both the M-15 peak, arising from loss of a methyl from the molecule, is present to a fair extent, i.e. m/e = 363 and 301 for the phenyl and methylboronate respectively. The two derivatives show different base peaks. The methyl boronate ester has its most abundant ion at m/e = 103, while in the phenylboronate ester this peak has an intensity of 90% of that of the base peak, m/e = 117, which is in turn present in the methylboronate ester with an intensity of 69% relative to m/e = 103. The former ion m/e = 103, would be formed by cleavage of the bond between carbons 5 and 6 (D) to give the ion XCVI, while the latter is presumably obtained by the fragmentation of the boronate ring (C) giving the fragment CXXIV containing carbons 5 and 6 and of m/e = 131. This latter would also result in the ion LXXVI of m/e = 105 and 43, or the base peak m/e = 117 (CXXV). Thus, the ease of formation of this last ion depends upon the ease of fragmentation of the boronate ester. Since the peak has a higher relative intensity in the spectrum of the phenyl boronate, the implication is that the phenyl group is destabilising the six-membered ring, under electron impact, with respect to the methyl group, as it was in the diboronates of methyl α-D-mannopyranoside.

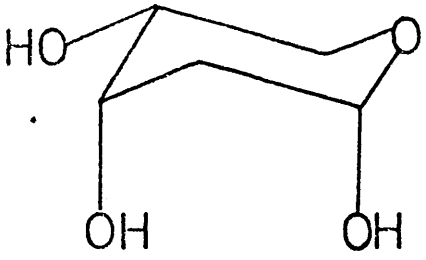
2-deoxy-D-ribose

There are two possible isomers of the boronate TMSi ethers of this sugar (CXXVI), the 3,4-boronate (CXXVII) and the 1,3-boronate (CXXVIII). After the reactions one methyl boronate and two phenylboronates, in the ratio of 6 : 1, are formed. Both the methylboronate TMSi ether and the first of the phenyl derivatives show large peaks due to the five-membered boronate ion (LXVII), at m/e = 84 and 146 respectively, and in fact, in the phenylboronate, this is the ion of greatest abundance (Table 16), implying that these are both the 3,4-boronate TMSi ethers (CXXVI) while the second phenylboronate is the 1,3-isomer (CXXVIII). This last compound does show the ion at m/e = 159 corresponding to the six-membered boronate ion LXXVa. In all the

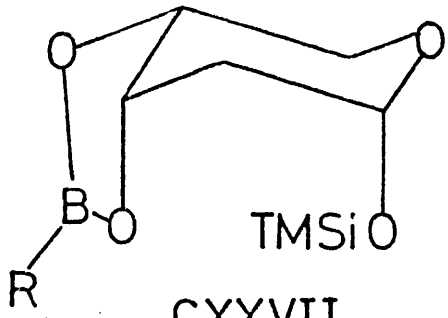
TABLE 16

2-deoxy-D-ribopyranose boronate TMSi ethers

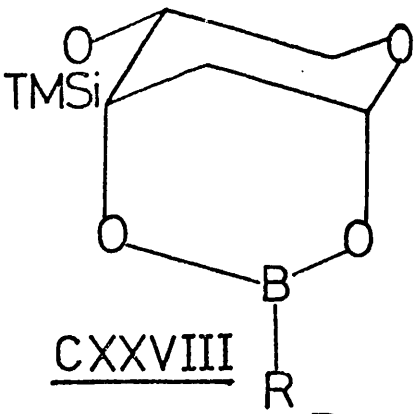
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 24	1255	230	101	2	215(9)	187(17)	185(29)
					145(23)	131(54)	116(65)
					97(21)	84(88)	43(62)
					42(23)		
Phenyl 25	1835	292	146	2	277(29)	249(10)	247(22)
					207(33)	159(16)	131(70)
					116(48)	105(45)	104(41)
					101(70)		
26	1855	292	132	1	277(45)	207(86)	159(28)
					131(27)	129(44)	116(44)
					105(40)	104(22)	101(57)



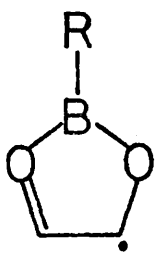
CXXVI



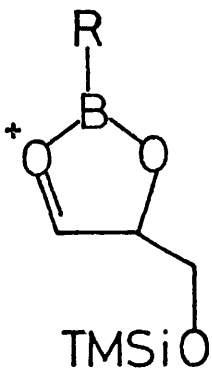
CXXVII



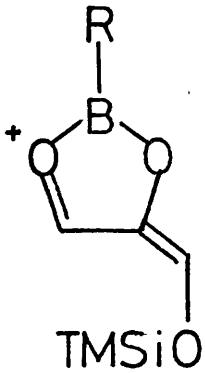
CXXVIII



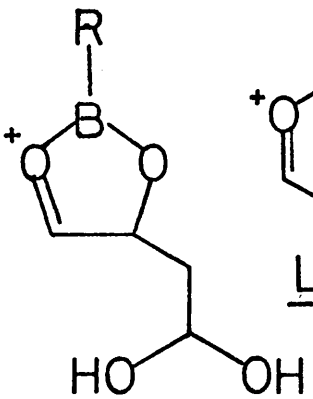
LXVII



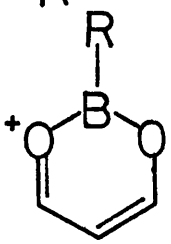
CXXIX



CVII

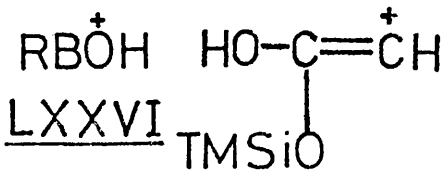


CXXX



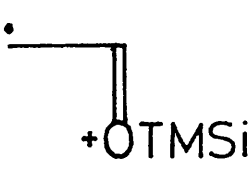
LXXV

CXXXI

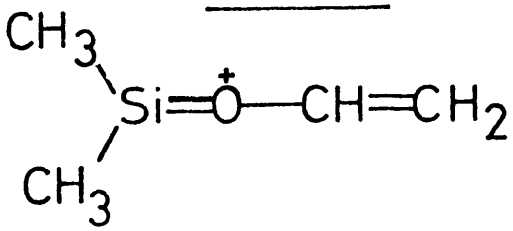


LXXVI

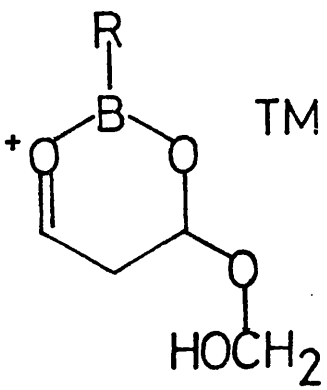
CXXXII



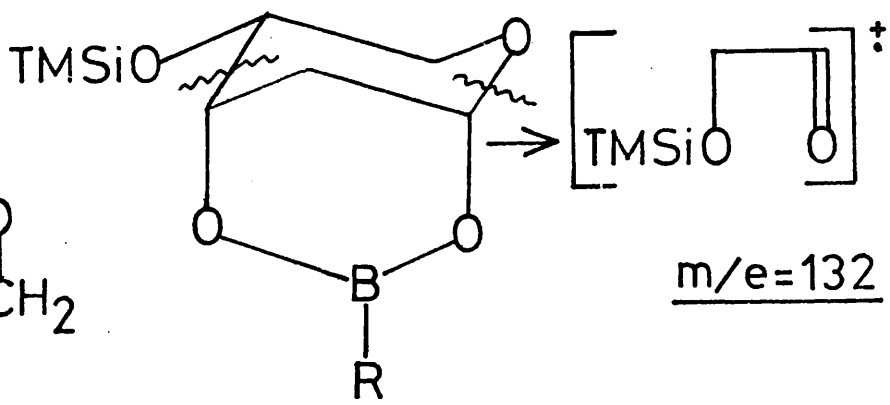
CV



CVI



CXXXIII



CXXVIII

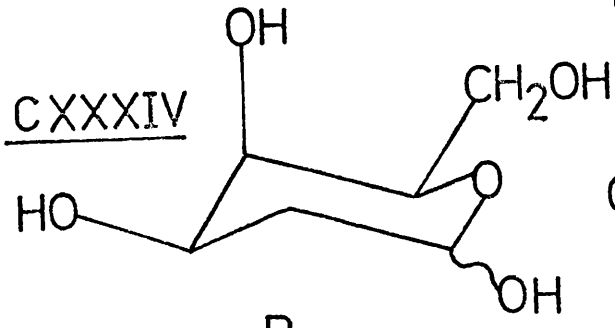
m/e=132

isomers the molecular ion is present to a very slight extent, while the M-15 peak at m/e = 277 and 215 is larger for the methylboronate ester, and very much larger for the phenylboronate esters.

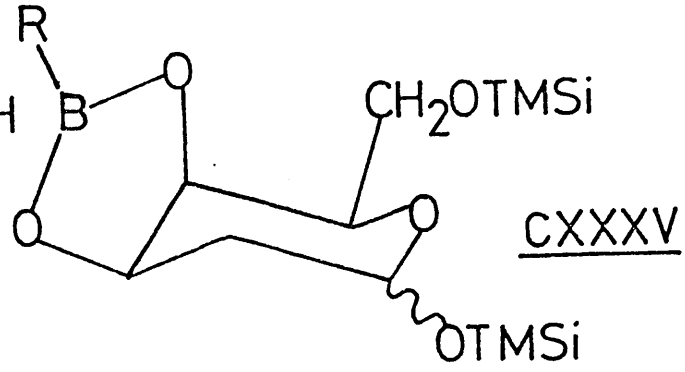
In both of the 3,4-boronates, migration of the TMSi ether group to carbon 2, followed by the cleavage of the sugar ring, would produce the ions of m/e = 249 and 187 (CXXIX) and 247 and 185 (CVII), while a slightly different breakdown of the ring, followed by loss of the $(CH_3)_3Si$- group would give the ions of m/e = 207 and 145 (CXXX). The fragmentation of the boronate ring would account for the two pairs of ions at m/e = 105 and 43 (LXXVI), and 104 and 42 (CXXXI) (114). The remaining significant ions are directly attributable to the breakdown and rearrangement of the TMSi ether, i.e. m/e = 131 (CXXXII), m/e = 116 (CV) and m/e = 101 (CVI), which is the most abundant ion in the spectrum of the methylboronate.

The 1,3-phenylboronate TMSi ether (CXXVIIIa) also shows a peak at m/e = 207, but in this case it either contains the six-membered cyclic ester and so probably has the structure CXXXIII, formed by migration of the TMSi ether, followed by the fragmentation of the sugar ring, and loss of the $(CH_3)_3Si$- group, or is a result of a rearrangement giving the ion (CXXX). The base peak, of m/e = 132, would arise by the cleavage of both of the bonds, in the sugar ring, α- to the boronate ring.

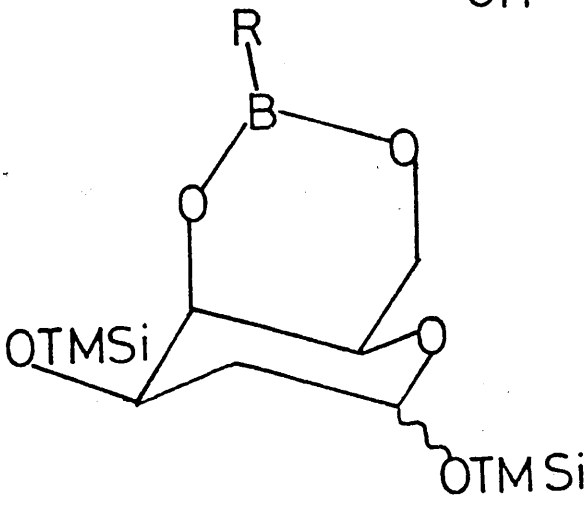
Apart from these ions and the cyclic boronate ion (LXXVa), the rest of the peaks in the spectrum are similar to those occurring in the spectra of the 3,4-boronates.



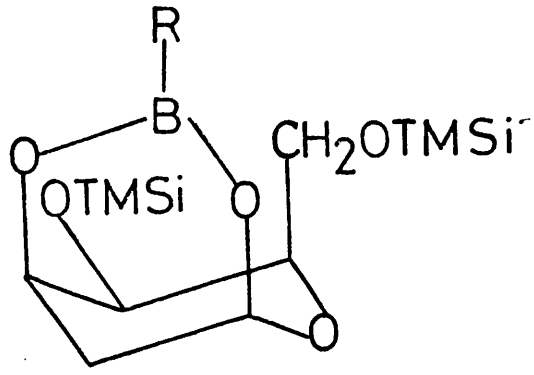
CXXXIV



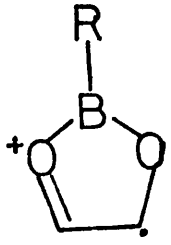
CXXXV



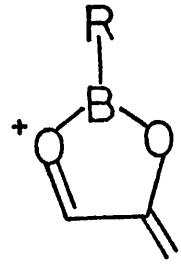
CXXXVI



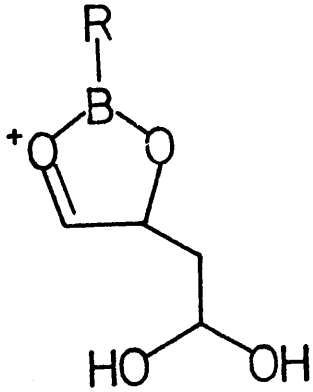
CXXXVII



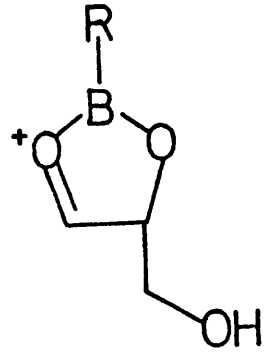
LXVII



LXXXV



CXXX



CXXXVIII

2-deoxy-D-galactose

The configurations of the four free hydroxyl groups in 2-deoxy-D-galactose (CXXXIV), result in the possibility of forming up to five boronate TMSi ethers, the α- and β-anomers of the 3,4-ester (CXXXV), the corresponding anomers of the 4,6-ester (CXXXVI) and the 1,3-ester (CXXXVII).

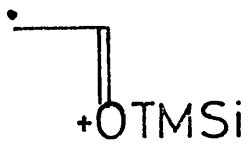
The reaction with methylboronic acid and the trimethylsilylating reagents produces three peaks in GLC, with the ratios of 2 : 1 : 1. Mass spectral examination shows that the last two peaks have almost identical fragmentation patterns, suggesting that they are one of the anomeric pairs, while the first compound is distinctly different from them, but similar enough to be one of the other isomers. The phenylboronate esters also number three, formed in the ratio 2 : 2 : 1, but each of these compounds shows a mass spectrum which is different from each of the others. Comparison of the two sets of spectra obtained from the methylboronate and phenylboronate esters shows similarities between the first compound in each case, and between the last phenylboronate and the pair of methyl boronates.

The cyclic boronate ion common to the first peaks of each set is the five-membered ring ion LXVII of m/e = 146 and 84, for while there are peaks in the methylboronate at m/e = 97 which might be due to a six-membered ring ion there is no corresponding peak in the phenylboronate, and so the ion probably has the structure shown (LXXXV), and hence these are probably the 3,4-boronate esters (CXXXV). If both anomers are present they are inseparable, but there may be only one anomer of each ester of this type formed by the reaction. Neither of the esters shows a molecular ion, the highest ion for the phenylboronate being m/e = 379, equivalent to a loss of a methyl group, and for the methylboronate, m/e = 259, due to loss of 73 mass units, i.e. loss of a $(CH_3)_3Si$- group. Both esters show peaks at m/e = 207 and 145, due to the ion (CXXX),

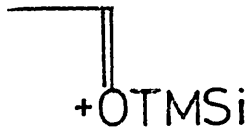
TABLE 17

2-deoxygalactopyranose boronate TMSi ethers

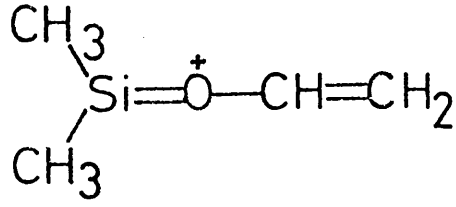
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 27	1325	332	116	-	259(1)	147(24)	145(20)
					129(32)	117(76)	101(56)
					97(11)	85(13)	84(10)
28	1350	332	129	-	242(13)	201(25)	171(53)
					147(20)	119(71)	117(87)
					116(65)	101(78)	98(53)
					97(42)	85(51)	
29	1390	332	129	-	242(25)	201(26)	171(52)
					147(20)	119(69)	117(85)
					116(65)	101(81)	98(53)
					97(42)	85(51)	
Phenyl 30	1930	394	116	-	379(2)	207(5)	191(6)
					147(24)	146(36)	129(29)
					117(95)	101(55)	
32	1940	394	101	2	304(32)	263(11)	233(25)
					160(75)	159(54)	147(80)
					146(30)	129(11)	119(59)
					117(95)	116(87)	
31	1975	394	129	1	304(21)	263(11)	233(19)
					160(70)	159(56)	147(65)
					146(23)	119(49)	117(70)
					116(64)	101(73)	



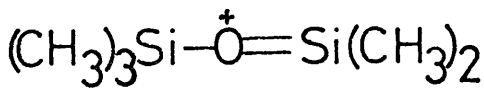
CV



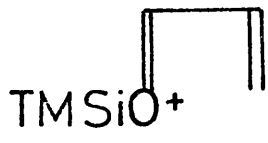
CXXV



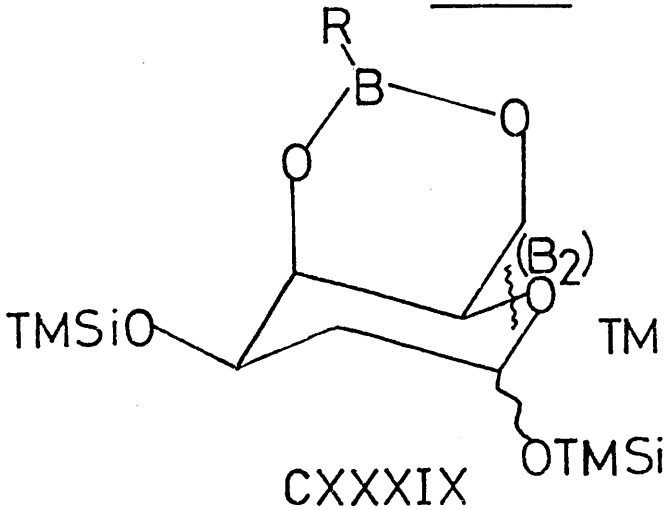
CVI



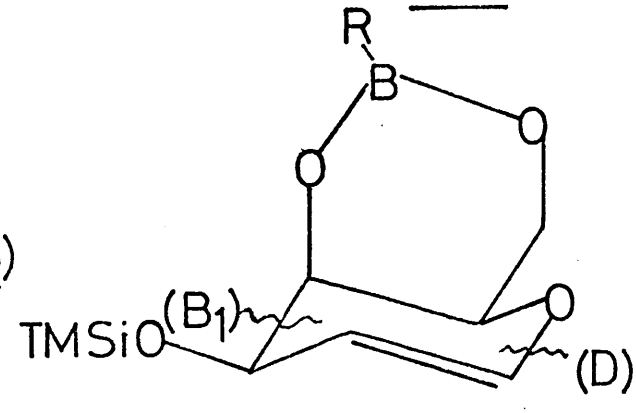
XCIII



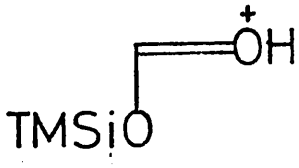
XCV



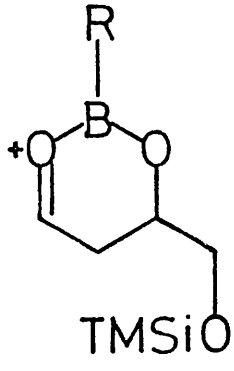
CXXXIX



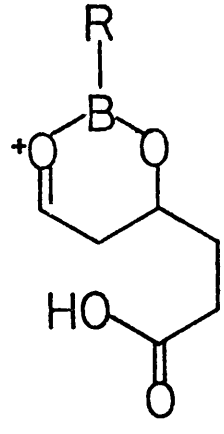
CXL



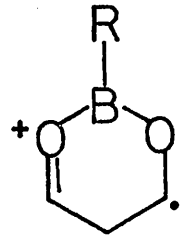
CXLI

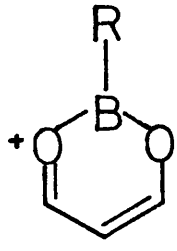


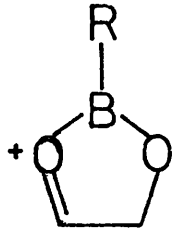
CXLII

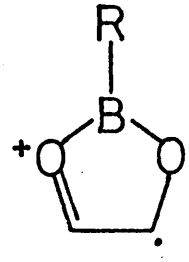


CXLIII







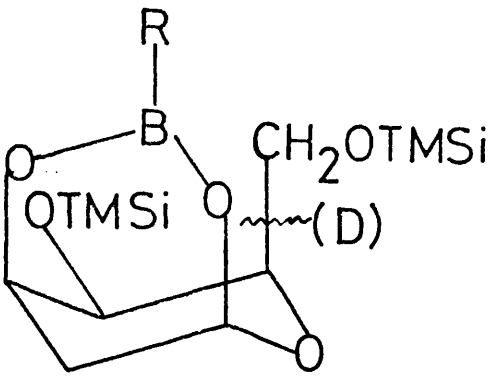


R=Ph	m/e= 160	159	147	146
Me	98	97	85	84

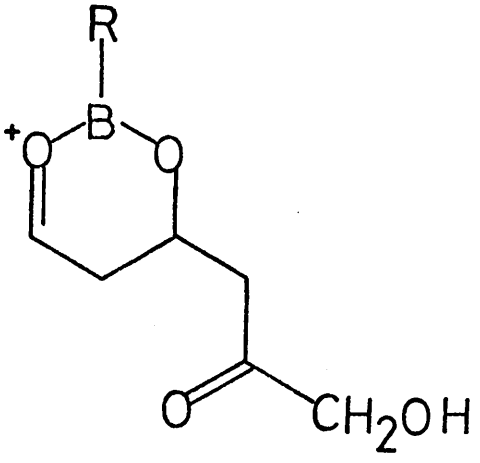
and at m/e = 191 and 129, due to CXXXVIII both of which arise by fragmentation of the sugar ring and subsequent loss of the $(CH_3)_3Si$- group.

The base peak, m/e = 116 (CV), the ion of m/e = 117, CXXV, and that of m/e = 101 (CVI), can arise directly by the elimination of the fragment containing carbons 4 and 5, while the peaks at m/e = 147 (XCIII) and m/e = 129 (XCV) require the rearrangement of the TMSi ether group during the fragmentation, hence the lower abundance of these last two peaks.

If the other pair of methylboronate derivatives are anomers as they seem to be, they, and the last eluted of the phenylboronate esters, would have to be the 4,6-isomers (CXXXIX), though in the latter case both the anomers are present they are indistinguishable by GLC on 1% They all have base peaks at m/e = 129, due to the ion (XCV) which could in this case be obtained directly, by cleavage of the sugar ring after the loss of one of the TMSi ether groups (CXL) and the freeing of the fragment containing carbons 1,2 and 3 (B, D). Of the other ions derived from the TMSi ether group, those at m/e = 119 (CXLI) m/e = 117 (CXXV) m/e = 116 (CV) and m/e = 101 (CVI) can be derived directly from the molecule, hence their relatively large abundance in the spectra, while the ion at m/e = 147 (XCIII) would be formed by a rearrangement process, giving a relatively low abundance. Only the phenylboronate ester shows the base peak, but all three show the M-90 ion due to loss of a TMSiOH group. Cleavage of the sugar ring α- to the boronate ring, between carbon 5 and the oxygen, (B_2) followed by further fragmentation would result in the two ions at m/e = 263 and 201 (CXLII) and at m/e = 233 and 171 (CXLIII). The basic boronate ring fragments, however, illustrate the possible ambiguities that can occur in these derivatives, since there are ions present in both spectra which might suggest the presence of both the six-membered cyclic ion, at m/e = 160 and 98, and m/e = 159 and 97, and the five-membered cyclic ion, at m/e = 147 and 85, and m/e = 146 and 84. Taking the suggestion, however, supported by the rest of the evidence



CXXXVII

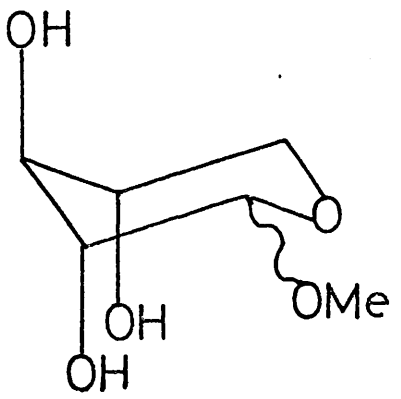


CXLIV

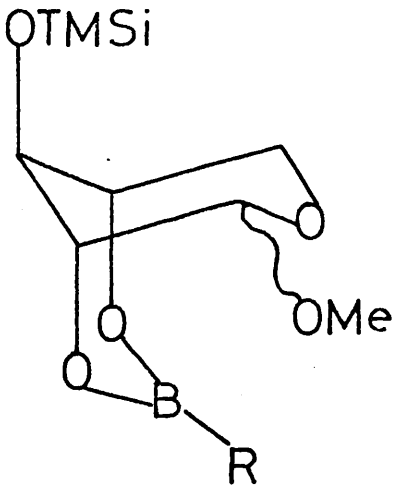
that it is the six-membered boronate ring which is present, the first ions will be due to fragments XC and LXVIII respectively, derived directly from the molecule, while the other ions will be due to a rearrangement process resulting in the fragments LV and LXVII.

The second of the three phenyl esters, which can only now be the 1,3-boronate TMSi ether (CXXXVII), also shows the same ambiguity where the basic boronate ions are concerned, and in fact has many similarities to the last set of esters, the main differences being the base peak at m/e = 101, presumably due to the ion CVI which can be easily formed by cleavage of the carbon 5 - carbon 6 bond, (D), and rearrangement of the $-CH_2OTMSi$ group, and the lower intensity of the peak at m/e = 233, which here would be the fragment CXLIV. The increase in intensity of the ions at m/e = 117 (CXXV) and 116 (CV) reflects the increase in ease of formation of these ions which are probably the result of cleavage of the sugar ring, freeing carbons 5 and 6.

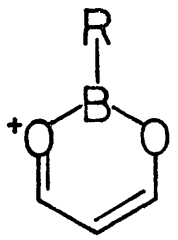
The ease of formation of the various isomers of this sugar, and their mass spectral similarities, highlights the difficulty in dealing with any sugar where the conformation of the hydroxyls allows the formation of several isomeric cyclic boronate esters.



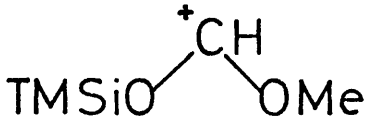
CXLV



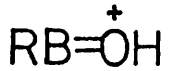
CXLVI



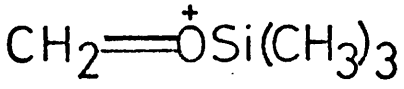
LXXV



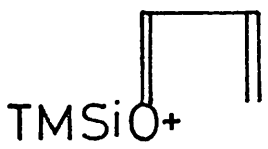
C



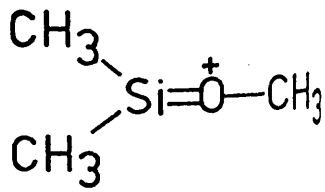
CXXXII



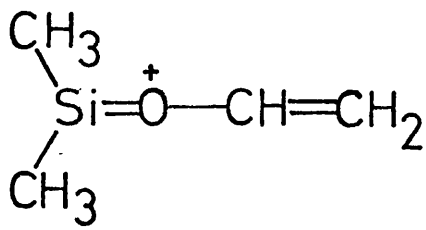
XCIV



XCV



CXLVII



CVI

Me D-xylopyranoside

Both the α- and β- anomers of this compound (CXLV), on treatment with the reagents, form the 2,4-boronate TMSi ethers (CXLVI) (94), and, after the reaction, the derivatives were examined by GC-MS (Table 18). The α- anomers of the methylboronate ester had longer retention time on both 1% SE-30 and 1% QF-1, than the β- anomer, unlike those of the phenylboronate pertrimethylsilyl ether derivatives of the Me D-glucopyranosides and the Me D-galactopyranosides (Tables 1 and 2). Inspection of a model shows that in the α- anomer the methoxy group is very close to the R- group of the boronate which may lead to some sort of deformation which is absent in the β- anomer, leading in turn to a change in retention index, especially in the case where R = Me and the interaction between the groups might be expected to produce a relatively larger effect.

All four compounds show similar mass spectra; the molecular ion appears in none of them, but each contains the M-15 peak due to loss of a methyl group, at m/e = 307 and 245, and the ion at m/e = 159 and 97, consisting of the six-membered boronate ion (LXXV). The base peak at m/e = 133 would be formed by migration of the Me_3SiO- group to carbon 1, which would then be liberated by cleavage of the sugar ring to give the ion C (71), while breakdown of the ester ring would result in the $RBOH^+$ ion of m/e = 105 and 43 (CXXXII), and if followed by a breakdown in the sugar ring, in the formation of the ion XCV of m/e = 129, by the liberation of carbons 1, 2 and 3, or carbons 3, 4 and 5. A similar process, liberating carbon 3 by itself would account for the ions at m/e = 103 (XCIV) and 101 (CVI).

The only appreciable difference in the spectra occurs in the intensity of the ion of m/e = 89, i.e. CXLVII. This ion is formed by the cleavage of the bond between carbon 3 and the ether oxygen. In both the β- anomers it is present with an intensity much higher than that with which it is found in the α- anomers (Table 18). If the close

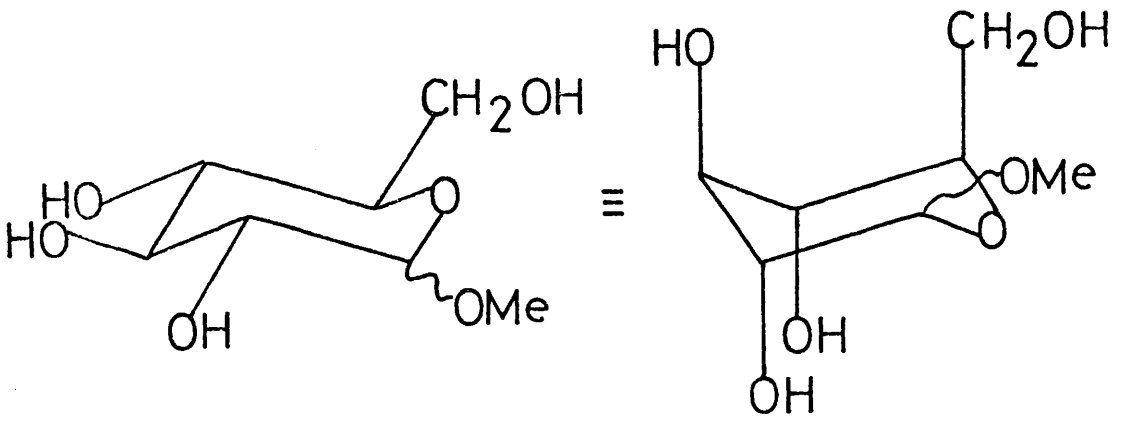
TABLE 18

Me α-D-xylopyranoside boronate TMSi ethers

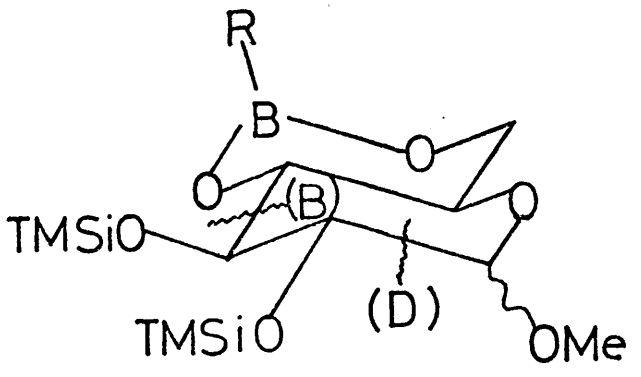
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl [33]	1415	260	133	-	245(3)	129(33)	103(39)
					101(18)	97(26)	89(6)
					43(23)		
Phenyl [34]	1980	322	133	-	307(2)	159(25)	129(26)
					105(14)	103(23)	101(9)
					89(4)		

Me β-D-xylopyranoside boronate TMSi ether

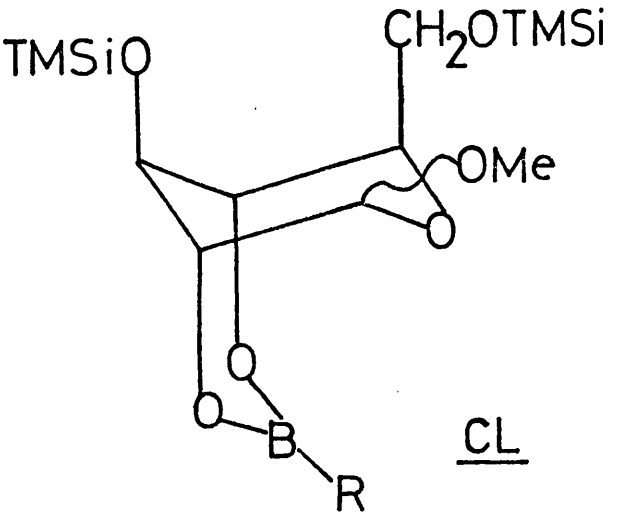
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl [35]	1350	260	133	-	245(1)	129(31)	103(33)
					101(15)	97(29)	89(19)
					43(21)		
Phenyl [36]	2005	322	133	-	307(1)	159(25)	129(23)
					105(12)	103(20)	101(8)
					89(12)		



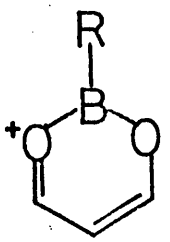
CXLVIII



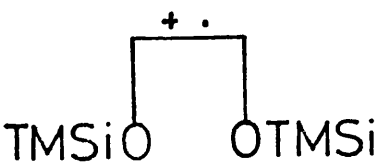
CXLIX



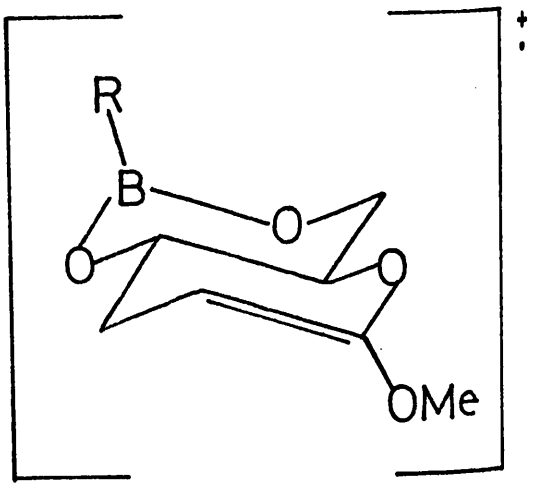
CL



LXXV



XXI



CLI

proximity of the methoxy group to the TMSi ether group in the β-anomer causes some steric strain which affects the ease of elimination of the TMSi ether group, this influence would not be present in the α- anomer and there would, therefore, be less of a driving force towards this elimination, and so a less intense peak in the spectrum. Whatever the mechanism, however, this difference in intensities does provide a means of distinguishing between the α-anomers and β- anomers of the methyl xylopyranosides.

Me D-glucopyranoside

Once again the derivatives of the α- and β- anomers of this sugar (CXLVIII) were made and examined by GC-MS (Table 19). The GLC properties of the anomers of these derivatives were similar to those of the pertrimethylsilyl ethers, in that the α- anomer has a lower retention index than the β- anomer (Tables 3 and 4).

The configurations of the hydroxyls in these sugars, make possible the formation of two derivatives of each anomer, the 2,4-boronate (CL) or the 4,6-boronate (CXLIX). Each of these isomers has a six-membered cyclic ester group, and this is supported by the occurrence in each of the spectra of the ion LXXV of m/e = 159 and 97 respectively. The base peak of all the compounds, at m/e = 204, is due to the ion XXI (71), which would be readily formed from the 4,6-boronate (CXLIX) by the breakdown of the sugar ring, liberating the fragment containing carbons 2 and 3 (B, D). In the 2,4-boronate (CL) the TMSi ether groups are situated on carbons 3 and 5, i.e. they are not cis- to each other, making the formation of the ion XXI a rearrangement process, which is more difficult, and less likely to give an ion which has the greatest intensity in the mass spectrum. The 4,6-ester of Me α-D-glucopyranoside has also been reported as being formed by the reaction of the sugar with phenylboronic acid (107, 116), and so it seems likely that the derivatives formed here are the 4,6-boronate ester TMSi ethers (CXLIX).

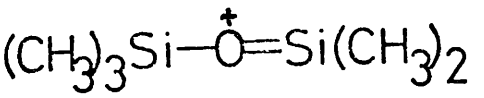
TABLE 19

Me α-D-glucopyranoside boronate TMSi ethers

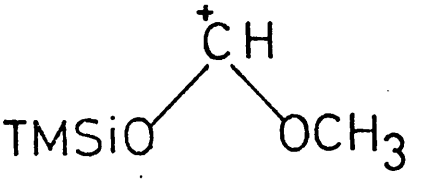
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 37	1690	362	204	-	347(6)	183(22)	147(39)
					133(32)	129(24)	117(17)
					97(7)	89(13)	
Phenyl 38	2280	424	204	-	409(45)	245(13)	159(12)
					147(14)	133(24)	129(18)
					117(12)	89(11)	

Me β-D-glucopyranoside boronate TMSi ether

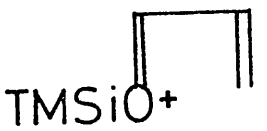
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 39	1715	362	204	-	243(1)	183(4)	147(15)
					133(15)	129(16)	117(12)
					97(7)	89(13)	
Phenyl 40	2310	424	204	-	305(1)	245(2)	159(6)
					147(19)	133(14)	129(10)
					117(8)	89(11)	



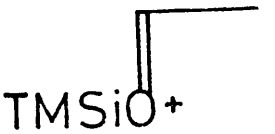
XCIII



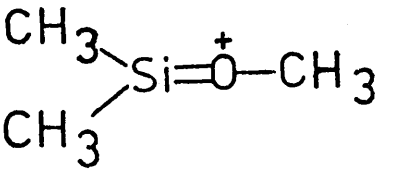
C



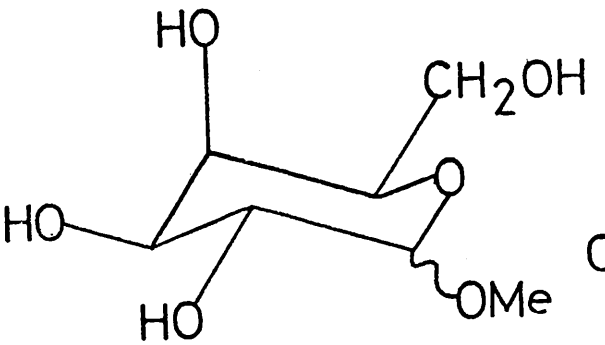
XCV



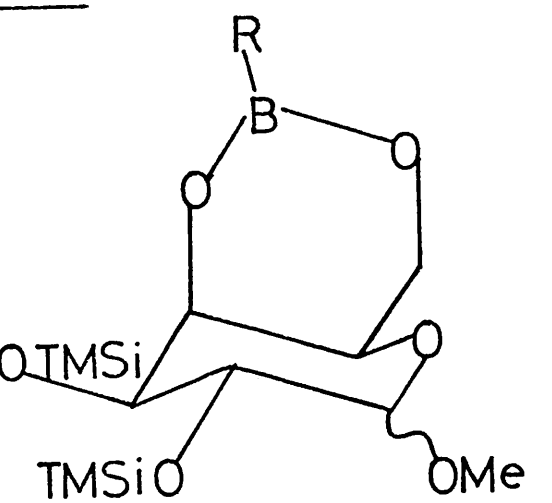
CXXV



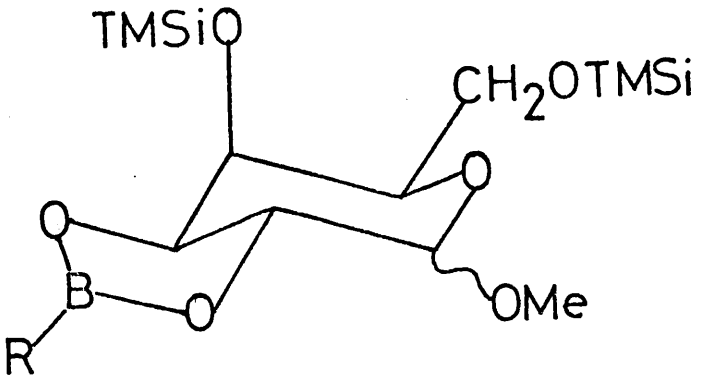
CXLVII



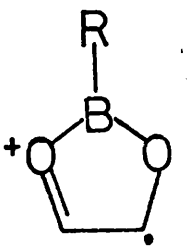
CLII



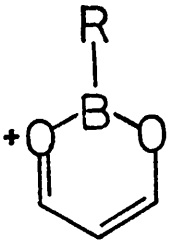
CLIII



CLIV



LXVII



LXXV

None of the esters shows a molecular ion, and while the α- anomers show peaks due to the M-15 ion, m/e = 409 and 347, the highest ion in the spectra of the β- anomers is at m/e = 305 and 243, arising from the elimination of one TMSiO- group and the MeO- group. Another difference between the anomers is shown in the intensity of the peaks at m/e = 245 and 183, which is much greater in the α- anomer; the ion (CLI) would arise by the loss of the two TMSi ether groups. Presumably the difference in conformation of the methoxy group leads to a difference in the stability of the ions. The intensities of the other ions in the spectra show, in general, a trend towards lower values relative to the base peak in the β- anomers than in the α- anomers, which may suggest, in common with the results above, that the sugar ring in the β- anomers is more unstable, and can more easily break down to give the base peak which is due to the ion XXI.

Apart from these differences the spectra of all the isomers are similar, each showing peaks due to the fragmentation and rearrangement of the TMSi ether groups, i.e. the ions at m/e = 147 (XCII), 133 (C), 129 (XCV), 117 (CXXV) and 89 (CXLVII). The differences are, however, a guide to distinguishing between the anomers.

Me D-galactopyranoside

The reaction of the α- and β- anomers of this sugar (CLII) results in the formation, for each, of two products. Examination of the structures of the sugar shows that these must be the 2,3- and 4,6- boronate TMSi ethers (CLIII, CLIV). Absolute identification of each is not possible from consideration of the basic boronate ring ions of m/e = 159 and 97 (LXXV) and m/e = 146 and 84 (LXVII), since both of these ions occur in each of the spectra, with enough similarity in intensity to prevent them from being diagnostic (Table 20). In this case more attention has to be paid to the other ions.

TABLE 20

Me α-D-galactopyranoside boronate TMSi ethers

	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Methyl 41	1635	362	115	-	259(2)	245(51)	218(69)
					204(43)	191(80)	147(64)
					133(15)	129(41)	97(55)
					89(53)	84(26)	
42	1655	362	133	-	347(1)	245(1)	218(2)
					204(44)	191(3)	147(35)
					129(15)	115(7)	97(11)
					89(12)	84(5)	
Phenyl 43	2130	424	177	-	377(4)	307(51)	218(92)
					204(45)	191(93)	159(30)
					147(57)	146(36)	133(23)
					129(36)	117(61)	89(71)
44	2165	424	133	-	218(30)	204(69)	191(33)
					177(39)	159(23)	147(60)
					146(21)	129(29)	117(39)
					89(45)		

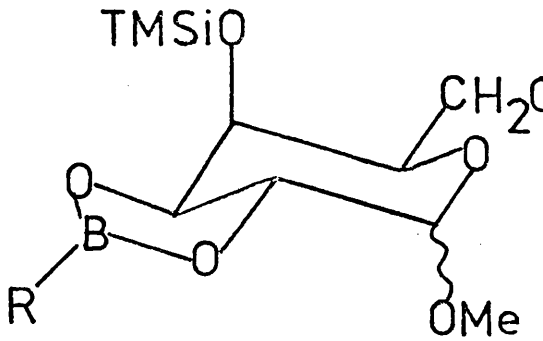
Me β-D-galactopyranoside boronate TMSi ether

Methyl 45	1615	362	115	-	259(1)	245(50)	218(64)
					204(42)	191(74)	147(53)
					133(16)	129(45)	97(42)
					89(31)	84(22)	
46	1655	362	133	-	245(1)	218(2)	204(45)
					191(3)	147(21)	129(11)
					115(5)	97(8)	89(12)
					84(22)		
Phenyl 47	2125	424	177	-	377(2)	307(49)	218(95)
					204(46)	191(97)	159(24)
					147(52)	146(20)	133(20)
					129(32)	117(48)	89(37)

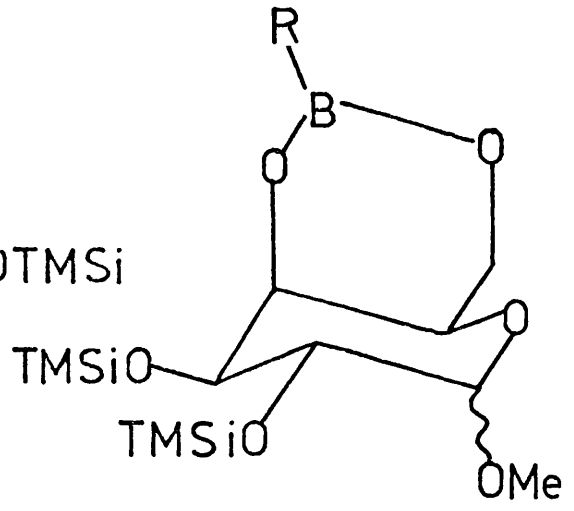
Contd.

TABLE 20 Contd.

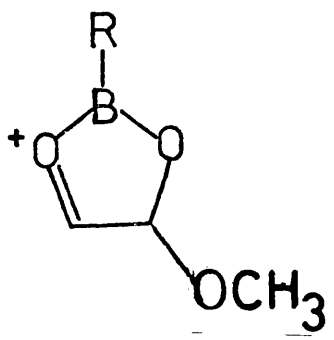
	$I_{1\%SE-30}$	M.W.	Base Peak	M(% of Base Peak)	Significant Ions (% of Base Peak)		
Phenyl [48]	2165	424	133	1	218(28)	204(49)	191(5)
					177(4)	159(7)	147(22)
					146(6)	129(8)	117(17)
					89(11)		



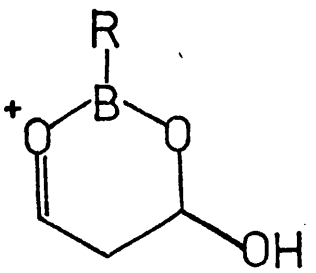
CLIV



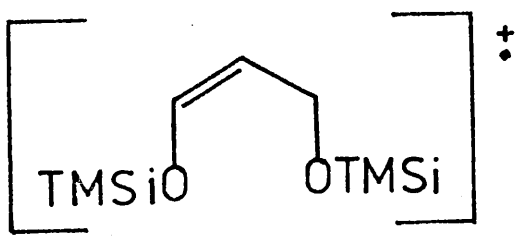
CLIII



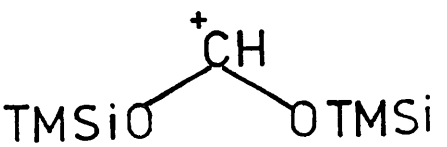
CLV



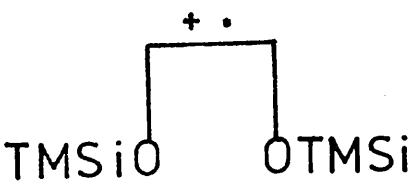
CLVI



XCII



XCIX



XXI

The derivatives fall into two sets, the first with a base peak at m/e = 177 and 115, the second with a base peak of 133. The latter is due to the ion C which could easily arise from the 4,6-boronate (CLIII) by a rearrangement process during the fragmentation of the sugar ring, while in the 2,3-boronate the larger separation between the TMSi ether and methoxy groups would make the process more difficult. The peak at m/e = 177 and 115, being boron-containing, would probably be due to the ion CLV which could only be derived from the 2,3-boronate (CLIV). The six-membered ion of that m/e value would have to have the structure CLVI, which in this case could only be formed if the carbon-oxygen bond α- to the boronate in the 4,6- ester (CXX) were retained in preference to the carbon-carbon bond α- to the ester, which is unlikely.

The first set of ions would therefore, seem to be due to the 2,3-boronate ester (CLIV) and the second set to the 4,6-ester (CLIII) and the other differences between the two sets can be explained on the basis of these structures. The 2,3-esters show a large peak at M-115, i.e. m/e = 307 and 245, which is probably due to cleavage of the sugar ring, with the subsequent elimination of carbons 5 and 6, a peak at m/e = 218 due to the liberation of carbons 4, 5 and 6 to give the ion XCII, and a peak at m/e = 191 (XCIX), which is probably caused by migration of the TMSi ether group on carbon 4 to carbon 6 which is then eliminated. None of these ions is present in the 4,6-ester which does not have the C-6 TMSi ether group, and so cannot break down in these ways. The only ion it shows which would involve both TMSi ether groups is that at m/e = 204 (XXX) which is formed by the liberation of carbons 2 and 3, while in the 2,3-ester it is probably the product of a rearrangement. The rest of the ions seen in the spectra of both boronates are the usual ones due to the fragmentation and rearrangement of the TMSi ethers.

The only apparent difference of any consequence between the anomers is shown in the intensity of the peak at m/e = 89 in the spectra of

the phenyl esters. In the α- anomers, particularly in the 2,3-ester it is much stronger than it is in the β- anomers, and in both cases it is stronger in the 2,3-esters than in the 4,6-esters. This difference is the only aid to distinguishing between these anomers, because all the other peaks in their spectra are very similar.

Table 21 gives a summary of the results obtained from the GC-MS data, and shows the common boron-containing ions present in the spectrum of each derivative.

In most of the cases examined, the formation of the boronate and hence of the boronate TMSi ether is unambiguous, and is determined solely by the availability of suitably orientated hydroxyl groups in the parent sugar.

The only sugar with two free hydroxyls was methyl 2,3-di-O-methyl- α-D-glucopyranoside in which the hydroxyls are in a suitable conformation, cis- to each other, allowing the formation of the 4,6-boronate.

Of the sugars with three free hydroxyls there are four in which the structure of the parent compound allows no choice in the formation of the boronate. Methyl β-L-arabinopyranoside, has only the 3- and 4-hydroxyls cis- to each other, while in methyl α-L-rhamnopyranoside only the 2- and 3- hydroxyls are cis- to each other, hence these compounds form the 3,4- and 2,3-boronate TMSi ethers respectively. Similarly the structures of both methyl α- and methyl β-D-xylopyranoside allow only the formation of the 2,4-boronate. In 1,2-isopropylidene-D-glucofuranose the positions of the hydroxyl groups seem to afford the possibility of the formation of either the 3,5- or the 5,6-boronate, but examination of a model of each compound shows that in the 3,5- isomer the five-membered sugar ring is distorted into a non-planar, puckered conformation, which is the preferred mode, as it reduces the interactions between substituents on the ring, and so the 3,5- boronate is formed preferentially to the 5,6- boronate which would not affect the structure of the sugar ring to such a large extent.

TABLE 21

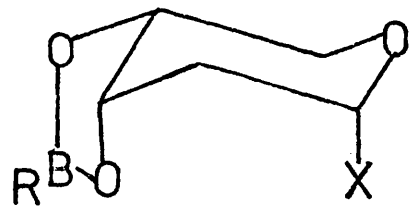
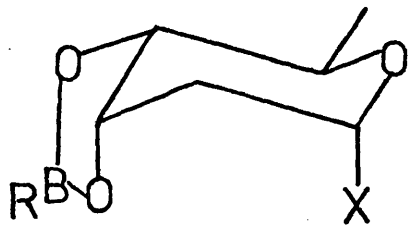
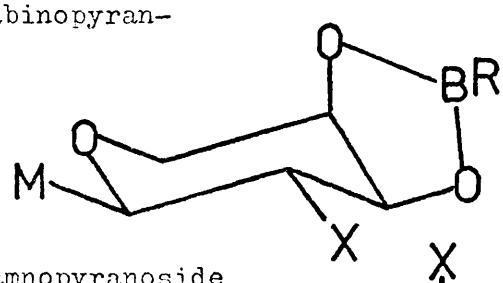
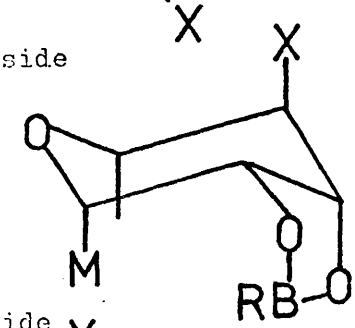
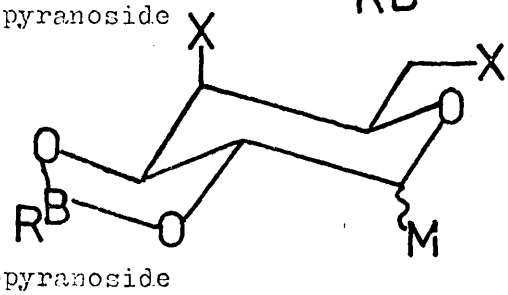
Compound	Derivative	% Present Me	% Present Ph	Significant Ions
2-deoxy-D-ribopyranose		100%	87%	A, C, F.
D-digitoxose		80%	50%	A.
Me β-L-arabinopyranoside		100%	100%	A, C, F.
Me α-L-rhamnopyranoside		100%	100%	A.
Me D-galactopyranoside		50%	50%	A, C, F.
Me α-D-mannopyranoside		100%	100%	A, B, C, F.

TABLE 21 Contd.

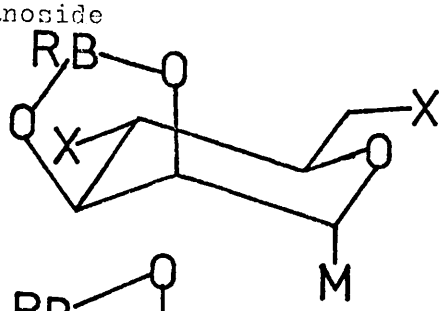
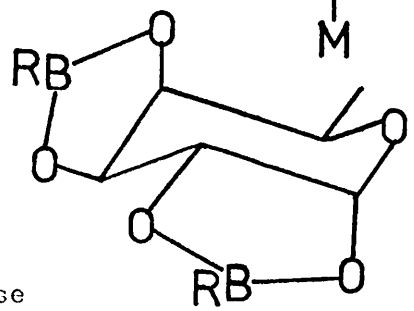
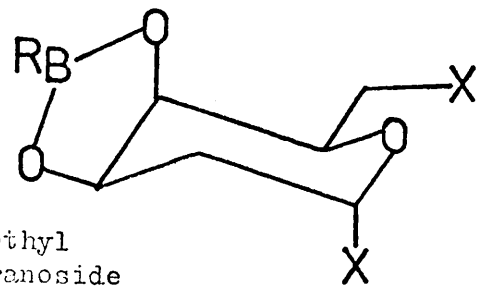
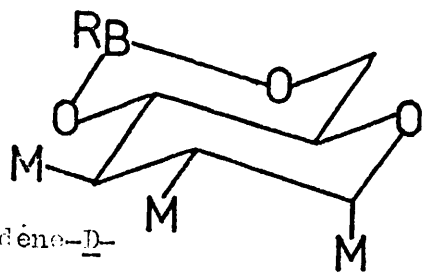
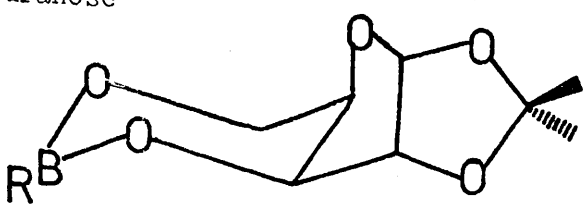
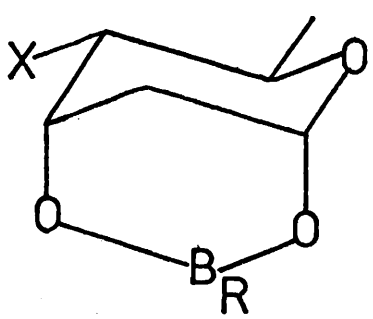
Compound	Derivative	% Present Me	% Present Ph	Significant Ions
Me α-D-mannopyranoside		100%	87%	B, C.
6-deoxy-galactose		100%	100%	A, C, E.
2-deoxy-D-galactose		50%	40%	A, C, F.
Me 2,3-di-O-methyl α-D-glucopyranoside		100%	100%	B, D, E.
1,2-isopropylidene-D-glucofuranose		100%	100%	B.
D-digitoxose		20%	50%	B, D.

TABLE 21 Contd.

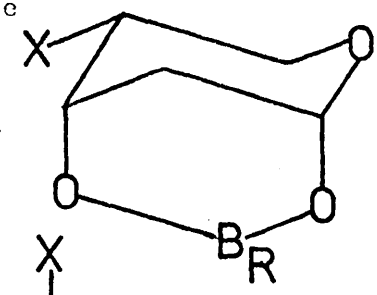
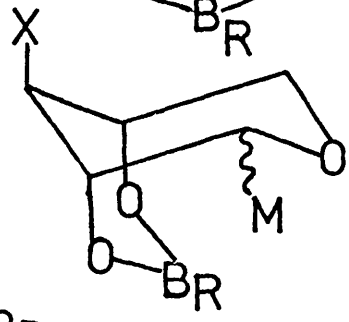
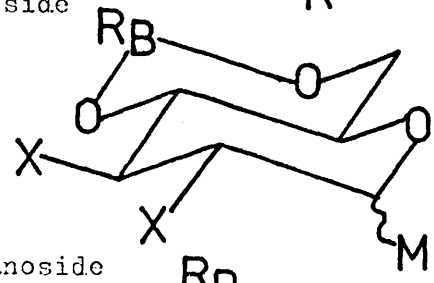
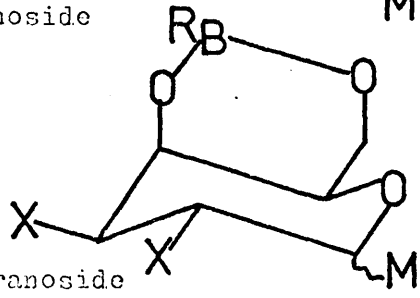
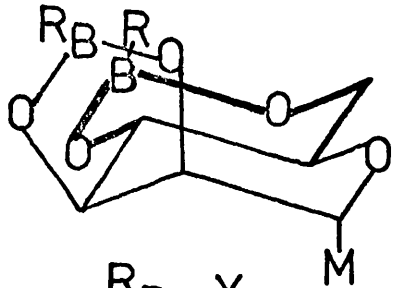
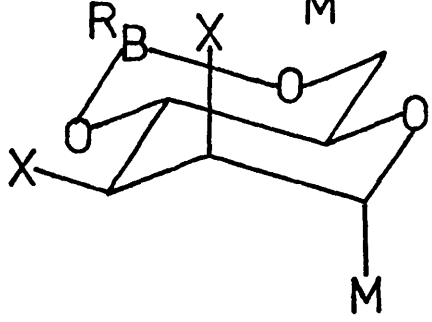
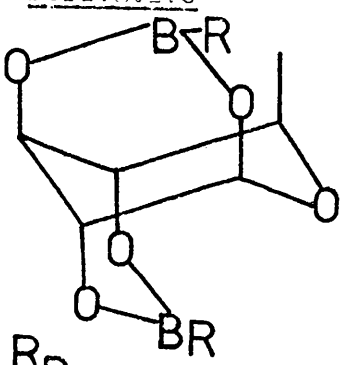
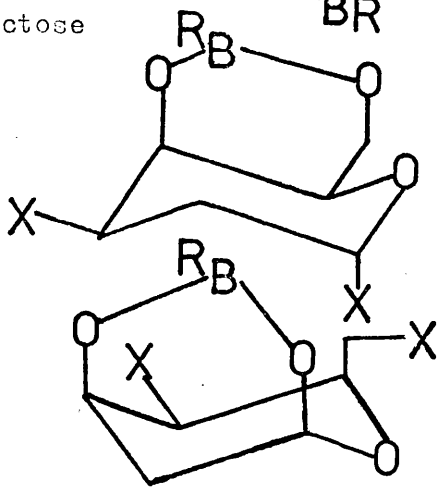
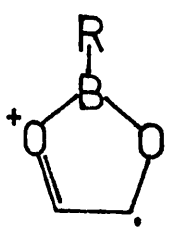
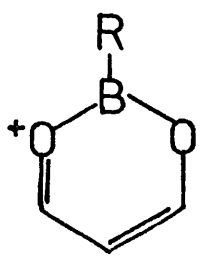
Compound	Derivative	% Present Me	% Present Ph	Significant Ions
2-deoxy-D-ribopyranose		-	13%	B, D.
Me-D-xylopyranoside		100%	100%	B, E.
Me D-glucopyranoside		100%	100%	B.
Me D-galactopyranoside		50%	50%	A, B, D.
Me α-D-mannopyranoside		100%	100%	A, B, C, E.
		-	13%	B, C.

TABLE 21 Contd.

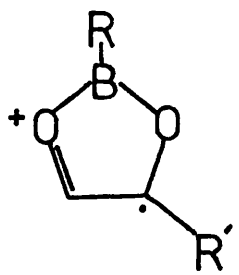
Compound	Derivative	% Present Me	% Present Ph	Significant Ions
6-deoxy-D-glucopyranose		100%	100%	B, D, E.
2-deoxy-D-galactose		50%	40%	B, D, G.
		-	20%	B, D, G.



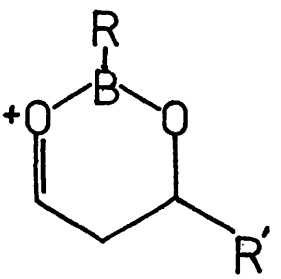
A



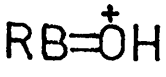
B



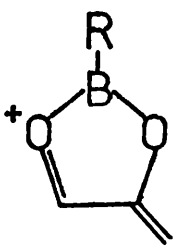
C



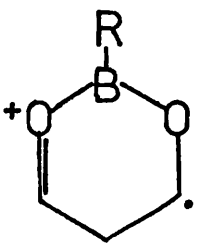
D



E

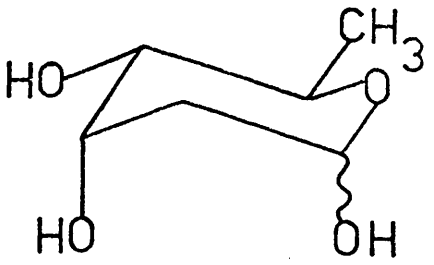


F

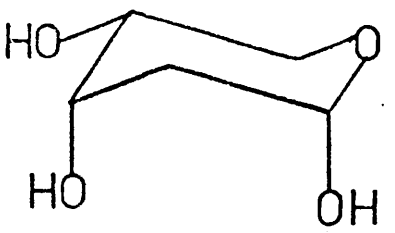


G

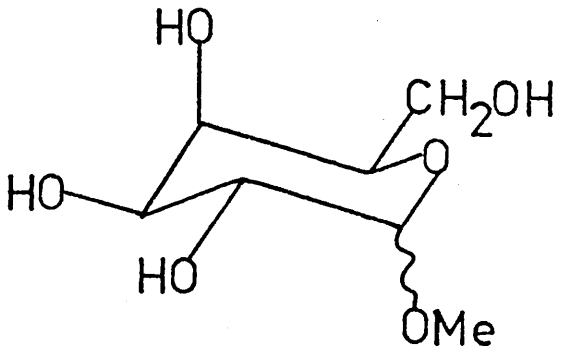
X = OTMSi M = OMe



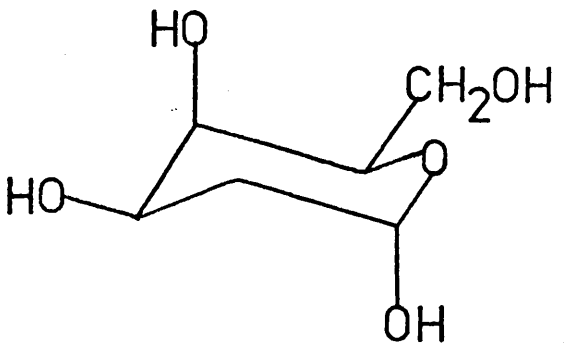
CI



CXXVI



CLII



CXXXIV

Of the sugars with four free hydroxyls there are three in which the hydroxyl configurations allows the formation of diboronates, vis: 6-deoxy-D-galactose, 6-deoxy-D-glucose and methyl ∝-D-mannopyranoside. This last compound contains both a five- and six-membered cyclic boronate ester. Of the remaining sugars in this group the only other which forms only one derivative is methyl-D-glucopyranoside, which forms the 4,6- boronate.

In the remaining cases the possibility of multiple derivative formation exists. In both D-digitoxose (CI) and 2-deoxy-D-ribopyranose (CXXVI) the three hydroxyls offer a choice between the formation of the 1,3-boronate or the 3,4-boronate. D-digitoxose forms the boronates in the ratio of 1 : 4 and 1 : 1 with the methyl and phenylboronic acids respectively, while 2-deoxy-D-ribopyranoside forms the 3,4-boronate exclusively with methylboronic acid, and both isomers in the ratio of 1:6 respectively, with phenylboronic acid. Methyl D-galactopyranoside (CLII) gives a 1 : 1 mixture of the 4,6-boronate, and the five-membered 2,3-boronate, and lastly 2-deoxy-D-galactose (CXXXIV) which can form three isomers, the 4,6-, the 3,4- or the 1,3-boronates gives a mixture of the three in the ratio of 1 : 2 : 2 respectively with the phenylboronic acid, and the 3,4- and 4,6- methylboronates in the ratio of 3 : 1 respectively.

These ratios of derivatives could be due to several processes. They may be the kinetic products of the boronation (though this is very unlikely as boronate esters are formed and equilibrated very easily) or the result of this equilibration process which allows the most stable compounds to predominate. The ratio of the original boronates themselves might also be altered by rearrangement of the ester groups during trimethylsilylation.

In an attempt to investigate this last possibility, larger samples of the methyl- and phenylboronates of both D-digitoxose and 2-deoxy-D-ribose were made and purified, where possible, by recrystallisation. These samples were then examined by NMR spectroscopy to determine the structure of the compounds present before trimethylsilylation.

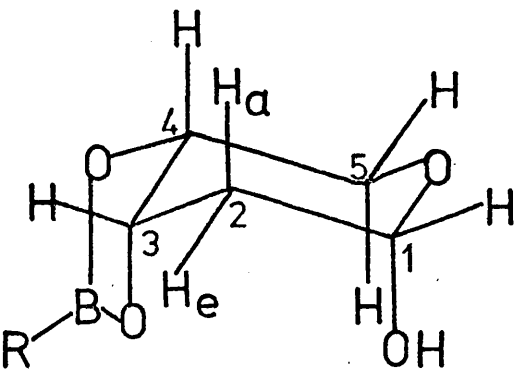


TABLE 22

Proton	Signal Type	δ	
		R = Me	R = Ph
C_1-H	Multiplet	5.14	5.22
C_2-He	Multiplet	1.24	1.83
-Ha	Multiplet	2.20	2.22
C_3-H		4.60	4.84
C_4-H	Doublet of Multiplets	4.39	4.61
		3.95	4.04
C_5-H	Doublet of AB System	3.58	3.62
O -H		5.22	-

The proton NMR spectra (Appendix II) of both boronates of 2-deoxy-D-ribose show the same pattern of peaks due to the sugar ring itself (Table 22), the major difference between them being the peaks due to the group attached to the boron. In the methylboronate there is a single peak at 0.48δ which integration shows to be due to three protons i.e. to the methyl of the boronate group, suggesting that in this case there is only one of the two possible isomers present. In the phenylboronate the peaks due to the phenyl group at 7.36 and 7.78δ are both multiplets, making it difficult to determine if there are both isomers present, however, the rest of the spectrum matches that of the methylboronate so closely that at least the predominant isomer present has the same form as the single isomer of the methylboronate, and if there are two isomers, the minor one must be present in less than 20% of the total to make it indistinguishable in the spectrum.

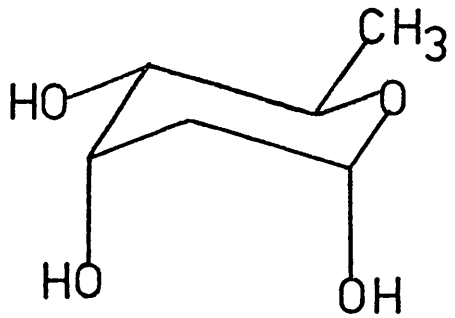
Double irradiation of the spectra, at the frequency of the C_1-proton, removed the coupling between it and the C_2-protons, allowing the identification of that set of peaks. Double irradiation at the frequency of the C_3-proton also caused sharpening of the C_2-proton signal, allowing the identification of the C_3-proton; this irradiation also removed the coupling with the C_4-proton, allowing the identification, both of it, and, by elimination, of the peak due to the C_5-protons. The coupling between the C_3- and C_4- protons was shown to be 8 Hz. Examination of models of each of the boronates shows that in the 1,3- isomer the angle between these protons is approximately 60°, while in the 3,4-boronates it is almost zero. The former situation would result in a coupling constant of 2-4 Hz while the latter would give about 8 Hz, implying that it is the 3,4- isomer which predominates.

The spectra of the TMSi ethers (Appendix II) show very similar patterns of peaks. The TMSi groups give large peaks at 0.35δ for both the methyl- and phenylboronates; in the former the TMSi and boronate methyl peaks are sharp singlets suggesting the presence of only one

isomer, but the TMSi ether signal in the phenylboronate consists of two sharp peaks in the ratio of 6 : 1. These results match the ratios obtained from the GC-MS examination of the boronate TMSi ethers.

The methylboronate of D-digitoxose proved to be a colourless oil, and attempts to crystallise it were unsuccessful. The NMR spectrum obtained from it showed a similar pattern to that of 2-deoxy-D-ribose methylboronate, except that one of the C_5- protons was replaced by a methyl group. The spectrum was, however, too complex to allow the certain assignment of any peaks apart from those due to the secondary methyl group at 1.3 and due to the boronate methyl group at 0.3 Both of these signals seemed to consist of a number of overlapping peaks, the boronate methyl signal containing three single peaks, and the secondary methyl containing two and possibly three pairs of peaks. The two doublets due to the secondary methyl which were certainly present were in a 1 : 1 ratio, and the peaks due to the boronate methyl seemed to be in a 2 : 2 : 1 ratio. Conversion of the boronate to the boronate TMSi ether only served to confuse matters, since the TMSi ether signals overlap with the boronate methyl signals. Thus it is impossible to correlate the peaks observed with any of the isomers, but it is possible to say that in the methyl boronate there were three isomers present in the ratio of 2 : 2 : 1, which is the ratio of methylboronate TMSi ethers found by GC-MS. This, and the result obtained from 2-deoxyribose, suggests that there is no rearrangement taking place during the trimethylsilylation, and the ratios of boronate TMSi ethers found by GC-MS are determined by the ratios of the boronates themselves before the reaction.

In order to examine whether the boronates present in a mixture before trimethylsilylation were the kinetic or the equilibrium products of the esterification, a sample of D-digitoxose was treated with methylboronic acid in pyridine and heated at 100°C, and samples were removed at fifteen minute intervals, trimethylsilylated and examined



CII

by GLC. The ratio of peaks present in the sample was found not to vary, so, if there is an equilibration reaction taking place, it is doing so very quickly, and consequently the products after fifteen minutes, which was the standard reaction time allowed for the esterification, must be the most stable isomers.

Examination of the boronates in this light suggests that the five-membered boronate ester group is more stable, in these compounds, than the six-membered ester, since in every case where the conformation of the sugar hydroxyls affords an apparently equal possibility of forming either of the isomers, in a situation where only one of them can be formed, eg. the 1,3,4-hydroxyl system of D-digitoxose (CII) the five-membered isomer is the major product. It would also seem that the phenyl group attached to the boron stabilises the six-membered ring with respect to the methyl group, for in several phenylboronates the ratio of five-membered to six-membered boronates is lower than in the corresponding methylboronates.

The stability difference is also borne out by the effect of trimethylsilylation on methyl α-D-mannopyranoside diboronate, in which both a five- and six-membered ester group are present. The major product of the reaction is the boronate TMSi ether with the five-membered ester group, implying that it is more stable than the corresponding six-membered group.

Another difference between the five- and six-membered boronates is shown by their retention indices on both SE-30 and QF-1. In all cases where both isomers of a sugar are present the five-membered boronate has a shorter retention time than the six-membered boronate.

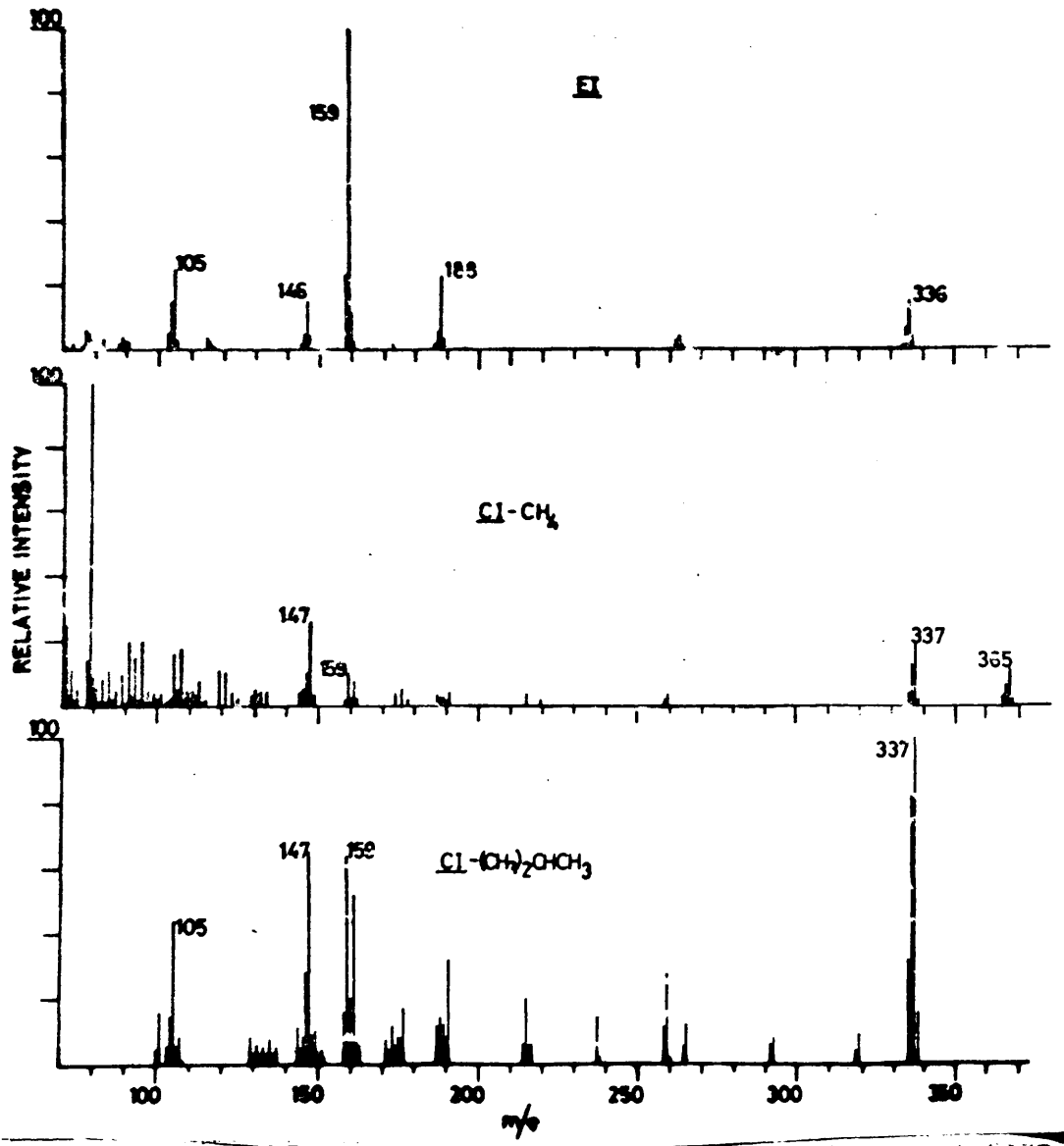
For GLC in general the boronates are easy to form and give as much information as, if not more than, the TMSi ethers. The multiple peak formation shown by any of them which have suitable hydroxyl conformations makes the examination of a mixture of a large number of

them difficult, but, in turn, it makes identification of one or two isomers simpler by increasing the number of parameters that can be used for the identification. This holds too for GC-MS, though in this field the difficulty is not so great. The sugar boronate TMSi ethers give spectra which are similar, containing the same sets of peaks in many cases, but they are different enough to be distinguishable from a consideration of the ions due not only to the boronate, but also to the TMSi ether, when it is present. For example in Me D-galactopyranoside 4,6-boronate TMSi ether a boron containing peak appears at m/e = 177 and 115 which does not appear in the corresponding derivative of Me D-glucopyranoside, a very similar compound. Similarly the peak at m/e = 218 in the galactopyranoside is totally absent in the glucopyranoside. The derivatives can also be used to distinguish between anomers of a sugar at least as successfully as the TMSi ethers and by a similar set of properties, in that while there is not, in any of these results, a single peak in one anomer of one sugar which is totally absent in the other anomer, several peaks do show intensity differences between the anomers. For example, the m/e = 89 peak is stronger in the α - anomer of Me D-galactopyranoside methylboronate TMSi ether than in the β - anomer, while in Me D-glucopyranoside phenyl- and methylboronate TMSi ethers the ion at m/e = 245 or 183 in the α- anomer is larger than in the corresponding β- anomer.

The boronate rings, by themselves, stabilise the sugar ring sufficiently for derivatives to show the molecular ion, for example, the diboronates of 6-deoxy-D-glucose, and 6-deoxy-D-galactose, but the presence of any other group at all results in the disappearance of the molecular ion, e.g. the diboronate of Me-D-mannopyranoside in which the highest ion is that of M-31, i.e. the result of the loss of the methoxy group.

The relative intensity of the molecular ion is increased in a chemical ionisation (CI) spectrum of the molecule. In this technique a light molecule such as methane or isobutane is ionised and then interacts

Fig XII


EI
159
105
146
189
336
Cl-CH4
147
159
337
365
Cl-(CH3)2CHCH3
147
159
105
337
RELATIVE INTENSITY
100
150
200
250
300
350
m/e

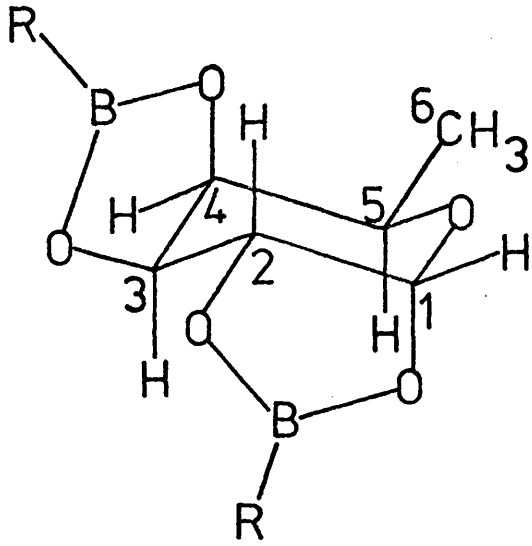


TABLE 23

Proton	Signal Type	Coupling (Hz)	δ	
			R = Me	R = Ph
C_6–H	doublet	$J_{5,6} = 6$	1.22	1.31
C_5–H	doublet of quartets	$J_{4,5} = 2$ $J_{5,6} = 6$	3.67	3.92
C_4–H	doublet of doublets	$J_{4,5} = 2$ $J_{3,4} = 8.5$	4.36	4.62
C_3–H	doublet of doublets	$J_{3,4} = 8.5$ $J_{2,3} = 2$	4.74	5.11
C_2–H	doublet of doublets	$J_{2,3} = 2$ $J_{1,2} = 6$	4.43	4.67
C_1–H	doublet	$J_{1,2} = 6$	5.77	5.83

with the molecule causing a fragmentation that is less violent than that shown under the electron bombardment of EI mass spectrometry. The CI spectra of 6-deoxy-D-glucopyranose diboronate with methane and with isobutane are shown (Fig. XII) along with the corresponding EI spectrum. As can be seen, the CI spectra are much simpler, though they still show characteristic ions arising from this particular compound.

The boronates can also be used as derivatives, in some cases, for NMR spectroscopy. They have the property of making the sugar soluble in a suitable medium for NMR. They can also simplify the spectra both by decreasing the number of available hydroxyl groups but also by making the sugar molecule rigid, and locking it into one conformation. The methyl and phenyl diboronates of 6-deoxy-D-galactopyranoside were prepared and the NMR spectra of these compounds were then obtained (Appendix II). The boronates gave very similar spectra: the peaks due to the sugar ring were almost identical, the major differences being due to the chemical shifts of the groups attached to the boron atom. The methyl boronate shows a pair of peaks at 0.4 δ which integrate as being due to six protons, and are the signals of the boron methyls present in the diboronate. The peaks in the phenylboronate arising from the phenyl group are much more complex, making it impossible to analyse them. The rest of the spectra were assigned as shown in Table 23, by studying the results of various decoupling experiments. The quartet at around 3.8δ would be due to the C_5-proton which would be coupled to both the methyl group and the C_4-proton. Double irradiation at the frequency of the C_5-proton, therefore, revealed the position of the C_4-proton and the coupling constant between these protons ($J_{4,5}$). Subsequent irradiation at the C_4- proton frequency revealed the position of the C_3- proton, and the process was continued until all the peaks were assigned.

The value of $J_{3,4}$ (8.5 Hz) is larger than would be expected for an interaction between an axial and an equatorial proton (139), and suggests that these hydrogens are almost aligned in the molecule.

The small value of $J_{2,3}$ (2 H_Z) is inconsistent with an angle between them of 180°, suggesting that this angle has been decreased. Both of these observations are consistent with a flattened structure in which the sugar ring is almost planar, similar to that suggested for the structures of the diboronates of xylose and arabinose (137).

A similar set of spectra for methyl α-D-mannopyranosides, however, gave less clear results. Both gave spectra containing broad, unresolved peaks (Appendix II). This may be the result of hydrogen bonding to the oxygen of the methoxy group. Thus the boronates can be said to be good derivatives for a number of purposes. They are readily formed and stable to the trimethylsilylation of any remaining hydroxyls on the parent molecule. They, or their TMSi ethers have good GLC properties and they give characteristic fragmentation patterns under electron impact, especially because of the stereoselectivity of the ester formation which is governed by the conformation and configuration of the parent sugar, allowing the possibility of distinguishing between both isomers and anomers. The boronates are also useful in some cases for locking the structure of a sugar for NMR spectroscopy.

Further work in this field should include an investigation of the differences in stability of the five- and six-membered cyclic boronates in compounds of this type, and also an examination of the rearrangement from a six- to a five-membered ring under electron impact, along with the possible reverse process which may also be taking place.

2 - 3 Experimental

2 - 3.1 GLC and GC-MS Procedures

All the GLC results were obtained on a Varian Aerograph Model 204 dual column gas chromatograph, fitted with flame ionisation-detectors, and modified by the removal of the metal injector blocks, allowing direct injection of the samples on to the column packing, rather than on to the metal walls of the flash heater as in the conventional assembly. The column packing was pre-coated with the stationary phase, 1% by weight, on to Gas-Chrom Q, 100-120 mesh, and was obtained from Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania. The phases used were SE-30, and QF-1. Columns were made up from Pyrex glass tubing, 3mm internal diameter, and 10 feet in length. The carrier gas used was nitrogen, at a flow rate of 35 ml/minute.

Mass spectra were recorded on the LKB 9000 combined gas chromatograph - mass spectrometer, the GLC conditions being set to match those of the original runs on the Aerograph instrument. The accelerating voltage was 70eV.

2 - 3.2 Boronate Formation and Trimethylsilylation

Dimethylformamide was dried by mixing it with benzene, in the ratio of 10 : 1, and distilling off the benzene-water azeotrope. Magnesium sulphate was then added to remove the remaining traces of moisture, and the solvent was distilled off. The dry solvent was stored in a Quickfit flask fitted with a drying tube filled with silica gel. Pyridine was dried by refluxing it overnight with calcium hydride, and then distilling it. The dry pyridine was stored over potassium hydroxide pellets.

Esterification was effected by mixing at room temperature a known weight of the sugar with a volume of a standard solution of the boronic acid in pyridine equivalent to double the number of moles of

the sugar, or four times the number for the formation of the diboronates, and then heating at 100°C for fifteen minutes. The standard solution was made up by dissolving 50mg. of each boronic acid in pyridine, purified as described, and stored in a flask fitted with a rubber septum.

Transfer of the requisite amount of the acid was effected by using a 100 ml syringe. After 20 such transfers, or sooner if it was thought to be necessary, the septum was changed.

The trimethylsilylation was carried out in a way adapted from that described by DeJongh et al. (67). An excess of each of the reagents, HMDS and TMCS, was added separately, in the ratio of 2 : 1, to the solution of the sugar or sugar boronate in pyridine at room temperature. The mixture was then heated to 100°C for fifteen minutes. For GLC, a sample could be injected straight into the chromatograph from the pyridine solution, but for GC-MS the mixture was purified by blowing off the pyridine under nitrogen, re-dissolving the soluble material in ethyl acetate to a concentration of 1 mg/ml, and filtering off the insoluble material. Samples of this solution (~ 1μl) were then injected into gas chromatograph.

NMR Spectroscopy of Boronates

In order to examine the boronates of some of the sugars by NMR spectroscopy, larger amounts of the derivatives were prepared by mixing one millimole of sugar with half a millimole of boronic acid in dry pyridine and heating at 100°C for fifteen minutes. The pyridine was removed under a stream of nitrogen and the reaction mixture extracted with AnalaR ether in which the excess sugar was insoluble. The boronates were recrystallised, where possible, from a mixture of acetone and n-pentane, a sample was removed for microanalysis, and the rest was submitted for NMR spectroscopy.

The NMR spectra were obtained on a Varian HA 100, 100H_Z NMR Spectrometer, in chloroform as solvent.

2-deoxy-D-ribopyranose

Both the methyl- and phenylboronates of this sugar were found to be crystalline.

Methylboronate - found C-45.59%, H-7.03%; required for $C_6H_{11}BO_4$, C-45.62%; H-7.02% M.pt. 75-80°C.

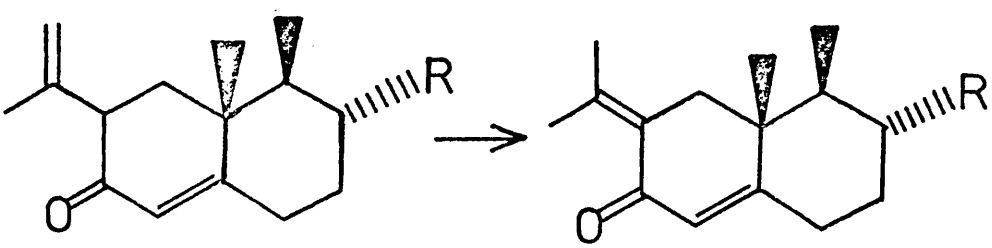
Phenylboronate - found C-60.05%, H-5.96%; required for $C_{11}H_{13}BO_4$, C-60.08%, H-5.94%. M.pt. 129-133°C.

6-deoxy-D-glucopyranose

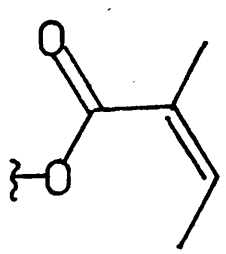
Phenylboronate - found C-64.35%, H-5.37%; required for $C_{18}H_{18}B_2O_5$, C-64.35%, H-5.37%. M.pt. 66-68°C.

Methyl α-D-mannopyranoside

Phenylboronate - found C-65.72%, H-5.33%; required for $C_{19}H_{20}BO_6$, C-65.70%, H-5.36%. M.pt. 112-114°C.



CLVII CLVIII

R = 

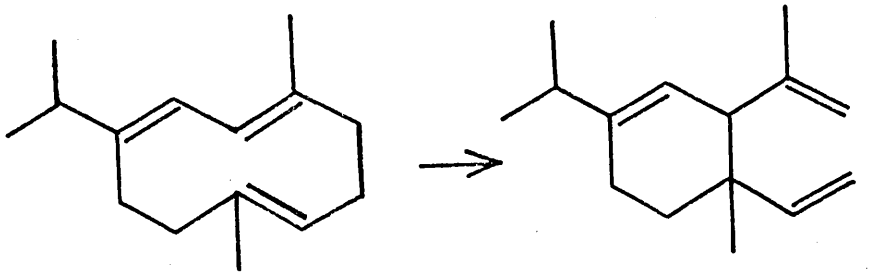
3. THE APPLICATION OF GLC AND GC-MS TO THE STUDY OF PLANT MATERIAL

3 - 1 Introduction

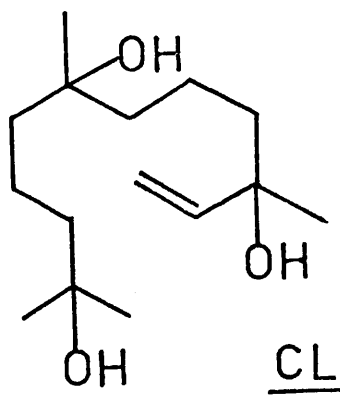
In recent years the amount of work done on the examination of plant material for naturally occurring terpenoid compounds has been increasing steadily. Aside from the classical, purely structural type of investigation, terpenes have been shown to be useful both for chemotaxonomy, where the familial relationship of plants is studied with reference to their chemical constituents, and for chemosystematic studies of, for example, the effects on their chemistry of cross-breeding two plants (140).

The classical techniques of obtaining pure samples of the compounds from plant material involve the extraction of large quantities of the plant in a suitable solvent, either at room temperature (141 - 143) under reflux (144, 145), or by steam distillation (140, 143). The extract obtained is concentrated by the removal of the solvent and then the individual components are separated by distillation under vacuum (141, 146), by chromatography on silicic acid (147), silica gel (142) or alumina (143-145, 148) or, where sufficient of the major component is present, by fractional crystallisation (150).

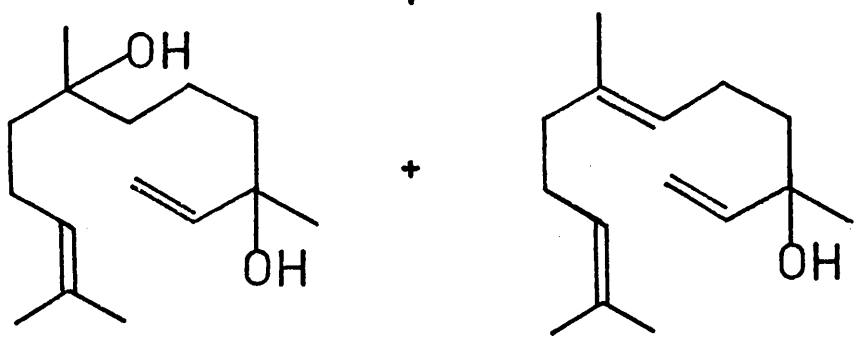
There are disadvantages attached to these techniques. The large amount of plant material originally required may be difficult to obtain unless the plant under study is readily available, and it also entails the use of large amounts of solvent. Extraction of the plant under reflux, or distillation of the extract, may both cause the decomposition or rearrangement of any unstable compounds present, resulting in artefacts which were not present in the original material, while it has been shown that certain compounds undergo changes in the presence of chromatographic material. Petasin (CLVII) rearranges to form isopetasin (CLVIII) in the presence of alumina or silica gel (151)



CLIX CLX



CLXI



CLXII + CLXIII

germacrene-C (CLIX) forms δ-elemene (CLX) at 100°C, or at room temperature in the presence of silica gel (152) and caparrapitriol (CLXI) suffers dehydration on an alumina column to give caparrapidiol (CLXII) and nerolidol (CLXIII) (153).

The separation of individual compounds from the extract is also difficult, since, although distillation and chromatography are efficient in dealing with the major products, the minor compounds may be obscured (140).

Some of these difficulties have been overcome by the introduction of the techniques of GLC and GC-MS. The size of sample required is much smaller, and after a careful extraction the material can be injected straight into the gas chromatograph without any intermediate operations (154), reducing the risk of artefact formation and allowing a much faster analysis of a much smaller amount of plant material than can be achieved by the classical methods. The sensitivity of the gas liquid chromatograph is sufficient to show peaks due even to minor components which might have been overlooked by the classical method, but this in itself is the cause of one of the main disadvantages of the method, the overlapping of peaks in a GLC trace (140). This is caused both by the occurrence of, for example, a number of compounds of a similar type, such as sesquiterpene hydrocarbons, or by the similarities in retention characteristics of compounds of two distinct types, such as oxygenated monoterpenes and sesquiterpene hydrocarbons (140). These difficulties can be partially overcome by adjusting the operating condition of the gas chromatograph to increase the resolution, for example, by programming a linear increase in temperature during the analysis (140), or by the use of suitable stationary phases. The major difficulty in this technique is the identification of individual compounds.

Although a correlation of structure with retention index has been shown to hold, not only for such simple molecules as n-alkanes, but also for those as complex as steroids (155), such a correlation has

not been found for sesquiterpenoids. In the case of a few sesquiterpenes, the structures of which can be regarded as being derived from those of corresponding monoterpenes by the addition of an isopropyl group, the retention indices have been shown to be divisible into a variable part from the monoterpene, plus a constant part from the isopropyl group. Thus from the retention indices of the monoterpenes, it is possible to predict roughly the indices of the corresponding sesquiterpenes (156).

Generally, however, this is not possible. There are a number of parent sesquiterpene skeleta, which can be modified by various stereochemical alterations, with the result that the total number of possible structures is fairly large. Although it is possible to group these structures according to their parent skeleta, the retention indices of members of each groups can be quite different (157).

Identification is more readily achieved by comparison of the retention data of the unknown compound with those of standards, but the difficulty here lies in amassing a suitable set of data. This would either have to be obtained by experiment on a large collection of suitable compounds, or from results quoted in the literature on the subject. The latter may, however, be of little use, since the GLC retention data reported have been obtained from a variety of stationary phases (157-161), and since the analysis of a complex mixture on a number of columns is very difficult, any data obtained from stationary phases other than the ones which are being routinely used cannot be easily utilised. In spite of these difficulties, however, the analysis of natural products by GLC alone is a useful technique (159, 160).

The addition of the mass spectrometer makes identification easier, though still not certain. The same problems of standard data still arise, but here they are less intractable. The data reported in

the literature can be used to a much greater extent and there are a number of sources from which information can be gleaned, viz: papers on individual sets of compounds (161-171) or on a general field (172, 173), or collections of mass spectra which can arise from compounds of the same type, but while absolute identification may not always be possible, the compounds observed can usually, at least, be classified according to their chemical types (154, 179), and while identification is possible even without the use of the retention characteristics (154), the combination of mass spectral and retention data increases the probability of any identification made.

Thus the application of GC-MS to the analysis of plant extracts has the advantages of requiring a low sample size, being quick, less likely to cause artefact formation than classical methods and able to give data on minor components of the mixture. Although it has the disadvantage that it may not be possible to identify each individual compound, they can at least be classified by chemical type. It would seem, therefore, that this technique is best suited for the initial analysis of a plant, to show if it is worthwhile isolating any of the compounds from a large scale extract, and as an aid to the identification of any such compounds isolated.

The method adopted for the extraction and examination of plant material is derived from that used by R.A.B. Keates in his studies on Petasites hybridus (180). The extract was made by macerating the plant in a suitable solvent, filtering, and re-extracting the residue in fresh solvent twice more in the same way. Further re-extraction of the plant showed that this method resulted in the extraction of most of the desired components.

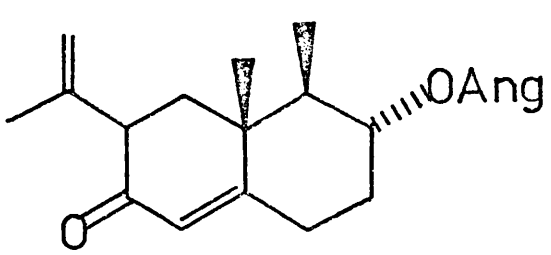
This total extract of the plant contains a number of compounds apart from the sesquiterpenoids which were to be studied, for example, tannins, pigments, glycosides and phospholipids, which are all involatile and thermally unstable. Injection of these compounds onto a gas chromatographic column at high temperatures would result in their

pyrolysis giving products which might interfere with the examination of other, more stable compounds, and which would cause rapid deterioration of the column. It therefore, seemed essential to separate these involatile compounds from the more volatile compounds which are suitable for analysis by GLC.

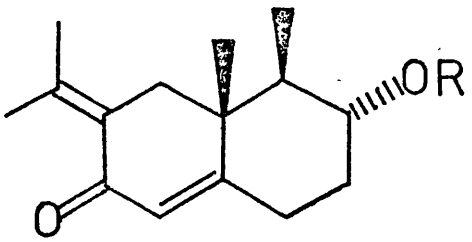
Both distillation and the use of TLC or column chromatography on silica or alumina were undesirable because of the possibilities of decomposition or rearrangement mentioned above, and any methods based on the polarity of the sample would only be partly effective, because of the variation in polarity of both the sesquiterpenoids and the interfering compounds. The major difference between the two groups of compounds lies in their molecular weights. Most of the interfering compounds have high molecular weights, i.e. greater than 600, compared to those of the sesquiterpenoids which range between 200 and 400, so a separation method based primarily on molecular weight or molecular size was needed. Such a separation can be obtained by using a liquid-gel column in which the polarity of the gel is approximately the same as the polarity of the solvent. Under these conditions, (gel filtration) the polarity of the individual compounds has no effect on their order of elution, this being governed totally by their molecular size (130).

Thus, by passing the extract through a gel-filtration column, the high molecular weight compounds were removed. Examination of the extract, by GLC, both before and after chromatography, gave identical results, showing that the compounds suitable for GLC analysis had all been eluted from the column, and that, as far as could be judged from their retention indices, they had been unaltered. The sample thus obtained was examined by GLC and then by GC-MS.

Such a preliminary investigation was first carried out on three plants, Degeneria vitiensis, Bailey and Smith; Petasites hybridus (L), Fl. Witt; and Petasites fragrans (Vill.) C. Presl.

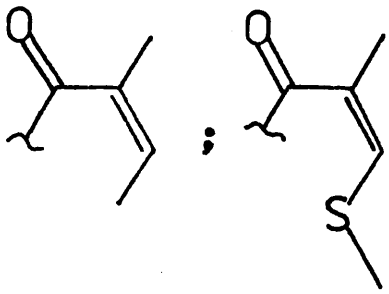


CLVII

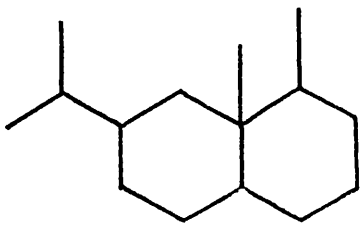


CLVIII

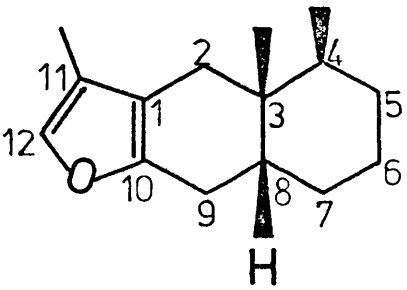
R = Ang =



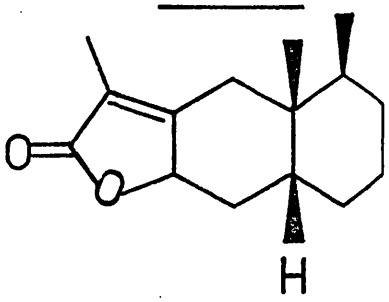
CLXIV



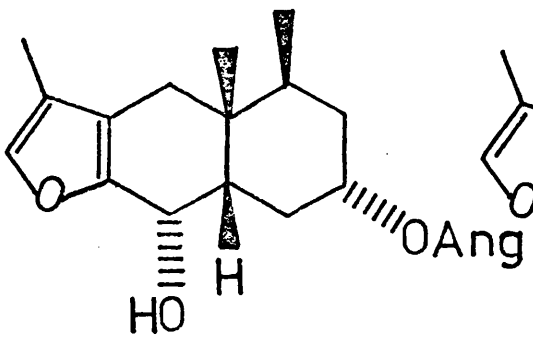
CLXV



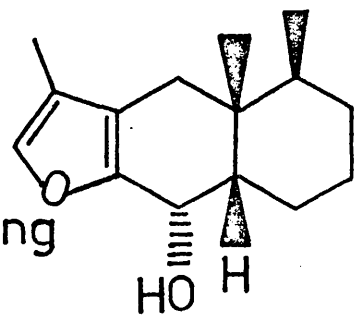
CLXVI



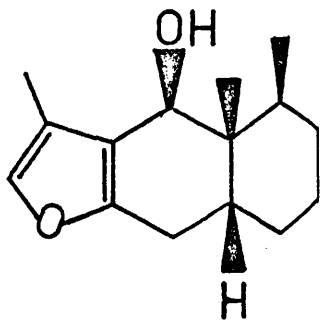
CLXVII



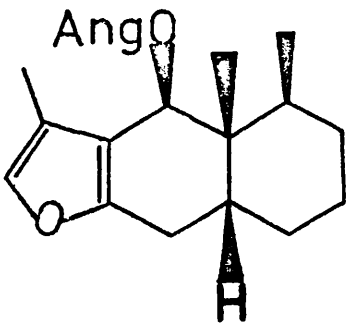
CLXVIII



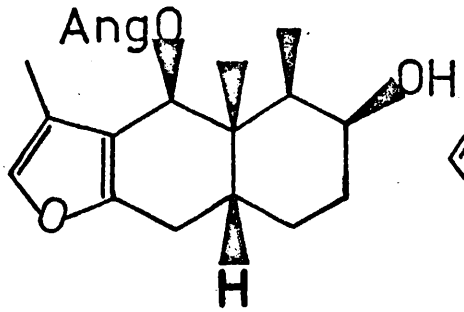
CLXIX



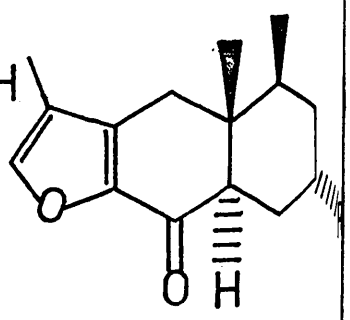
CLXX



CLXXI



CLXXII

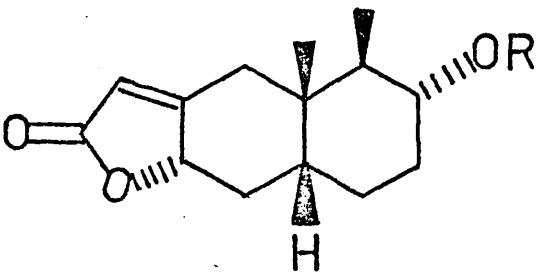


R = OAng

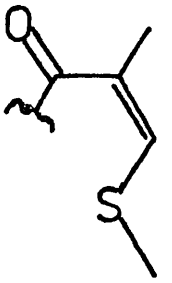
CLXXIII

Degeneria vitiensis is a shrub found in the Fiji Islands and is related to the genus Drimys (181), plants of which have been found to be rich in terpenoids (182-184). It was examined previously by Pojer and co-workers (185), who extracted a sample of leaves and twigs with light petroleum (b.p. 40-60°C) at room temperature. The solution obtained in this way was extracted with, in turn, 5% solutions of hydrochloric acid, sodium bicarbonate, sodium carbonate, and sodium hydroxide. These extracts proved to be intractable mixtures except for that from the bicarbonate which yielded, on chromatography on silica gel, an acid, identified as 2-hydroxy-6-methoxybenzoic acid. From the neutral fractions remaining after the extractions, chromatography on alumina separated β-sitosterol and a paraffin wax.

Plants of the genus Petasites have been intensively studied since the discovery of the sesquiterpenoid ester petasin (CLVII) (186), and the subsequent determination of its structure (151) and absolute configuration (187,188). Isopetasin (CLVIII) and S-isopetasin (CLXIV) were also found to co-occur in Petasites hybridus with petasin, all three of these compounds being based upon the eremophilane skeleton (CLXV). A number of compounds derived from furanoeremophilane (CLXVI) and eremophilenolide (CLXVII) have also been isolated from plants of this genus (189, 190). A variety of Petasites hybridus was found to contain furanopetasin (CLXVIII) (191), and other furanoeremophilanes oxygenated at C-9 (CLXIX) though it did not contain petasin or any of the non-furanoid compounds; P.albus and P.japonicus were found to contain furanoids oxygenated at C-2 such as petasalbin (CLXX) and its angelate ester (CLXXI) (192, 193), at C-2 and C-5 (CLXXII) (193) and at C-6 and C-9 (CLXXIII) (193), though as in P.hybridus there were found to be two varieties of P.albus one containing furanoids and the other containing non-furanoids (194). The petasitolides (CLXXIV, CLXXV) have been isolated from P.officinalis (189) which has been shown to be a form of P.hybridus (195). These compounds occur with the furanoeremophilanes but neither type has



CLXXIV R = Ang

CLXXV R = 

been isolated from a plant which produces petasin. The two sub-species of P.hybridus which are distinguishable by the occurrence of furanoid and non-furanoid sesquiterpenoids have been found in various locations all over western Europe (194), so the difference in the chemistry of these plants is not a purely geographical effect.

The pattern of occurrence of these compounds throughout the genus has been used in systematic studies of the relationships of individual plants within the genus, attempts being made to construct a family tree for these plants (193).

Petasites hybridus was also studied in Glasgow by R.A.B. Keates, as a preliminary to his examination of the biosynthesis of petasin, using a similar method to that outlined (180), so an examination of this plant was expected to serve as a check of the analytical techniques used in this study.

6 ft 1%SE-30 80-270°C, 2C°/min. N_2-30ml/min.

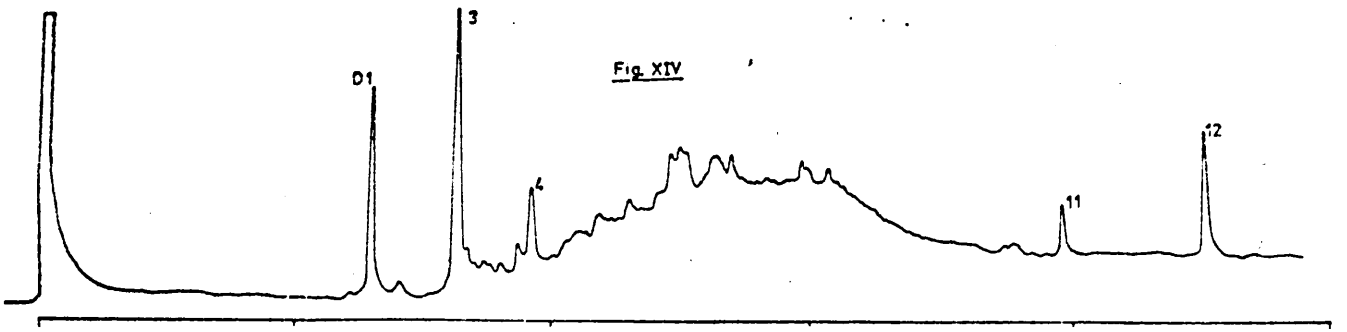


Fig. XIV

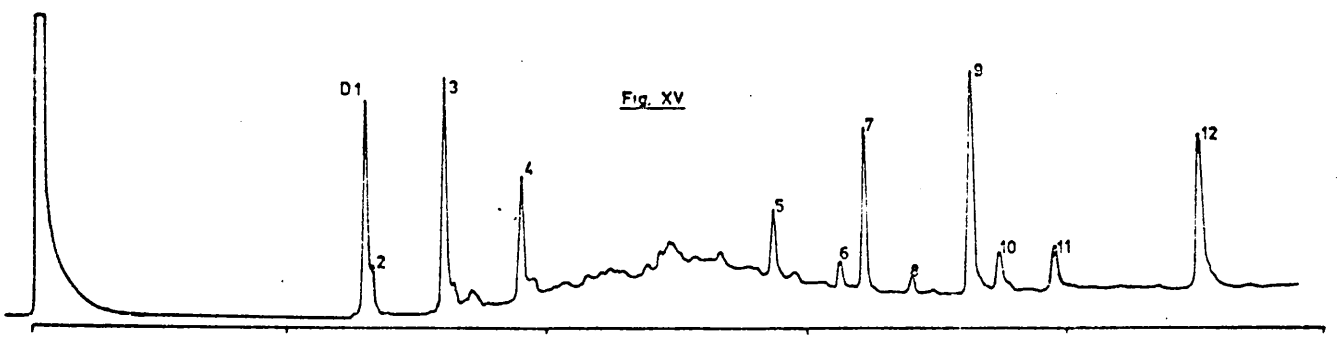


Fig. XV

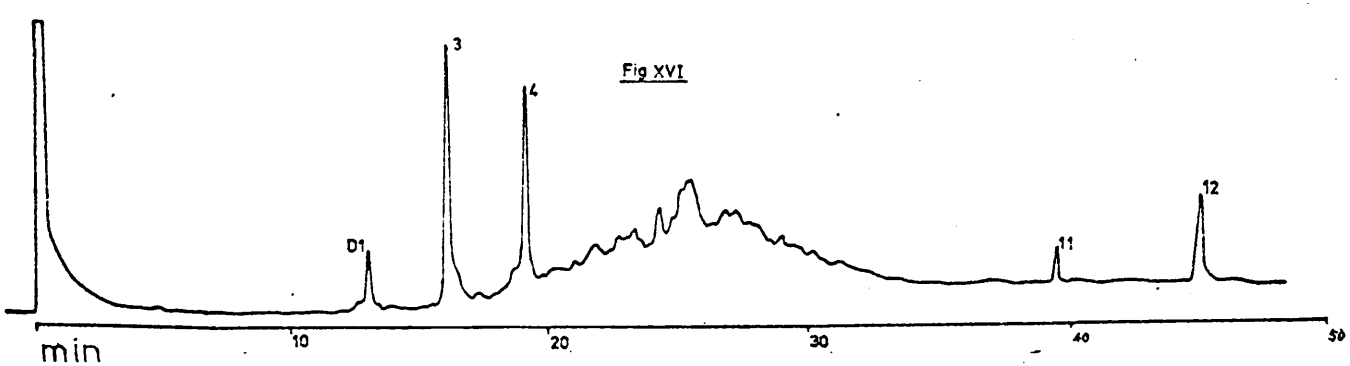


Fig XVI

3 - 2 Results and Discussion

3 - 2 - 1 Degeneria vitiensis

Samples of twig and leaf material of Degeneria vitiensis were provided by Professor M. Martin-Smith, in November of 1971. Three 10g portions of each type of plant material were extracted with three different solvents, ethanol, ethyl acetate and n-heptane and the solutions obtained were concentrated and examined by GLC on 1% SE-30 after gel filtration to remove any high molecular weight compounds present.

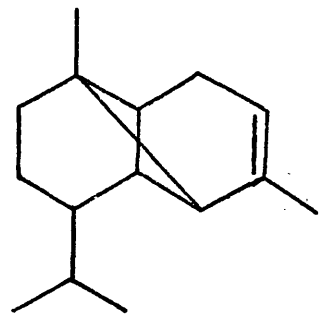
The ethanol and ethyl acetate extracts of the twig material gave similar GLC traces (Fig. XIV) containing a large amount of unresolved material in the region where peaks due to sesquiterpenoid compounds might be expected, and a few well resolved peaks. The heptane extract, however, (Fig. XV), showed much less of the interfering material, the same well-defined peaks and some which were not present in the other traces. The extracts of the leaves were all very similar and showed the same pattern as the ethanol and ethyl acetate extracts of the twigs (Fig. XVI). All the major peaks observed in the GLC traces of each of the extracts were found to occur in the trace of the heptane extract of the twig material and so it was decided to examine this extract by GC-MS. The retention indices and mass spectral properties of the major peaks are shown in Table 24, in which the compounds are labelled according to their order of appearance on the GLC trace. Extraction of a second sample of twig material in n-heptane was found, by the same technique, to contain compounds D1-D4, D7, D10 and D11.

TABLE 24

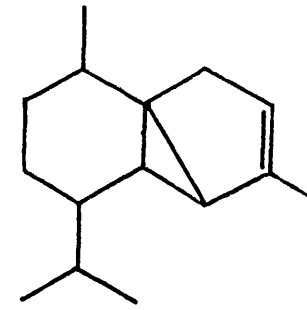
Compound	$I_{(1\%SE-30)}$	Apparent M^+ (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
D1	1375	204 (20)	105	119(98)	161(42)	93(65)
				41(63)	81(45)	91(42)
D2	1380	204 (4)	81	93(96)	68(76)	41(67)
				67(53)	107(51)	55(46)
				91(30)		
D3	1460	204 (62)	105	93(97)	107(96)	81(86)
				79(86)	67(72)	121(71)
				91(70)		
D4	1540	220 (4)	43	41(81)	93(57)	79(57)
				69(54)	108(50)	55(45)
D5	1845	270 (7)	88	43(60)	101(55)	41(40)
				55(38)	57(33)	69(25)
				157(11)		
D6	1940	284 (8)	88	43(77)	101(54)	55(51)
				41(49)	57(37)	69(35)
				157(10)		
D7	1975	284 (7)	88	101(50)	43(37)	41(24)
				55(24)	57(20)	69(14)
				157(9)		
D8	2060	298 (6)	55	43(86)	41(78)	69(72)
				88(71)	57(60)	83(50)
				85(20)		

TABLE 24 Contd.

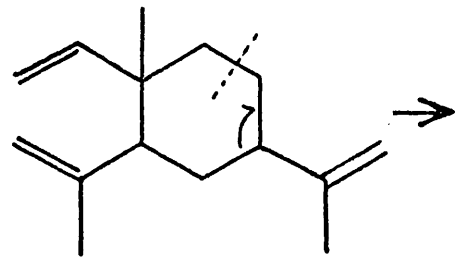
Compound	$I_{1\%\ SE-30}$	Apparent M^+ (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
D9	2145	308 (8)	67	81(85)	55(76)	41(64)
				95(61)	54(42)	82(41)
D10	2180	310 (6)	55	43(96)	41(94)	69(94)
				57(70)	83(69)	88(47)
				85(22)		
D11	2205	350 (3)	235	237(65)	165(36)	236(17)
				212(11)	239(11)	199(10)
				81(10)		
D12	2450	390 ( )	149	57(40)	167(36)	71(26)
				70(24)	41(23)	279(10)



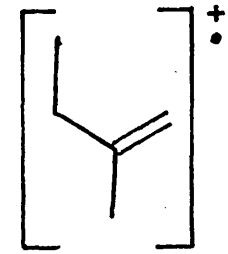
CLXXVI



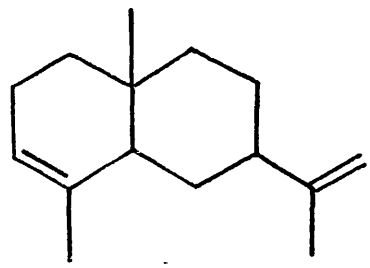
CLXXVII



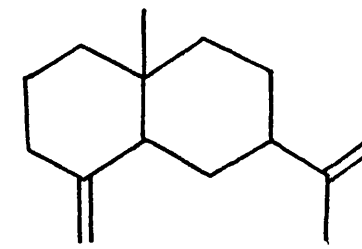
CLXXVIII



m/e = 68



CLXXIX



CLXXX

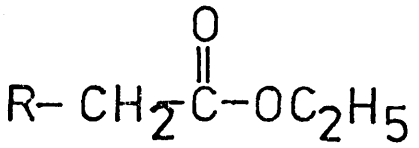
Compounds D1 - D3

Each of these compounds has a molecular ion of m/e = 204, classifying them as sesquiterpene hydrocarbons, with the formula $C_{15}H_{24}$. From the tables of retention indices and mass spectra compiled from literature and experimental data (Appendix III) a tentative identification of each of them can be made.

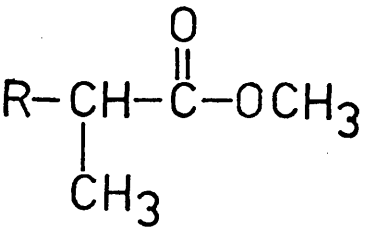
There are two compounds which have similar GC-MS properties to compound D1. The first is α-copaene (CLXXVI) which has a retention index on 1% SE-30 of 1379 (158) and a mass spectrum which contains the same major peaks as that of compound D1 (173). There are differences in the relative intensities of corresponding peaks in both spectra, but these may be due to a variation in the conditions under which the spectra were obtained, for though the accelerating voltage was 70eV in each case, the instruments were different and this could lead to such a difference in intensities, though not to a difference in the m/e values. The second compound is α-cubebene (CLXXVII) which also has a similar mass spectrum (173). While its retention index on 1% SE-30 is not known, comparison of its retention characteristics on other stationary phases with those of sesquiterpenes which have been run on SE-30 suggests that its retention index would be similar to that of D1 (158).

Compound D2 is almost perfectly matched by β-elemene (CLXXVIII). Again a comparison of the retention characteristics of β-elemene on different stationary phases suggests that on this phase the retention index would be very similar (158), while the mass spectra closely resemble each other, especially in the occurrence and high relative intensity, in each, of the ion of m/e = 68 (172, 173).

The last of the hydrocarbons, D3, has a mass spectrum which closely resembles those of α- and β- selinene (CLXXIX, CLXXX) (173), though the retention indices of these compounds while not known on 1% SE-30 may be higher than that of D3 (158). Once again both of these



CLXXXI



CLXXXII

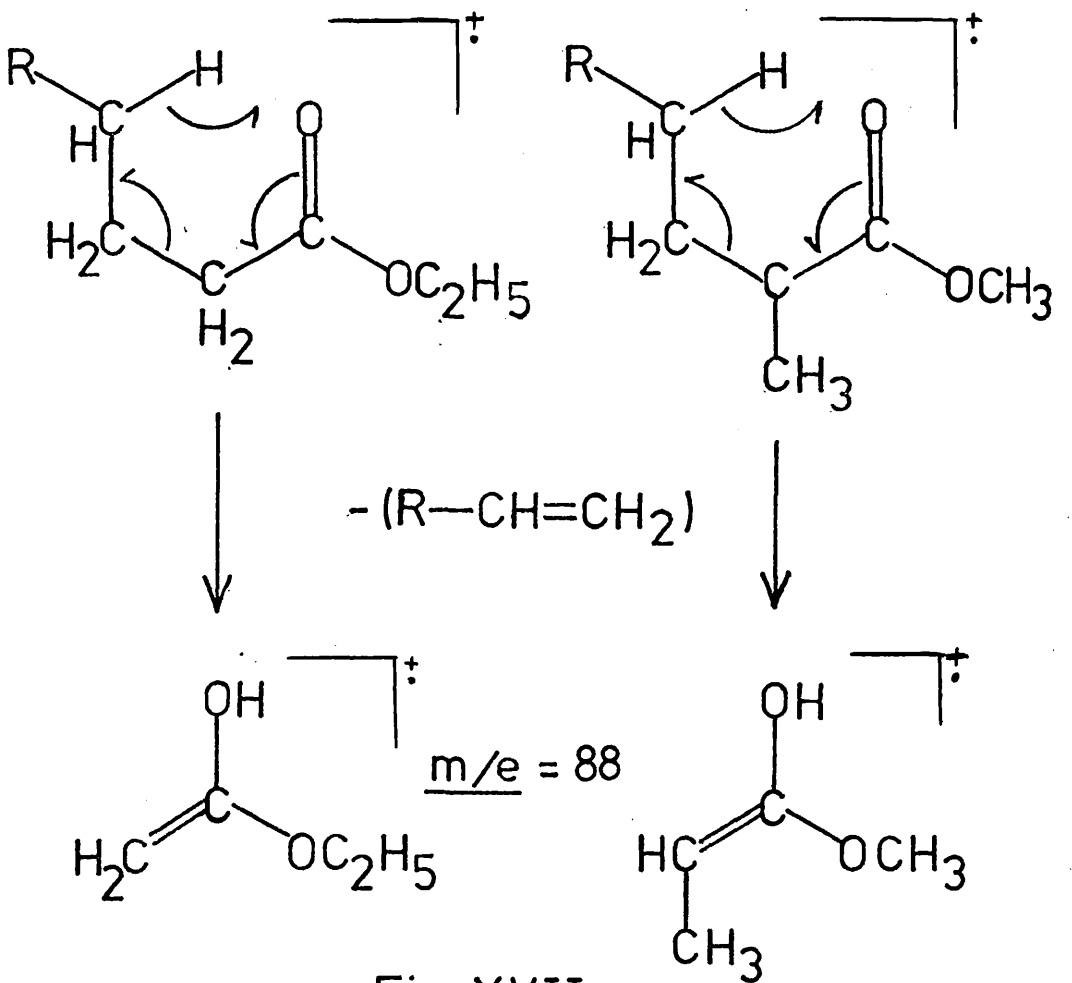


Fig. XVII

compounds give the same ions under electron impact as D3 though the relative intensities are different. Of the two, the α-isomer (CLXXIX) is perhaps the more likely as it shows a much weaker peak due to the molecular ion than does β-selinene (CLXXX).

Compound D4

This compound has a molecular ion of 220 which suggests that it has the formula $C_{15}H_{24}O$. Treatment of a sample of the extract with silylating reagent, bis(trimethylsilyl)acetamide (BSA) and trimethylsilylimidazole (TSIM) in the ratio 1 : 1, and rechromatography on 1% SE-30 showed no change in this peak, suggesting that this is not an alcohol. A similar test for a carbonyl group performed by treating a sample of the extract with methoxylamine hydrochloride in pyridine to form a methyl oxime also proved unsuccessful, implying that the compound is either an ether, or that it is very unreactive.

Compounds D5-D10

The mass spectra of these compounds are very similar to one another and the pattern of peaks and the molecular in weights suggest that they may be fatty acid esters (196), since the larger peaks are those of low m/e implying that they have been formed by the breakdown of an acyclic compound. The base peak in each of the first three compounds has an m/e value of 88 which could arise from either an ethyl ester (CLXXXI) or a methyl ester of an α-methyl fatty acid (CLXXXII) by a McLafferty rearrangement as shown (Fig. XVII) (77, 78, 196). In each spectrum, however, the highest ion apart from the molecular ion, at m/e = 270, 284 and 284 respectively, is that due to a loss of 45 mass units from the molecule, i.e. at m/e = 225, 239 and 239 respectively. This ion would be due to the loss of the $-OC_2H_5$ ion in each case. A similar loss of an $-OCH_3$ group would result in an ion of m/e value

CLXXXIII CLXXXIV

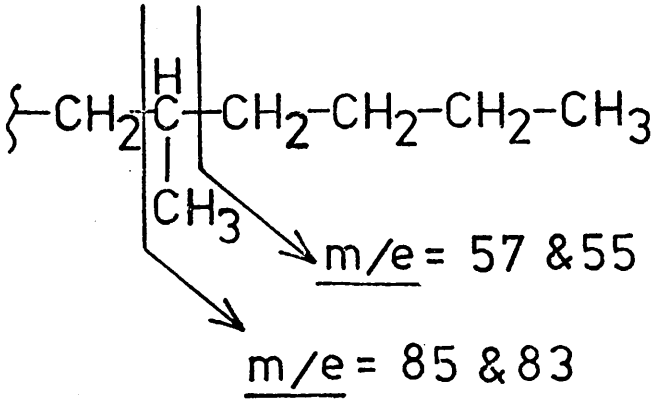
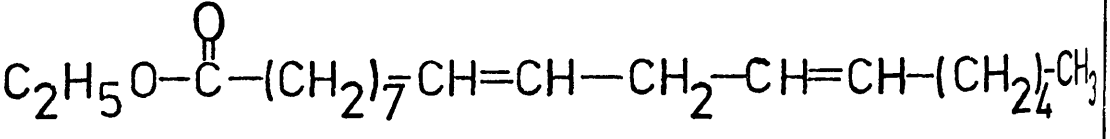


Fig XVIII



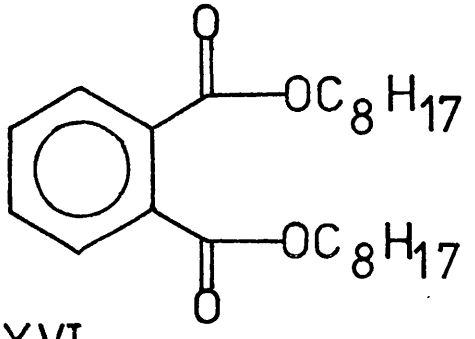
CLXXXV

equal to that of the molecular ion minus 31 mass units (196). Since this ion is not observed in any of the spectra it suggests that the compounds present are all ethyl esters of fatty acids.

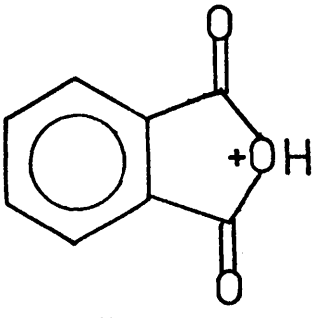
The spectra do not match those of straight chain acid esters in which the molecular ion would be expected to be much larger (196), so they are probably branched chain compounds, with compounds D6 and D7 being isomers of the same molecular formula.

Compounds D8 and D10 also show a peak at m/e = 88, though in these spectra it is no longer the base peak, which now has the m/e value of 55. This peak arises from the loss of a four carbon unit in the form of the ion of m/e = 57 (CLXXXIII) which loses two hydrogens to give the ion CLXXXIV of m/e = 55 (196). If the fatty acid were branched as shown in Fig. XVIII, the result would be an enhancement of the relative intensity of the ions at m/e = 55 and 57, which would explain the change in the base peak, and in the intensities of the ions of m/e = 85 and 83 (196), which is also observed in these spectra, suggesting that compounds D8 and D10 are branched in this way. The molecular weight of D8, 298, implies the formula $C_{19}H_{38}O_2$ which, in common with D5, D6 and D7, would make it the ethyl ester of a saturated fatty acid, but the formula of D10, $C_{20}H_{38}O_2$ suggests that the fatty acid in this ester is unsaturated.

In compound D9 there are still peaks at m/e = 88 and M-45, i.e. 263 though their intensities are small compared to those of the other peaks in the spectrum. The molecular weight, 308, implies the formula $C_{20}H_{38}O_2$, which would possess two double bond equivalents. The spectrum of this compound is similar to that of methyl linoleate $C_{19}H_{36}O_2$ (CLXXXV) (196), the major differences being the peaks due to the presence of the ethyl group in place of the methyl group in the ester, and a much smaller molecular ion. These differences suggest that D9 might be an isomer of ethyl linoleate.



CLXXXVI



CLXXXVII

Compound D11

The molecular ion of this compound, m/e = 356, suggests a number of possible formulae, such as $C_{25}H_{40}O$, $C_{24}H_{36}O_2$ or $C_{23}H_{48}O_2$, which are only a few of the possibilities, depending on the degree of oxidation of the compound. Attempts at derivative formation produced a reaction with the trimethylsilylating reagents, the peak due to compound D11 disappearing from the GLC trace, and a new one appearing at I_{1SE-30} = 2370, suggesting the presence of a hydroxyl group. Apart from this, very little information can be obtained from the data.

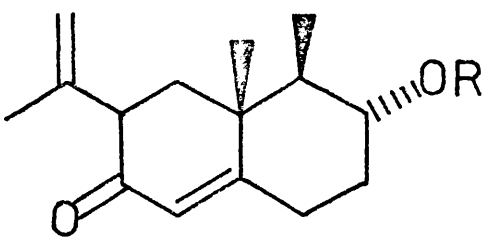
Compound D12

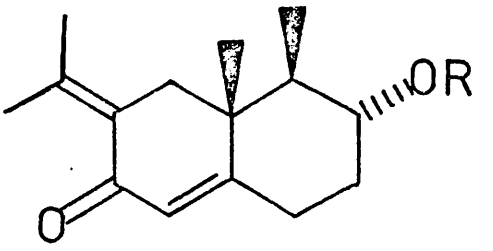
The mass spectrum and retention index of this compound match that of di-octyl phthalate (CLXXXVI) (197). The large base peak at m/e = 149 is due to the ion CLXXXVII. This compound is a plasticiser and might have entered the samples from the plastic bags in which they were packed, or from the solvents used for the extraction or the gel filtration (197).

It was decided that none of the compounds found in the plant samples warranted further large scale studies, so the examination was concluded at this point.

Of the three compounds previously reported in the plant, only the β-sitosterol and possibly the paraffin wax, if it possessed a suitable number of carbon atoms, might be expected to appear in the GLC trace under the conditions used. The former has a retention index on 1% OV-1 of 3220 (180) and would be expected to have a similar value on 1% SE-30. No such peak was, however, observed, nor was there any sign of the paraffin wax.

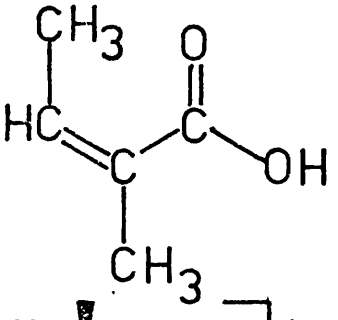
Some of the fatty acid ethyl esters occur in extracts made of other





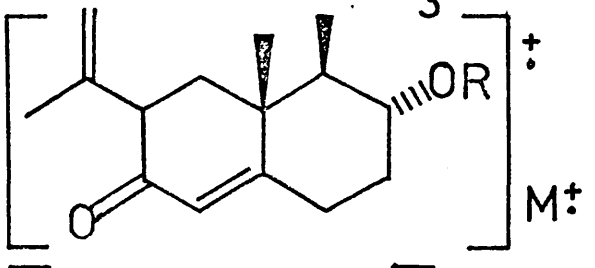
CLXXXVIII R=H CLXXXIX

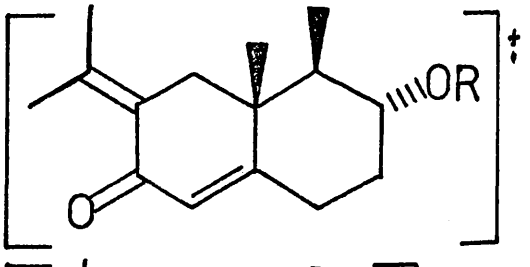
CLVII R=Ang CLVIII

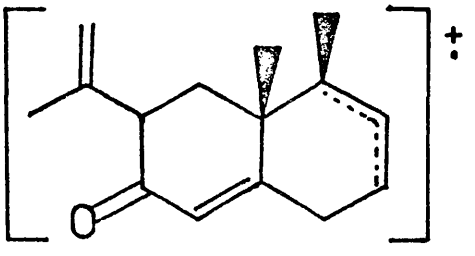


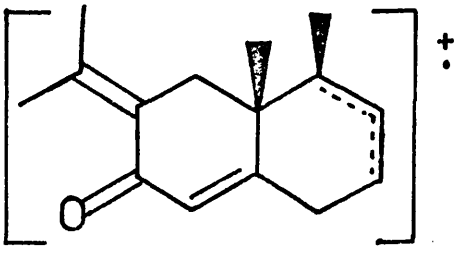
CXC

(≡HOAng)

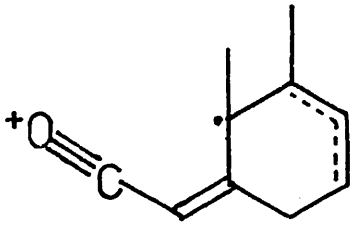




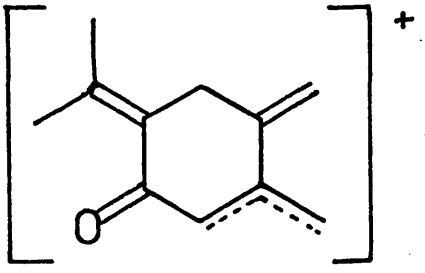




m/e = 216



m/e = 148



m/e = 161

Fig. XX

plants (Chapter 4) and so they may well be impurities, introduced into the plant material before, or into the extract during the extraction procedures.

3 - 2 - 2 Petasites hybridus and Petasites fragrans

In order to fulfil a request from Dr. L. Novotný of the Czechoslovak Academy of Science in Prague, for a sample of petasin (CLVII), extracts were made of the rhizomes of Petasites hybridus and Petasites fragrans, both of which were obtained from sources in England, the former from Braughing in Hertfordshire, and the latter from Arrington Bridge, Royston, Herts. Since both samples were available it was decided to compare the petasin esters occurring in each plant. These compounds are esters of petasol (CLXXXVIII) and isopetasol (CLXXXIX) with a variety of acids, for example, angelic acid (CXC) with which they give petasin and isopetasin (CLVIII).

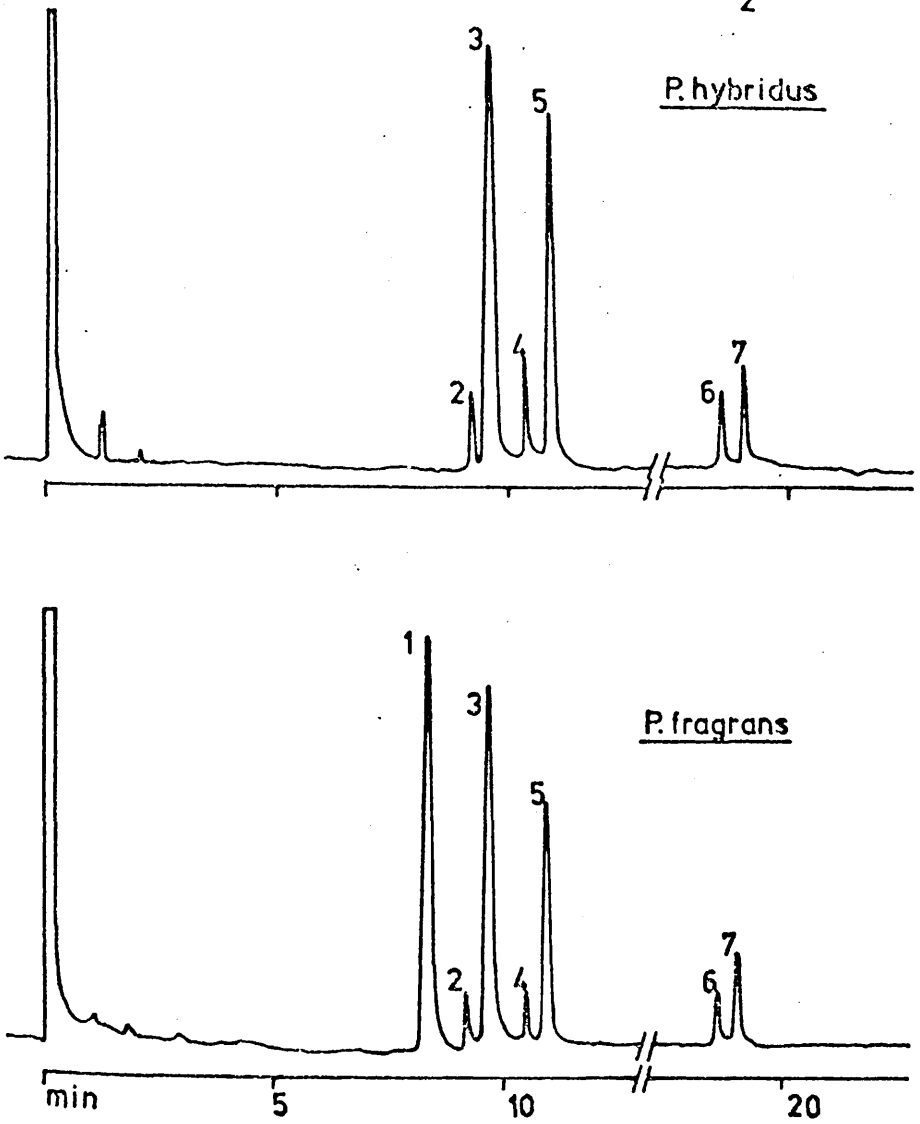
The rhizomes were extracted with light petroleum (b.p. 60–80°) and the solutions obtained were purified for GLC by gel filtration. The extracts were then examined by GLC, and by GC–MS with the results summarised in Figure XIX and Table 25.

Each of the compounds shows an ion at m/e = 216 derived from the molecular ion by ester elimination (Fig. XX), and so the molecular weight of the acyl moiety can be determined from the molecular weight of the ester. The esters of petasol and isopetasol can be distinguished by their different base peaks, both of which are formed from the m/e = 216 ion (180); the base peak in petasin esters is the ion of m/e = 148, formed by the loss of isoprene from the m/e = 216 ion, while cleavage, in this ion, of ring A results in the base peak of the isopetasin esters, of m/e = 161 (Fig. XX).

From these facts it can be seen that compounds 1, 2, 3 and 6 are

Fig XIX

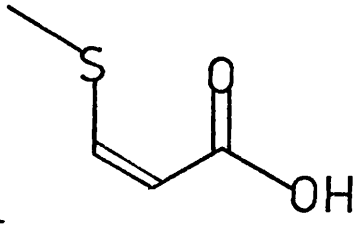
6ft 1%SE-30 100-275°C
6C°/min N_2-30ml/min



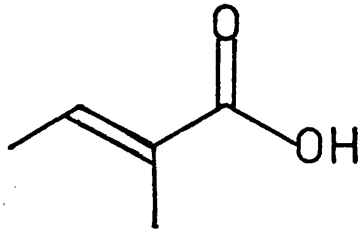
6' 1% SE-30, N_2 - 30ml/min., 100-275°C @ 6C°/min.

TABLE 25

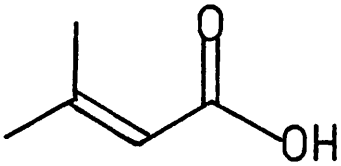
Compound	$I_{(1\%\ SE-30)}$	M	Base Peak	Strongest Ions					
1	2255	316	148	55	105	83	41	161	216
2	2285	316	148	55	83	161	216	105	41
3	2300	316	148	83	55	105	216	161	41
4	2330	316	161	55	216	201	105	41	83
5	2345	316	161	55	148	83	41	216	105
6	2640	354	148	101	216	105	91	41	161
7	2700	354	161	216	101	41	91	105	201



CXCI



CXCIII



CXCII

TABLE 26

		$I_{1\%\ SE-30}$	Ratio	
P.hybridus	Acids			
		1015	1	Angelic
		1080	2	Tiglic
	Me Esters	950	1	Me angelate
		1010	2	Me tiglate
P.fragrans	Acids	1015	1	Angelic
		1060	2	Dimethylacrylic
		1080	2	Tiglic
	Me Esters	950	1	Me angelate
		1010	2	Me tiglate
		1015	2	Me dimethylacrylate

esters of petasol, 4, 5 and 7 of isopetasol. The molecular weights suggest that compounds 1-5 are esters of unsaturated C_5 acids such as angelic acid (CLX) while 6 and 7 are esters of β-methylthioacrylic acid (CXCI) (151, 180), i.e. S -petasin and S -isopetasin.

Comparison of the retention indices of these compounds with those previously reported (180) shows that compounds 3 and 5 are petasin and isopetasin respectively. Compounds 1 and 2 must be petasol esters of acids isomeric with angelic acid, i.e. dimethylacrylic acid (CXCII) or tiglic acid (CXCIII), while compound 4 is an isopetasol ester of one of these acids. Since the possibility exists of isomerisation from angelic to tiglic acid, compounds 2 and 4 might be the esters of tiglic acid formed during the extraction. Compound 1 would then be the ester of dimethylacrylic acid. Esters of angelic and dimethylacrylic (senecioic) acids have been shown to co-exist in plants of the genus Ligularia (198).

Each extract was hydrolysed by treatment with mild base, and after the reaction the basic solutions were extracted with ether to remove the alcohols. The remaining aqueous solution was acidified with dilute hydrochloric acid, and extracted with ether. The ethereal solutions were examined by GLC, a comparison being made with authentic samples of angelic acid, tiglic acid and dimethylacrylic acid. The acids were methylated, by treatment with diazomethane, and the methyl esters were also examined by GLC. The results are summarised in Table 26.

The hydrolysis of the petasin esters from P.hybridus gave a much smaller ratio of angelic to tiglic acid than might have been expected from the original ratio of the esters, which suggests that there has been some isomerisation of angelic acid to tiglic acid during the hydrolysis. The effect was also noted during the hydrolysis of an authentic sample of isopetasin. Taking this into account, the ratios of the three acids derived from the esters in P.fragrans are consistent with the ratios of the original esters if compound 1 was the petasol ester of dimethylacrylic acid.

The composition of the petasin esters of P.hybridus, shows very little change from the results obtained by R.A.B. Keates during his study of the plant in this department (180) which suggests that the extraction and analytical procedures are satisfactory, while the close family relationship of the two plants examined is demonstrated by the presence of petasin type esters in both of them.

3 - 3 Experimental

3 - 3 - 1 Extraction of Plant Tissue

The plant material was cut into small pieces, immersed in ten times the volume (w/v) of solvent at room temperature, and shredded in a Townson and Mercer 'Top Drive' macerator for about a minute, reducing the sample to small fragments and destroying the fibrous structure. This was followed by filtration of the suspension, and re-extraction of the residue twice more in the same way. This procedure was shown to be sufficient by the re-extraction of the residue; this last extract showed no trace of the compounds which were to be examined.

The combined extracts were then evaporated to small bulk ($\sim$ 5ml/100g of tissue) at room temperature in a Büchi Rotary Evaporator. In the preliminary experiments a sample of this solution was examined by GLC before the separation of the high molecular weight compounds was attempted, and was found to give a GLC trace identical to that obtained after the separation. An equal volume of benzene was added to the extract which was then evaporated to dryness, at room temperature, in the Büchi Rotary Evaporator, any water in the extract forming an azeotrope with the benzene which was evaporated. The residue was then weighed. To this residue was added a mixture of benzene and isopropanol in the ratio of 3 : 1 (5ml/100g fresh tissue), and the resulting suspension was filtered to remove any non-lipids extracted by the large bulk of the initial solvent.

Aliquots of the filtrate of up to 4ml, equivalent to 80g of fresh tissue, were then reduced to a volume of about 0.5ml in preparation for gel filtration. This was done on a column containing the substituted gel N1114-50%-LH20 with benzene/isopropanol, 3 : 1 v/v, as solvent. The bed volume was measured as 260ml. Using the term Standard Elution Volume (SEV) where:

$$SEV = \frac{\text{elution volume} \times 100}{\text{bed volume of column}} \qquad (180)$$

two fractions were taken from the column, A (SEV 30–46), collection being started when 80ml of solvent had eluted and stopped when 120ml had passed, and B (SEV 46–77), taken from the end of A, till a total of 200ml had eluted. Fraction A was found to be free of any volatile sesquiterpenoids, all the compounds giving peaks on the GLC trace, under the required conditions, being found in Fraction B. GLC data were obtained using the Aerograph 204, fitted with hydrogen flame-ionisation detectors and modified to allow the use of glass columns with direct injection of the samples onto the packing and without any contact with the metal walls of the flash heater. Most of the samples were examined by means of a programmed temperature rise of from 2 to $6^{\circ}C$/min. Under these conditions the detector heater oven was set at a temperature approximately $25^{\circ}C$ higher than the maximum temperature of the programme. If this was not done, high molecular weight compounds, eluted at high temperatures, tended to condense slightly on the way through the detector head to the flame, resulting in broad, tailing peaks on the GLC trace, and a build-up of material in the detector which showed as a gradual rise in the baseline of the trace. All the mass spectra were obtained by using an LKB 9000 combined gas chromatograph and mass spectrometer, with the GLC conditions matching those of the Aerograph, and the ionising voltage of the mass spectrometer set at 70eV.

3 - 3 - 2 Degeneria vitiensis

Three 10g lots of leaf material and three 10g lots of twig material were separately extracted with ethyl acetate, ethanol, and n-heptane (Table 27).

TABLE 27

		Wt. of Total Extract (g)	Wt. of Fraction B (g)
Ethyl Acetate	Leaf	0.819	0.279
	Twig	0.309	0.115
Ethanol	Leaf	0.485	0.156
	Twig	0.413	0.107
n-Heptane	Leaf	0.209	0.093
	Twig	0.076	0.054

Trimethylsilylation

Trimethylsilylation was carried out by evaporating to dryness a sample of the extract (~5mg), redissolving it in dry pyridine (0.25ml) and adding a large excess of a freshly prepared mixture of HMDS and TMCS in the ratio of 2 : 1 (0.1ml). This was then heated for ten minutes at 100°C, the pyridine and excess reagents were evaporated under a stream of nitrogen, and the residue was dissolved in ethyl acetate to a concentration of about 1mg/ml for GLC.

Methyloxime Formation

The formation of the methyl oximes of any carbonyl groups present was performed in a similar way, except that in this case the pyridine solution was treated with methoxylamine hydrochloride and the subsequent procedure followed.

3 - 3 - 3 Petasites

Both P.hybridus and P.fragrans were extracted with light petroleum (b.p. 40-60°C). 61g of the former yielded 1.053g of total extract and 0.34g of fraction B, while 45g of the latter yielded 0.917g of total extract and 0.25g of fraction B.

Ester Hydrolysis

The hydrolysis of the petasins was carried out by dissolving about 10mg of the purified extract in a minimum of benzene (~0.1ml). This solution was treated with 0.2ml of a mixture of five parts by volume of ethanol to two parts, by volume, of 33% aqueous potassium hydroxide (w/v). The reaction mixture was heated at 65°C for 30 min. in a Reacti-Vial. After cooling, the mixture was extracted with ether to remove any non-acidic material, such as the alcohols, and

the residue was acidified by the addition of 10% (v/v) hydrochloric acid until a test with pH paper showed that it had a pH of 6. This solution was then re-extracted with ether to remove the organic acids. The ether solution was dried over magnesium sulphate, filtered and examined by GLC (6' 1% SE-30, 50°C, N_2-30ml/min).

Methylation of the Acids

The methyl esters of the acids were prepared by treating the ether solution with a freshly prepared ether solution with diazomethane, for preparation see (4-3-4), the addition being made slowly until the yellow colour of the reagent persisted, showing that the reaction was complete. The excess reagent was then evaporated at room temperature under a stream of nitrogen, the residue redissolved in ethyl acetate and examined by GLC under the same conditions as before.

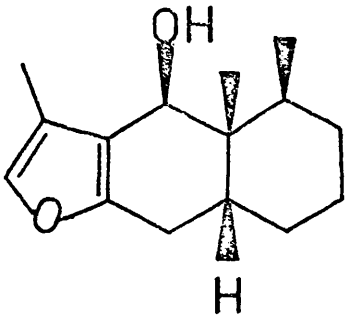
4. CHEMICAL STUDIES OF LIGULARIA

4 - 1 Introduction

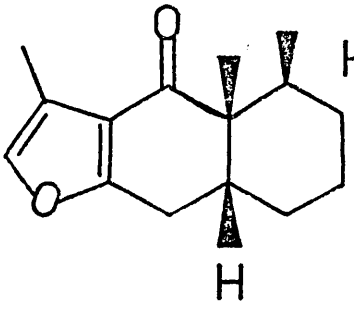
The genus Ligularia is a member of the tribe Senecioneae of the family Compositae, so it is related to the genus Petasites, which is also a member of the Senecioneae. The genera Ligularia and Senecio are very similar, and there is still some disagreement as to whether they are actually separate, but this distinction is gradually becoming accepted (199). In many sources, therefore, particularly in older ones, the two genera may be treated under either name. The confusion has also led to the further segregation of additional genera from Ligularia for example, Cremanthodium and Farfugium. This latter is becoming re-integrated with the original genus, for example, Farfugium Japonica is now equated with Ligularia tussilaginea (199), but again it is still referred to in some places by the older name.

Apart from L.sibirica which has an extensive geographical range, reaching as far west as France, the Ligularias are Asiatic in origin, with many species in China, Japan and Siberia, and some in India. They have, however, been imported into many western countries by gardeners, and some hybrids owe their origins to the experiments of western nurserymen. For example, Ligularia 'Gregynog Gold' which is a hybrid of L.clivorum and L.veitchiana, was first grown at Gregynog Hall in Wales. The number of species now included in the genus ranges from estimates of 35 - 150 (199).

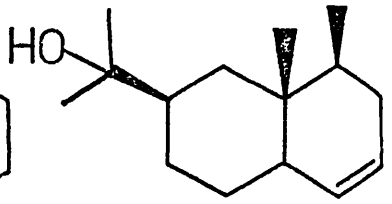
The chemical constituents of a number of species have been examined, especially by Japanese and Eastern European workers who have ready access to large supplies of the plants which grow in their respective regions. Some of these papers were published before, and some after, this study which was commenced in January, 1973.



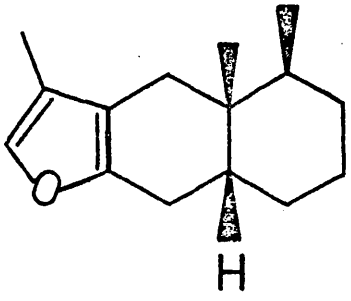
CLXX



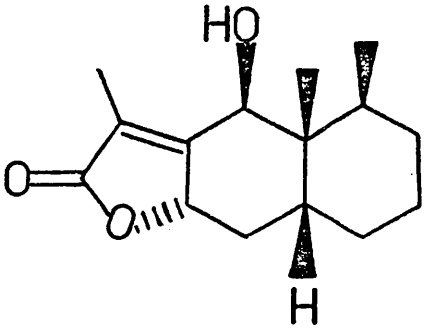
CXCIV



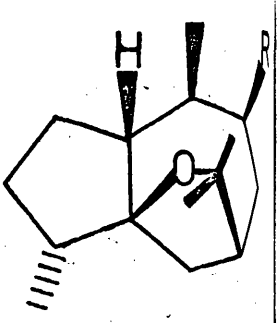
CXCV



CLXVII



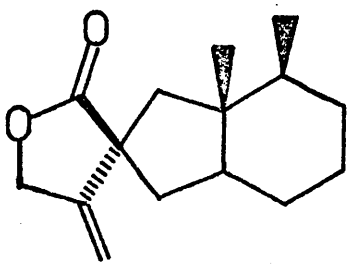
CXCVI



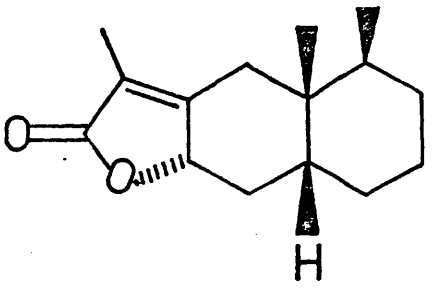
R=H CXCVII

=OH CXCVIII

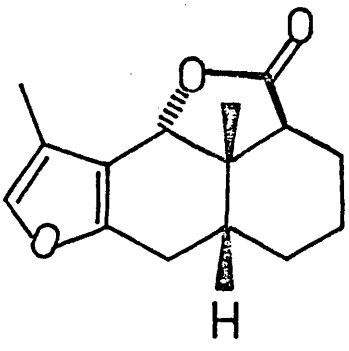
=OAc CXCIX



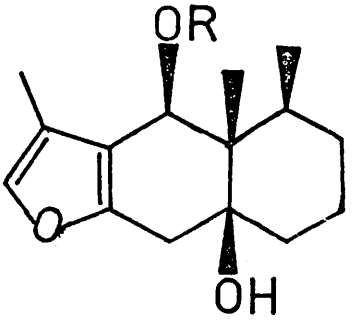
CC



CLXVI

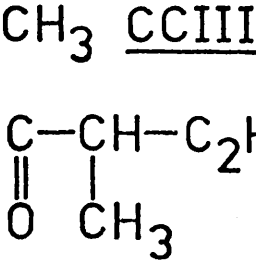


CCI

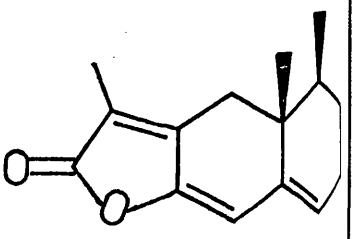


R H CCII

CH_3 CCIII



CCIV

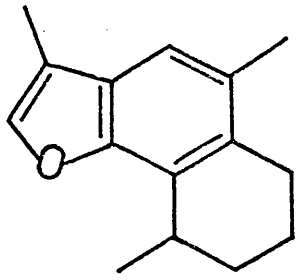


CCV

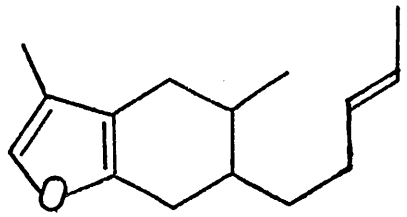
Both L.sibirica and L.fischeri, which is a cultivated variety of L.sibirica, have been shown to contain ligularol, which has been identified with petasalbin (CLXX), and ligularone, the oxidised form (CXCIV) (200, 201). The latter plant has also yielded a number of other sesquiterpenoids, viz; eremoligenol (CXCV), furanoeremophilane (CLXVI) and 2β -hydroxyeremophilenolide (CXCVI) (201), as well as three based on the guaiane skeleton, liguloxide (CXCVII), liguloxidol (CXCVIII) and liguloxidol acetate (CXCIX) (202, 203).

L.hodgsonii was shown to contain furanoeremophilane (CLXVI) bakkenolide-A (CC) and eremophilenolide (CLXVII) (204), as well as a new furanosesquiterpenoid, furanoeremophilan-4β-, 2α -olide (CCI) (205), which has also been isolated from L.fauriei and L.angusta (206), while three other furanosesquiterpenoids (CCII-CCIV) have been found in L.japonica (207). A further compound based on the eremophilenolide structure is ligularenolide (CCV), isolated from the Chinese drug San-Shion, which is derived from a root of a Ligularia species (208). Examination of the rhizomes of Farfugium Japonicum (=L.tussilaginea) produced the compounds farfugium A (CCVI) and B(CCVII) (209), as well as another set of furanosesquiterpenoids (CCVIII-CCXII) (198).

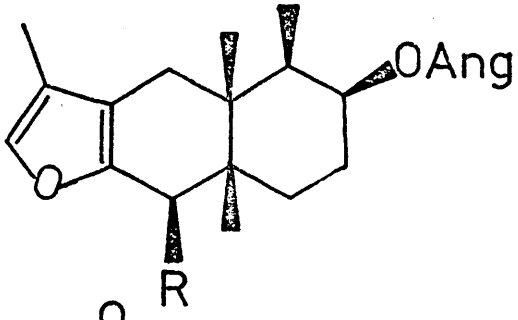
The presence, in so many of the Ligularias examined, of compounds derived from the furanoeremophilane and eremophilenolide skeletons, argues a close similarity between these plants and those of the genus Petasites, in which the same types of compounds are found (189-192). In the latter genus and particularly in P.hybridus the species occur in two forms, one which contains furanoid compounds and the other containing non-furanoids (194). Such a distinction also seems to occur in the Ligularias, for all the plants mentioned so far, with the exception of L.fischeri, would fall into the former class, containing only furanosesquiterpenoids. L.kaialpina, however, which like L.fischeri is a subspecies of L.sibirica (210) contains no furanoid compounds, its main sesquiterpenoid constituent being fukinone

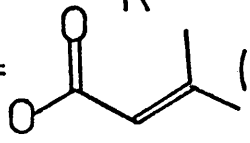


CCVI



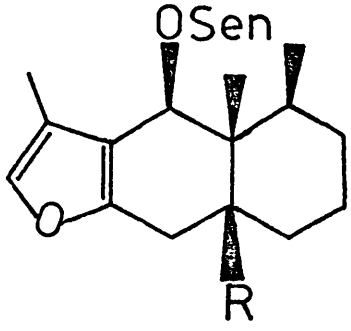
CCVII



R =  (≡ OSen) CCVIII

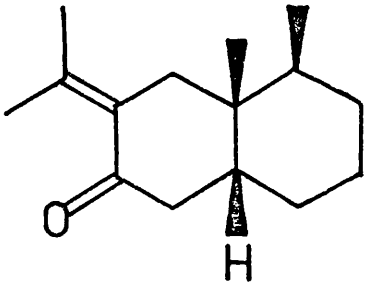
= OH CCXII

= H CCIX



R = OH CCX

= H CCXI

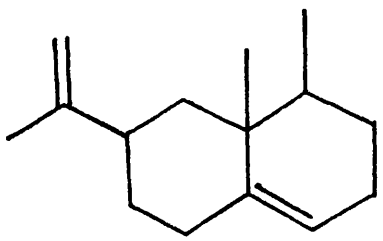


CCXIII

(CCXIII) (211). This argues a situation similar to that found in Petasites, the only exception being L.fischeri which has been reported as containing both types of compounds (201-203). This may be an exception to the rule, or may result from the mixing of both types of plants when the samples were collected.

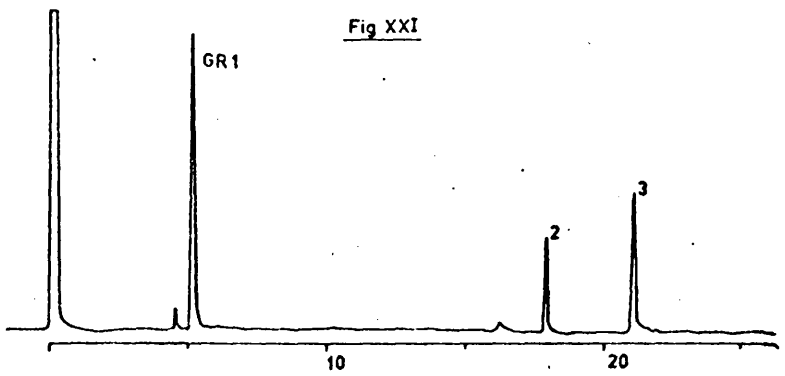
A number of alkaloids have also been isolated from Ligularias. Investigation of L.clivorum resulted in the alkaloid clivorine (212, 213) the structure of which has been determined by X-Ray Crystallography (214). L.brachyphylla and L.macrophylla have also been examined along with L.clivorum and found to yield a number of these compounds (215).

The plants examined in this thesis were found growing in Logan Botanic Gardens near Ardwell in Wigtownshire. These were L.clivorum (Maxim), L.clivorum "Desdemona" (referred to as L.'Desdemona'), which is a cultivated variety of L.clivorum (199), L.'Gregynog Gold', a cross between L.clivorum and L.veitchiana (Hemsley) (199), L.veitchiana itself, L.tangutica and L.tussilaginea 'aureo-maculata' a cultivated variety of the original L.tussilaginea distinguished by the yellow spots on its leaves (199).



CCXIV

Fig XXI



4 - 2 Results and Discussion

4 - 2 - 1 Plant Material

The plants used in this study were originally collected from Logan Botanic Gardens, and samples of these were transplanted into the gardens of the Botany Department of Glasgow University, at the Garscube Estate. A preliminary examination was made of root and, where possible, leaf tissue from these plants and in the cases which suggested the profitability of a more extensive study, larger samples were obtained, once more, from Logan Gardens.

The extraction procedure used was similar to that previously described, the solvent in this case being a mixture of benzene and isopropanol in the ratio of 3 : 1, which is the mixture used for gel filtration. Apart from this the extraction and purification of the compounds from the tissue was performed in the same way, and they were again examined by GLC and GC-MS.

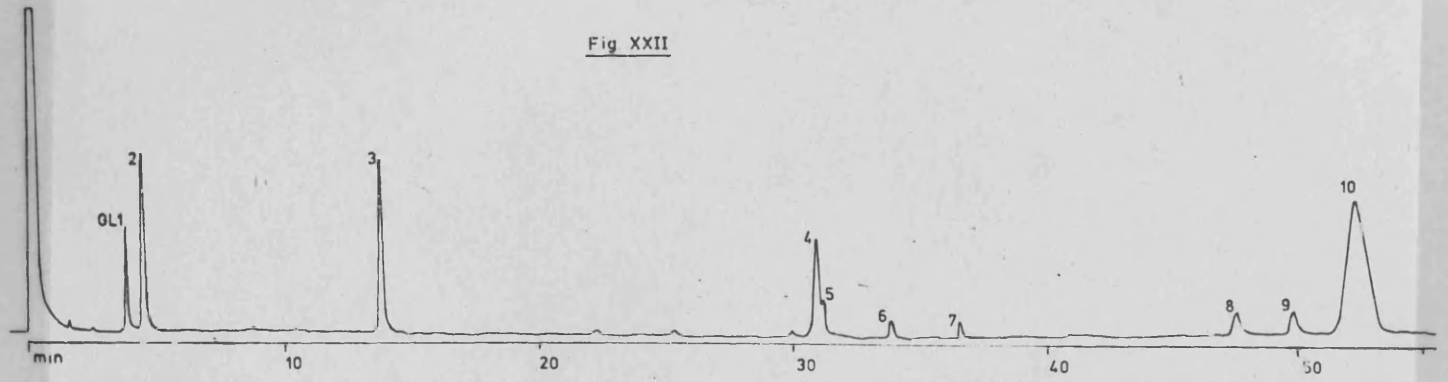
4 - 2 - 2 L.clivorum and L.'Gregynog Gold'

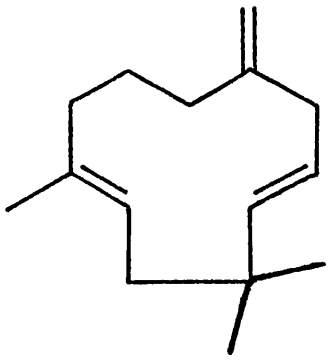
The root extracts of these two plants gave very similar GLC traces (Fig.XXI), containing the same three major compounds. GC-MS confirmed the GLC result, showing that the compounds examined were the same in each plant (Table 28). Compound GR1 has a molecular weight of 204, suggesting that it is a sesquiterpene hydrocarbon. Comparison of its mass spectrum with those on record (Appendix III), showed that it closely resembled that of eremophilene (CCXIV) (180), while its retention index on 1% SE-30, 1465, compares favourably with the recorded value of 1460 on 1% OV-1 (180), a similar stationary phase. Rechromatography of the sample on 1% OV-1 resulted in a value of 1465 $\pm$ 3 units for the compound increasing the probability that

TABLE 28

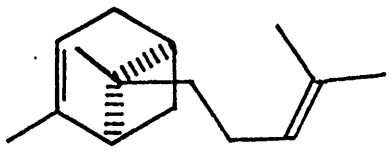
Compound	$I_{1\% \text{ SE-30}}$	M (% of Base Peak)	Base Peak	Strongest Ion (% of Base Peak)	
GR1	1460	204 (30)	93	41(95)	91(79)
				79(71)	105(68)
				161(55)	107(52)
GR2	1980	284 (5)	88	101(54)	43(38)
				41(27)	55(25)
				57(22)	157(10)
GR3	2150	305 (4)	67	81(77)	55(63)
				41(60)	95(59)
				82(41)	54(41)

Fig XXII

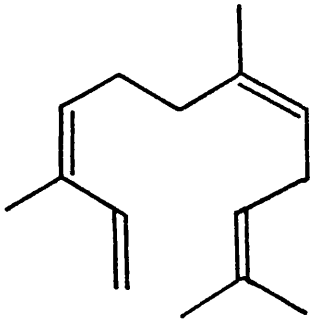




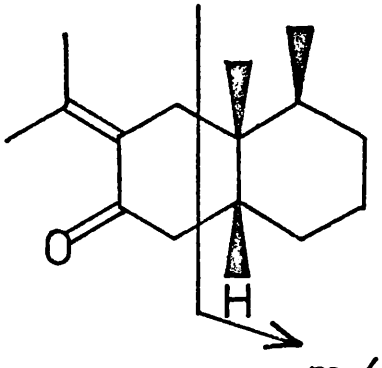
CCXV



CCXVI

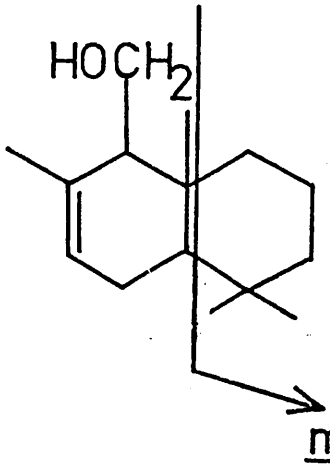


CCXVII



m/e =109

CLXII



m/e =109

CCXVIII

the identification is correct.

Compounds GR2 and 3 seem to be fatty acid ethyl esters and strongly resemble two of the compounds found during the extraction of Degeneria vitiensis, D7 and D9. This, and the fact that they are found in many of the extracts from different plants, suggests that they are impurities, introduced into the sample during the extraction procedure.

An extract of leaf tissue from L.'Gregynog Gold' gave the GLC trace shown in Figure XXII, and mass spectra of the major peaks are summarised in Table 29. Both compounds GL1 and 2 are sesquiterpene hydrocarbons, formulae $C_{15}H_{24}$, and comparison of their spectra with those of known compounds suggests that the former is β-humulene (CCXV) (180), while the latter is α-bergamotene (CCXVI) (175). The only other compound with a mass spectrum similar to that of compound GL2 was α-farnesene (CCXVII) (175), but the retention index of this compound, while not known, would be similar to that of β-farnesene which is 1367 (158).

Compound GL3 has a molecular weight of 220, and so the formula, $C_{15}H_{24}O$. As well as the peaks shown, the mass spectrum contains a peak at m/e = 202, i.e. $M^+ - 18$, which suggests that it is an alcohol, and that the peak is due to loss of H_2O. Trimethylsilylation of the extract resulted in the disappearance of this peak and the appearance of a new peak at 1690 index units confirming the suggestion. The base peak at m/e = 109 might arise in a number of ways, for example, by cleavage of a ring system as it does in fukinone (CLXII) (177) or in drimenol (CCXVIII) and there are no distinctive ions in the spectrum to facilitate identification. None of the standard spectra (Appendix III) match this compound.

Compounds GL5, 6, 7, 8 and 9 all show very similar mass spectra, and their retention indices suggest that they are related. The mass

TABLE 29

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
GL1	1480	204 (10)	161	43(82)	41(72)	105(50)
				91(51)	81(50)	57(48)
				119(40)	79(39)	
GL2	1490	204 (5)	93	41(93)	119(83)	69(64)
				55(59)	79(39)	43(37)
				107(32)		
GL3	1640	220 (4)	109	41(98)	43(89)	79(69)
				91(63)	81(60)	55(60)
				67(51)		
GL4	2400	288	104	91(79)	288(57)	184(49)
				80(48)	155(46)	79(40)
GL5	2400	-	93	57(95)	55(86)	83(76)
				69(73)	97(61)	41(57)
GL6	2500	-	43	57(47)	55(87)	83(78)
				69(70)	97(65)	41(56)
GL7	2600	-	69	43(70)	57(67)	81(65)
				55(59)	83(40)	97(35)
GL8	3000	-	43	73(97)	55(81)	41(79)
				57(79)	69(68)	81(45)
GL9	3100	-	73	43(87)	55(85)	41(79)
				57(72)	69(59)	81(50)
GL10	3235	408	218	43(53)	55(52)	141(44)
				69(43)	81(33)	45(32)

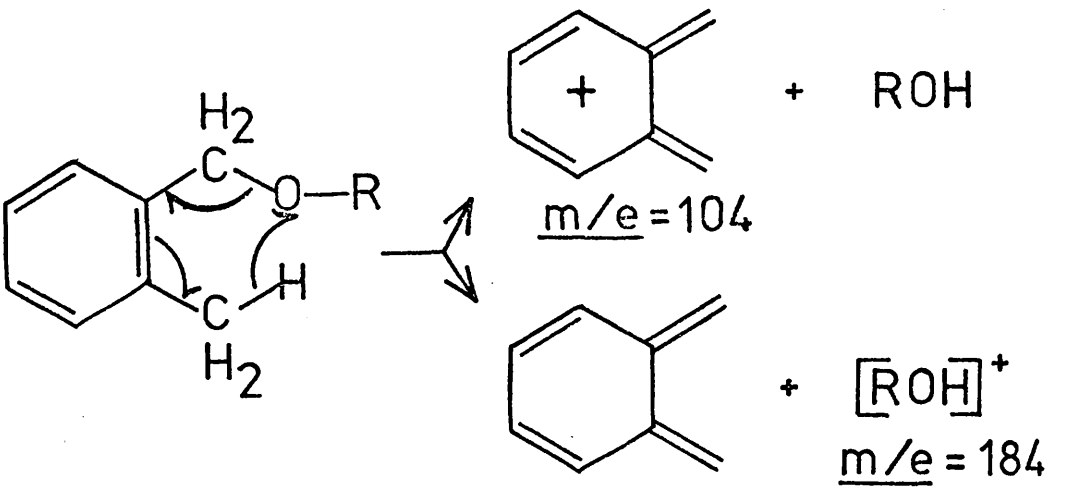
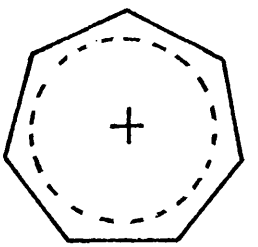
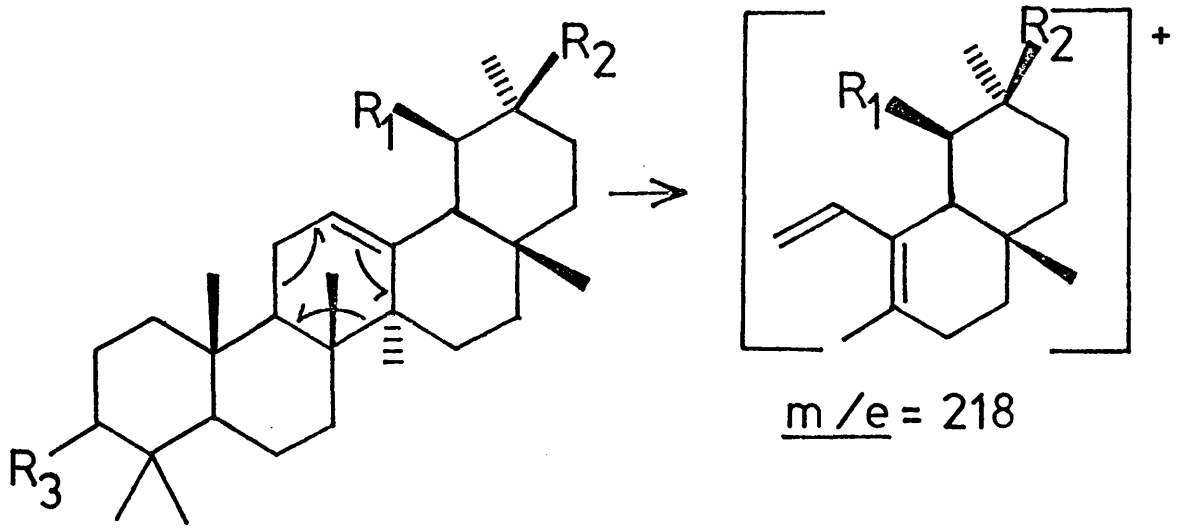


Fig. XXIII



CCXIX



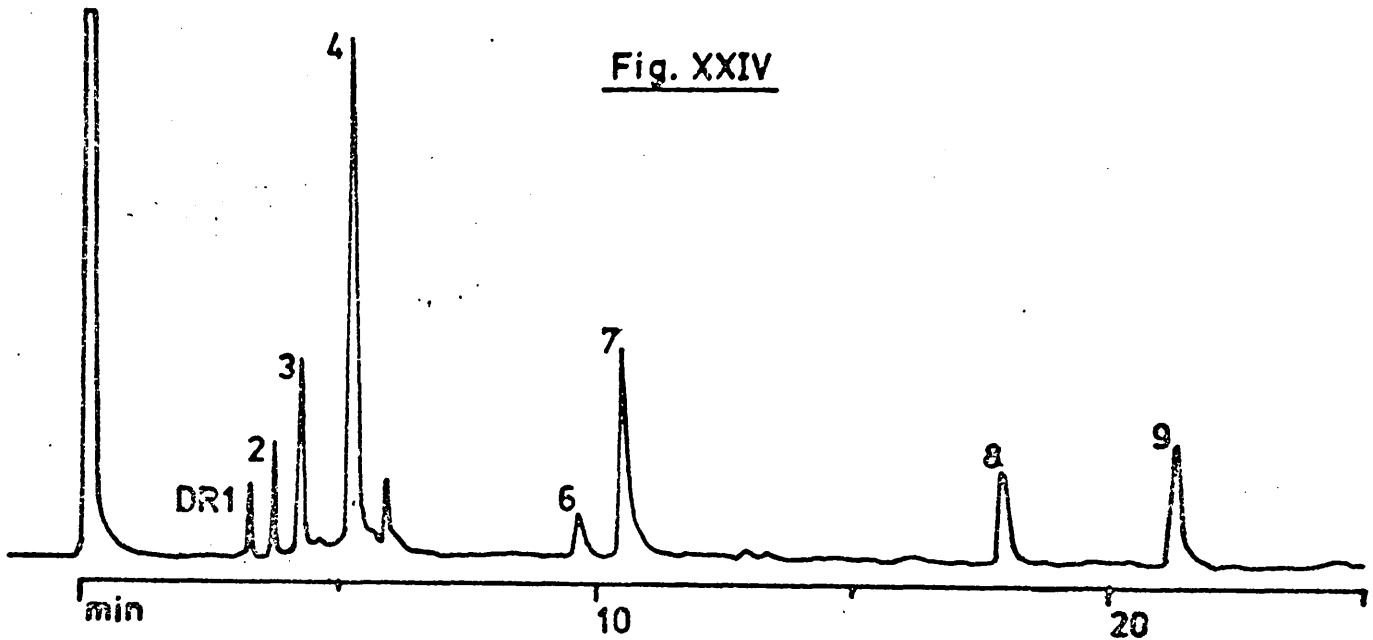
$R_1=CH_3$	$R_2=H$	$R_3=H$	CCXX
H	CH_3	H	CCXXI
CH_3	H	OH	CCXXII

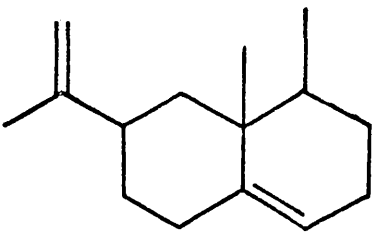
spectra suggest the fragmentation of long chain compounds and so they seem to be either straight chain hydrocarbons or straight chain alcohols of the general formula $CH_3(CH_2)_nCH_2OH$ (216). When a TLC plate, developed in chloroform, was run of the extract, these compounds were found in a band of R_f 0.3 - 0.4, along with GL3. This suggests that the compounds are alcohols. Although the molecular ions were not observed the formulae can be arrived at from the knowledge that $C_{24}H_{49}OH$ has a retention index on 1% OV-1 of 2600 (180). Thus the formulae can be assigned as GL4 - $C_{22}H_{45}OH$, GL5 - $C_{23}H_{49}OH$, GL6 - $C_{24}H_{49}OH$, GL8 - $C_{28}H_{57}OH$ and GL9 - $C_{29}H_{59}OH$. These compounds are common components of leaf waxes (216).

Compound GL4 has a molecular weight of 288. It is unaffected by attempts to trimethylsilylate it or to form a methyl oxime. It has an R_f of 0.9, suggesting that it is either a hydrocarbon, or an extremely non-polar oxygenated compound such as an ether, giving the alternative formulae $C_{21}H_{36}$ or $C_{20}H_{32}O$. The base peak at m/e = 104 and the M^+ - 104 peak at m/e = 184, might arise from the fragmentation as shown in Figure XXIII (217). The presence of an aromatic ring is further supported by the ion at m/e = 91 (CCXIX) which arises by rearrangement of the ring (217b).

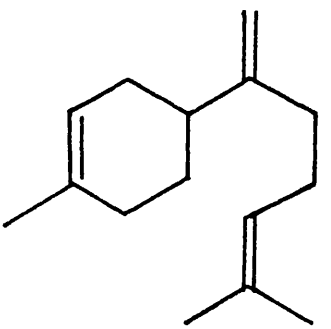
The last compound, GL10, has a molecular weight of 408 and a base peak of m/e = 218. This latter ion is characteristic of unsaturated triterpenes, such as α-(CCXX) and β-amyrene (CCXXI) or α-amyrin (CCXXII) (218), arising from a retro Diels-Alder reaction to give the ion as shown. This fragmentation is specific for triterpenes having a double bond in this position (218, 219).

Fig. XXIV





CCXIV



CCXXIII

4 - 2 - 3 Ligularia 'Desdemona'

Extraction of the roots of this plant gave a solution which was examined by GLC and GC-MS with the results summarised in Figure XXIV and Table 30.

Compounds DR1, 2 and 3 are all sesquiterpene hydrocarbons of the formula $C_{15}H_{24}$, and have mass spectra which are similar, particularly in the occurrence of the ion at m/e = 189, which is due to the loss of a $-CH_3$ group from the molecular ion.

Compound DR4 is another sesquiterpene hydrocarbon with the formula $C_{15}H_{24}$. The retention index and mass spectrum of this compound match those of eremophilene (173, 177, 180) (CCXIV), which was also present in both L.'Greenmog Gold' and L.clivorum.

The last of the sesquiterpene hydrocarbons DR5 has a mass spectrum which matches that of β-bisabolene (CCXXIII) (172, 175) which has a retention index of 1497 (158). While not exactly the same, the retention indices lie within the limits of experimental error, hence the identification is almost certain.

Compound DR6 has a molecular weight of 220. The mass spectrum shows ions due to the loss of 15 and 18 mass units from the molecular ion i.e. to loss of $-CH_3$ and H_2O respectively, implying that the compound is an alcohol. Trimethylsilylation results in the disappearance of the peak and the appearance of a peak at 1690 index units. The compound does not form a methyl oxime, and so it is an alcohol, formula $C_{15}H_{24}O$.

Compound DR7 gives no response to trimethylsilylation, but it does form a methyl oxime with a retention index of 1760, therefore it contains a carbonyl group. Its molecular weight is 222, so its formula is presumably $C_{15}H_{26}O$.

TABLE 30

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
DR1	1310	204 (44)	41	43(97)	91(86)	105(79)
				147(73)	55(63)	189(60)
DR2	1340	204 (18)	189	41(33)	119(32)	162(27)
				147(27)	105(23)	91(23)
DR3	1370	204 (23)	41	69(84)	93(67)	101(58)
				91(52)	189(48)	55(49)
DR4	1460	204 (26)	93	41(94)	91(60)	55(63)
				79(60)	77(50)	105(50)
				107(45)		
DR5	1480	204 (15)	69	41(78)	93(69)	67(30)
				43(28)	79(27)	94(25)
DR6	1640	220 (16)	43	133(64)	41(62)	93(59)
				69(47)	91(44)	95(42)
DR7	1670	222 (5)	179	43(10)	161(7)	180(6)
				222(5)	69(4)	41(3)
DR8	1980	284 (6)	88	101(52)	43(42)	41(28)
				55(27)	57(23)	73(17)
DR9	2150	308 (6)	67	81(84)	55(69)	95(52)
				79(47)	41(45)	54(40)

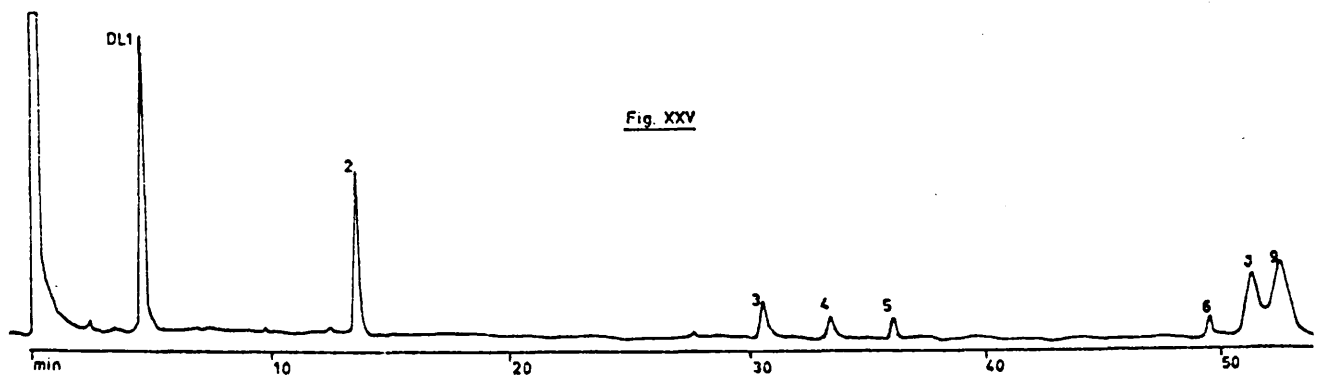
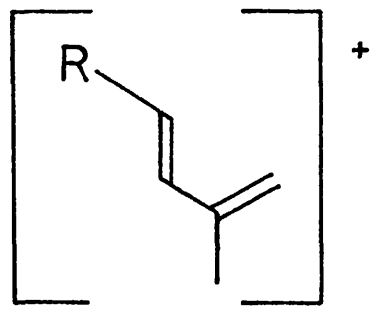


Fig. XXV



CCXXIV

R = H m/e = 68

CH_3 82

The base peak m/e = 179 is very large with respect to the rest of the spectrum, suggesting a very stable ion. It is formed by loss of 43 units from the molecular ion, which could be caused either by the loss of an isopropyl unit, $HC(CH_3)_2$, or an acetyl radical $CH_3CO^{\bullet}$.

The two compounds DR8 and 9 have the same GLC and GC-MS properties as the two fatty acid ethyl esters, found in the extracts of L.clivorum and L.'Gregynog Gold'. The presence of these esters in these three, and in subsequent extracts suggests that they may be impurities introduced into the sample at some stage of the extraction procedure. A fresh extract of L.'Desdemona', made under the same conditions as before, showed no trace of these compounds, though all the others were present, so it would seem that they can be disregarded as impurities.

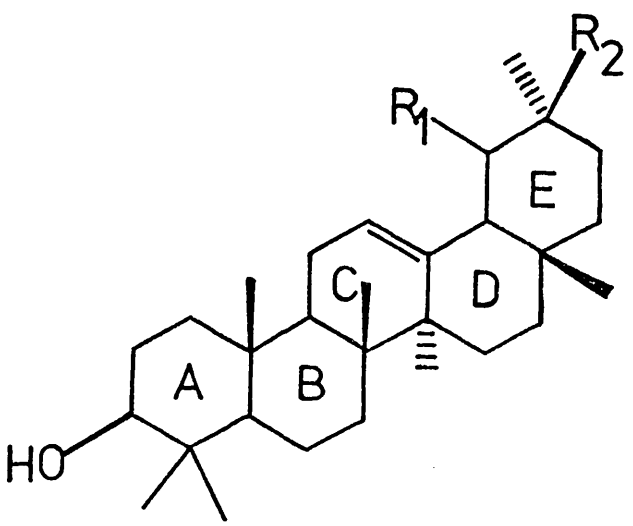
Examination of the leaf extract gave the data summarised in Figure XXV and Table 31. The first compound examined, DL1, has a molecular weight of 222, which implies the formula $C_{15}H_{26}O$. The mass spectrum shows no $(M-18)^+$ peak due to loss of H_2O, and attempted trimethylsilylation and methoximation were unsuccessful. Thus it is unlikely that the compound is either an alcohol or a ketone.

The same is true of compound DL2, from which no derivatives were obtained. This compound has a mass spectrum in which the low mass ions predominate, suggesting that it may be a long chain hydrocarbon. The molecular weight, 278, would then suggest the formula $C_{20}H_{38}$. The base peak m/e = 68 and the m/e = 82 peaks may be due to the ions shown (CCXXIV).

Compounds DL3, 4 and 5 have the retention indices and mass spectra of the long chain alcohols $C_{22}H_{45}OH$, $C_{23}H_{47}OH$ and $C_{24}H_{49}OH$ which were found in the leaf extract of L.'Gregynog Gold'. Compound DL6 however, has a mass spectrum which does not match that of the alcohol with retention index 3100, $C_{29}H_{59}OH$. Examination of the extract by TLC, with chloroform as mobile phase showed that while compounds DL3, 4 and 5 had an R_f of 0.25, compound DL6 showed an R_f of 0.85. Thus

TABLE 31

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
DL1	1480	222 (6)	207	41(96)	55(82)	43(79)
				81(63)	69(48)	95(48)
DL2	1810	278 (2)	68	43(86)	57(49)	41(72)
				55(71)	82(68)	95(63)
DL3	2400	-	43	57(95)	55(89)	83(75)
				69(71)	97(63)	41(55)
DL4	2500	-	43	57(93)	55(87)	83(75)
				69(70)	97(65)	41(52)
DL5	2600	-	69	43(69)	57(69)	81(64)
				55(56)	83(42)	97(37)
DL6	3100	-	43	57(72)	221(67)	178(60)
				55(52)	41(47)	71(46)
DL7	3220	414 (30)	43	55(75)	41(56)	57(55)
				81(54)	95(51)	69(46)
DL8	3230	424(6)	218	55(33)	43(32)	41(28)
				69(20)	95(26)	81(22)


R2
R1
E
C
D
A
B
HO

compound DL6 is probably the n-alkane, $C_{31}H_{64}$.

While the more intense peaks in the mass spectrum of compound DL7 occur at fairly low m/e values, there are a number of peaks in the region above m/e = 200 which are diagnostic, namely the molecular ion m/e = 414, and peaks at m/e = 399, 396, 329, 303, 273, 255 and 213. All of these peaks match those of β-sitosterol (220) as does the retention index, which, for the sterol, is 3220 on 1% OV-1 (180).

Compound DL8 has the m/e = 218 base peak which is a characteristic of the α- and β-amyrins (213, 219). The molecular weight, 424 implies that this is an oxygenated compound, having one oxygen more than the amyrenes, in the form of a hydroxyl since the peak disappeared on trimethylsilylation, though no new peak appeared. The substitution has not affected the base peak so it cannot be on rings C, D or E. Thus the compound is substituted on Ring A or B, and is probably α-amyrin (CCXXII) or β-amyrin (CCXXV).

CCXXII $-R_1 = CH_3$ $R_2 = H$

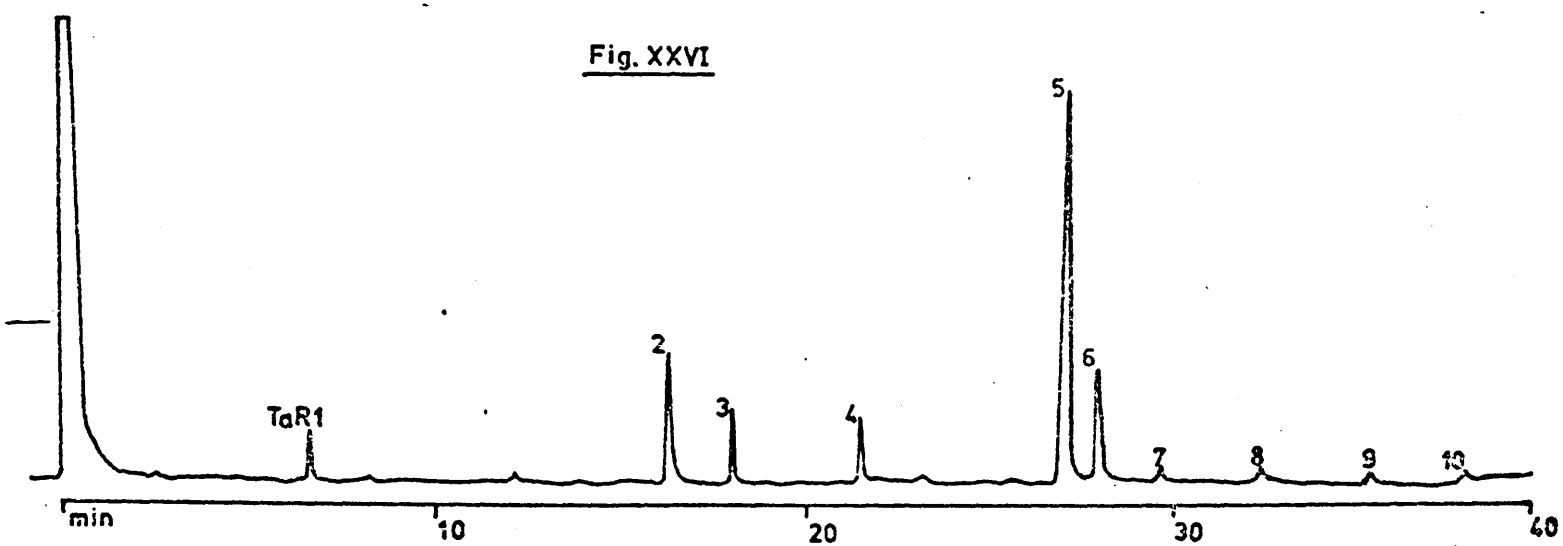
CCXXV $-R_1 = H$ $R_2 = CH_3$

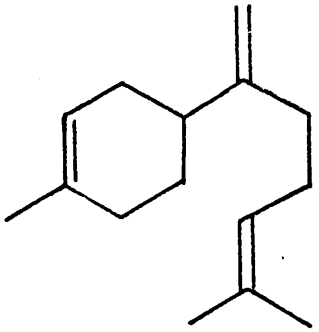
The retention index matches that of α-amyrin.

The relationship between L.'Desdemona', L.clivorum and L.'Gregynog Gold' shows in the similarity of their chemical components, and especially by the fact that the major compound present in the root extract of each of the plants is eremophilene. This compound is also known to occur in various members of the family Senecioneae (221), though not in any of the other Compositae, so the familial relationship of the genus Ligularia to the genera Petasites and Euryops can be established both by taxonomy and chemotaxonomy.

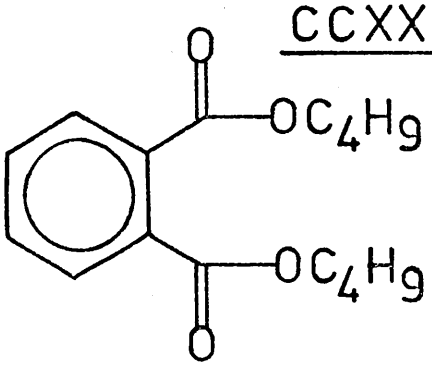
There are a number of compounds present in these extracts which could not be identified by GLC and GC-MS. Determination of the structures of these, might however be possible by larger scale extraction of the plant tissue, and purification of a sufficient sample of each compound to allow spectroscopic analysis.

Fig. XXVI

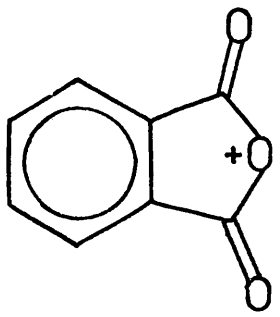




CCXXIII

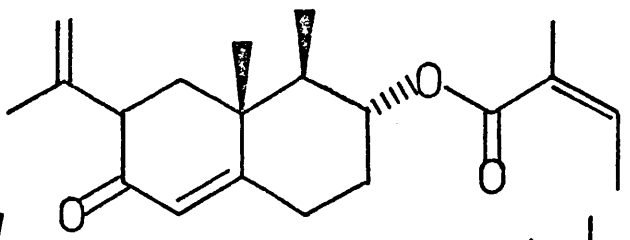


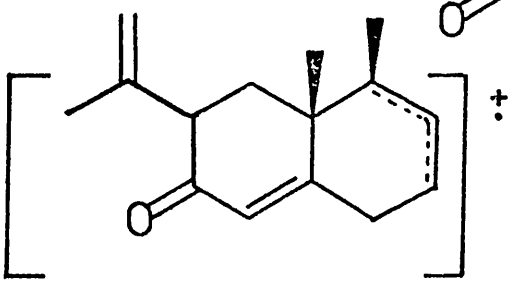
CCXXVI



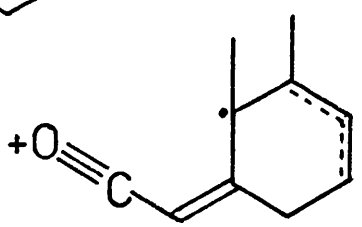
CLXXXVII

CLVII



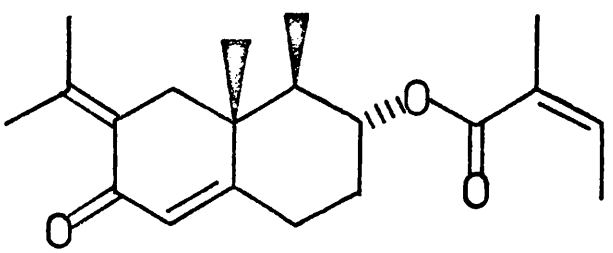


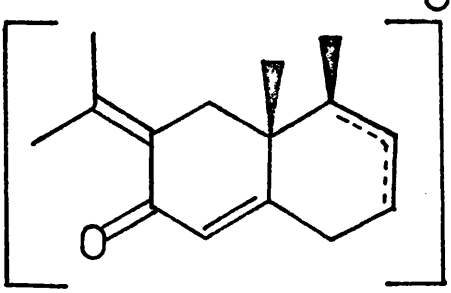
CCXXVII



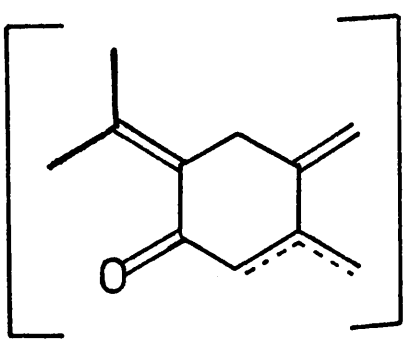
CC XXVI

CLVIII





CCXXIX



CCXXVIII

4 - 2 - 4 L.tangutica

At the time when samples were collected there was very little leaf growth on this plant, so only root material was collected and extracted. This gave the results summarised in Table 32, and Figure XXVI.

Compound TaR1 is a sesquiterpene hydrocarbon, formula $C_{15}H_{24}$. The ions at m/e = 69, 67, 81 and 109 form a distinctive pattern that is not matched exactly by any of the recorded spectra (Appendix III), but it does closely resemble the spectrum of bisabolene (CCXXIII) (173), while its retention index is close to that of β-bisabolene which is 1497 (158).

Compound TaR2 is di-butyl phthalate (CCXXVI), the base peak in the mass spectrum being due to the ion CLXXVII, and the $(M + 1)^+$ ion giving a peak at m/e = 279 (197). The retention index of the compound also matches that of di-n-butyl phthalate, which is 1910 on 1% SE-30 (197).

Compounds TaR3 and 4 are the fatty acid ethyl esters which have been found, as impurities, in other extracts.

Compound TaR5 has a molecular weight of 316 and a base peak m/e = 148 (CCXXVI), characteristic of petasin (CLVII) (180). The ion at m/e = 216 (CCXXVII) is due to loss of the elements of the acid from the ester. The retention index of this compound also suggests that it is petasin, the retention index of which has a value on 1% SE-30 of 2305 (180). Similarly, compound TaR6 has the retention index and mass spectral properties of isopetasin (CLVIII) (180). The base peak m/e = 161 is due to the ion CCXXVIII and the m/e = 216 ion again arises from the loss of the elements of the acid (CCXXIX).

TABLE 32

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
TaR1	1500	204 (12)	41	43(86)	69(73)	67(67)
				55(56)	109(54)	81(49)
				79(46)	93(41)	
TaR2	1900	279(M+1) (7)	149	67(59)	41(52)	81(51)
				43(47)	95(39)	
TaR3	1985	284 (9)	88	101(55)	43(39)	46(28)
				55(29)	57(23)	
TaR4	2160	308 (6)	55	67(98)	41(87)	81(83)
				95(64)	43(68)	
TaR5	2300	316 (13)	148	55(59)	57(48)	43(43)
				71(32)	161(28)	216(23)
TaR6	2345	316 (10)	161	55(73)	148(67)	83(65)
				41(45)	216(43)	105(59)
TaR7	2400	338 (1)	57	43(77)	71(64)	85(43)
				55(41)	41(37)	69(23)
				83(23)		
TaR8	2500					
TaR9	2600					
TaR10	2700					

Compound RaR7 shows a mass spectrum in which the ions of high intensity are of low m/e value, suggesting a straight chain hydrocarbon. The molecular weight, 338, and the retention index of 2400 suggest that the compound has the formula $C_{24}H_{50}$. Further peaks of retention index 2500, 2600 and 2700 which are probably the C_{25}, C_{26} and C_{27} n-alkanes suggest that the homologous series may be impurities introduced either during the extraction, or onto the plant material, in, for example, pesticides or fertiliser. The last three of these compounds were not scanned in the mass spectrometer, because of the small amount of each present in the extract.

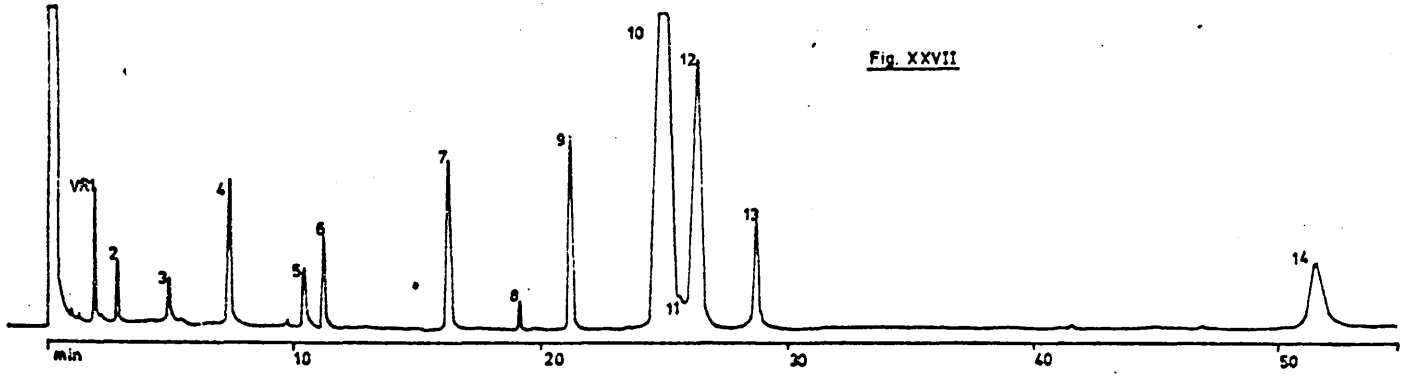
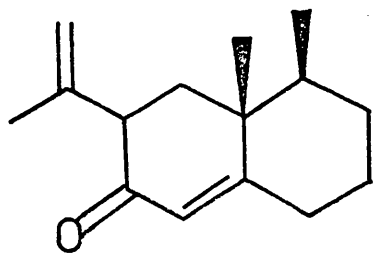
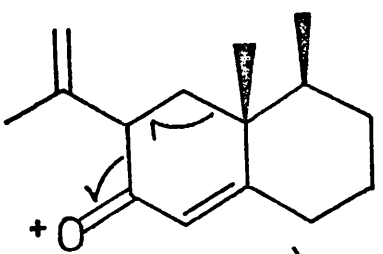


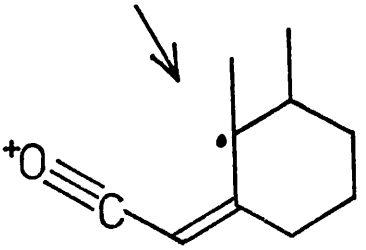
Fig. XXVII



CCXXX

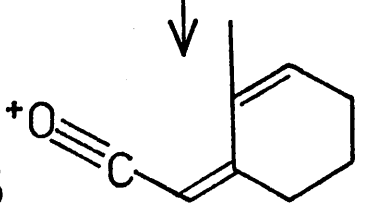


M+

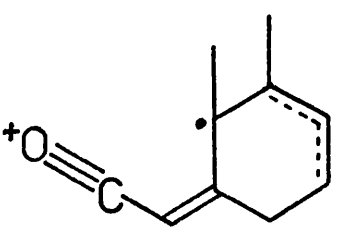


m/e=150

m*=121.8



m/e=135



CCXXVI

Fig. XXVIII

4 - 2 - 5 Ligularia veitchiana

Both root and leaf material of this plant were available, so both were extracted and examined. The results obtained for the preliminary extract of the root material are summarised in Figure XXVII and Table 33.

Compound VR1 is a sesquiterpene hydrocarbon, formula $C_{15}H_{24}$, and has a retention index and mass spectrum which match those of compound DR2, found in the root extract of L.'Desdemona'.

Compound VR2 is also a sesquiterpene, and again the retention index and mass spectrum match those of a hydrocarbon which occurs in L.'Desdemona' root material, namely compound DR5, which was tentatively identified as β-bisabolene.

Compound VR3 gives a mass spectrum in which the highest ion is at m/e = 204, but its retention index seems too high for a sesquiterpene hydrocarbon. Trimethylsilylation of the extract results in the disappearance of the peak due to this compound on the GLC trace, and the appearance of a new peak at 1650 index units. Methoximation proved unsuccessful. The compound is, therefore, a sesquiterpene alcohol, and the peak at m/e = 204 is due to the $(M - 18)^+$ ion, making the molecular weight 222 and the formula $C_{15}H_{26}O$. The base peak, m/e = 59 occurs in the spectra of both eudesmol and guaiol (175), but the rest of the spectra of these compounds do not match that of compound VR3.

Compound VR4 has a molecular weight of 218 which suggests the formula $C_{15}H_{22}O$. The mass spectrum of the compound matches that of eremophila-9, 11-dien-10-one (CCXXX) (177), the differences in relative intensity of a number of peaks resulting from the fact that the original mass spectrum was obtained with an accelerating voltage of 20eV, while that of compound VR4 was obtained at 70eV. The fragmentation pattern of the molecular ion (Fig. XXVIII) parallels that of

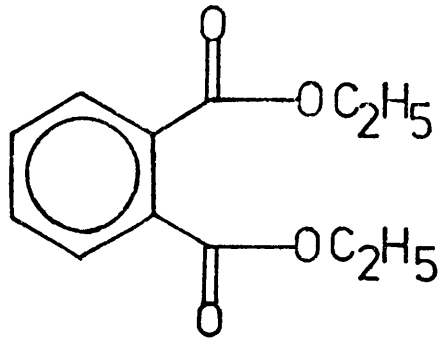
TABLE 33

Compound	$I_{1,SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
VR1	1345	204 (23)	189	119(46)	41(45)	91(39)
				43(38)	147(34)	162(30)
				161(27)		
VR2	1485	204 (22)	69	41(63)	93(62)	43(51)
				55(44)	204(22)	94(21)
VR3	1590	-	59	67(67)	43(60)	41(45)
				81(34)	55(33)	95(29)
VR4	1695	218 (24)	135	150(47)	41(32)	55(22)
				69(19)	91(18)	79(17)
				203(13)		
VR5	1760	223 (M+1) (7)	149	57(30)	41(15)	69(11)
VR6	1890	-	118	146(91)	214(37)	90(23)
				41(20)	89(17)	
VR7	1985	284 (8)	88	101(53)	43(38)	41(29)
				55(27)	57(26)	157(15)
VR8	2090	290 (11)	148	57(53)	41(43)	43(41)
				55(39)	105(30)	161(18)
VR9	2150	308 (5)	67	81(77)	41(72)	55(72)
				95(56)	68(42)	79(41)
				59(41)		
VR10	2255	316 (7)	148	55(64)	105(34)	41(24)
				161(23)	216(20)	83(13)
VR11	2280					
VR12	2300	316 (9)	148	83(55)	55(49)	161(37)
				216(30)	105(29)	41(24)
VR13	2400	288 (48)	104	91(79)	184(46)	155(45)
				79(35)	41(34)	80(34)
VR14	3220	414 (20)	43	55(75)	57(67)	41(60)
				81(51)	69(47)	95(44)

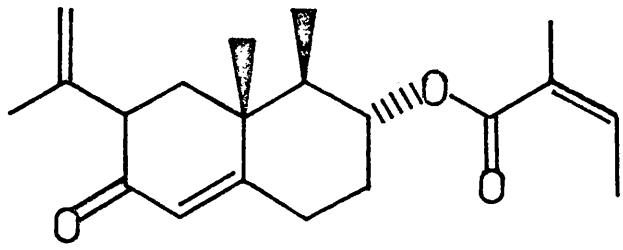
TABLE 33 Contd.

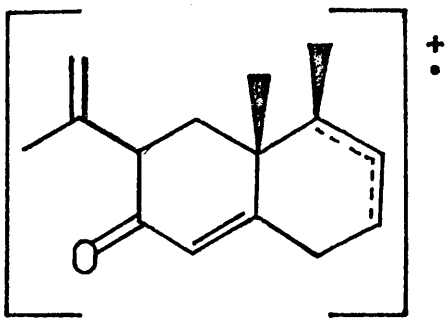
Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
VR12	2300	316 (9)	148	83(55)	55(49)	161(37)
				216(30)	105(29)	41(24)
VR13	2400	288 (48)	104	91(79)	184(46)	155(45)
				79(35)	41(34)	80(34)
VR14	3220	414 (20)	43	55(75)	57(67)	41(60)
				81(51)	69(47)	95(44)

CCXXXI

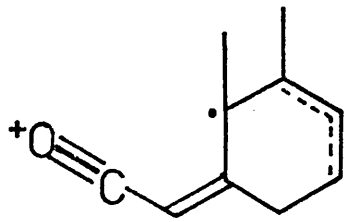


CLVII

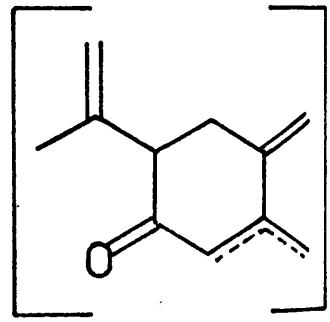




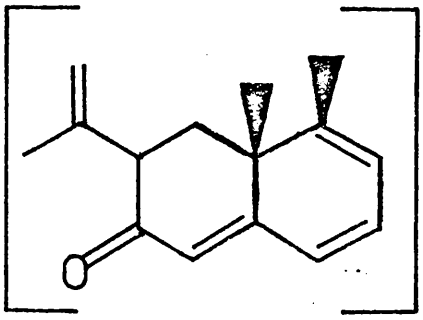
CCXXVII



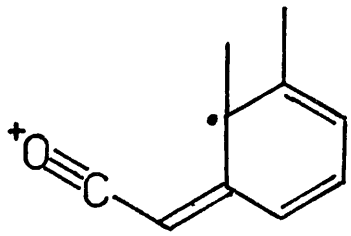
CCXXVI



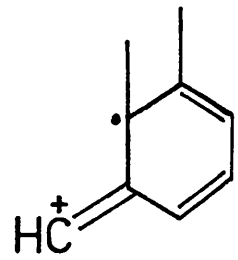
CCXXVIII



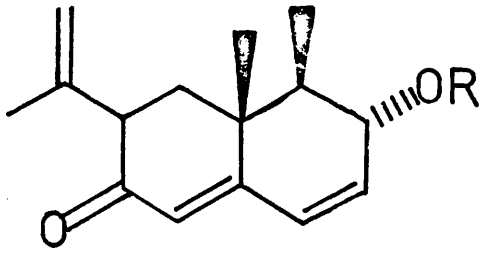
CCXXXII



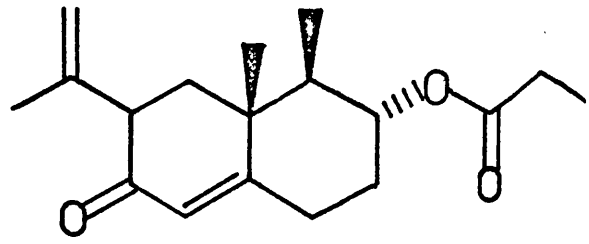
CCXXXIII



CCXXXV



CCXXXIV



CCXXXVI

petasin, giving a similar ion, at m/e = 150, to the most intense ion in petasin at m/e = 148 (CCXXVI). The breakdown of this m/e = 150 ion to give the intense ion at m/e = 135 (Fig. XXVIII), is supported by the presence of a metastable ion at m/e = 121.8 in the spectrum. This compound was reported as being a constituent of P.hybridus (180).

The base peak of compound VR5, m/e = 149, is characteristic of phthalates (197). The highest ion in the spectrum, at m/e = 223 is due to the $(M + 1)^+$ ion, indicating that the compound is di-ethyl phthalate (CCXXXI), or an isomer.

In the mass spectrum of compound VR6 the highest ion observed is that of m/e = 214, while there is also a major ion at m/e = 146. These two ions suggest the fragmentation of petasin (CLVII), where there are ions at m/e = 216 (CCXXVII) and 148 (CCXXVI) (180). To maintain the difference of 2 mass units between the corresponding peaks of the compounds, compound VR6 would have to have a double bond between carbons 6 and 7 as shown in structures (CXXXII) and (CCXXXIII), and so the original structure would be that shown (CCXXXIV). The base peak, m/e = 118 (CCXXXV) would arise by loss of CO from the m/e = 146 ion, and would probably be much more stable than the corresponding m/e = 120 ion of petasin through rearrangement to an aromatic form. The extra double bond in ring A would also interfere with the breakdown of this ring to give the ion corresponding to that of m/e = 161 (CCXXVIII). Since the molecular ion was not observed, the acyl group cannot be identified, but judging from the retention index it had a low molecular weight, and may have been due to either formic or acetic acid.

Compounds VR7 and 9 are the fatty acid esters encountered in almost all the extracts, and discounted as artefacts because of this.

Compound VR8 has the base peak m/e = 148 (CCXXVI), and a peak at m/e = 216 (CCXXVII) distinctive for petasin esters. The molecular ion, m/e = 290, suggests that the compound is the ester of propionic acid (CCXXXVI). The difference between the retention index of this

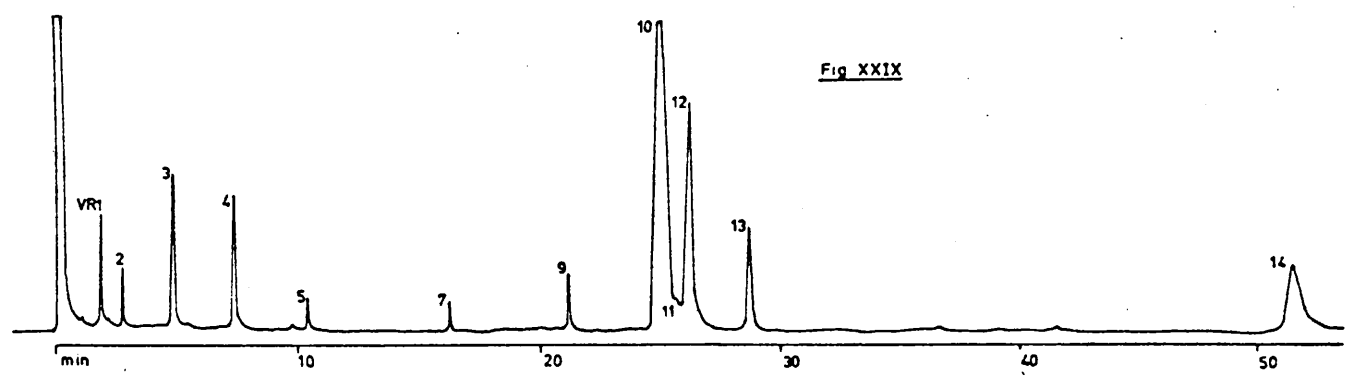
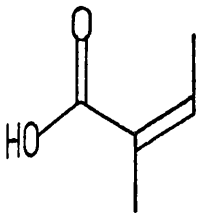
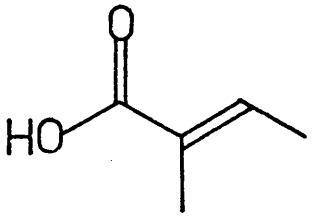


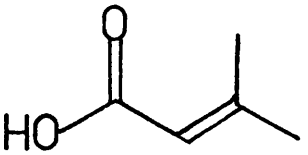
Fig XXIX



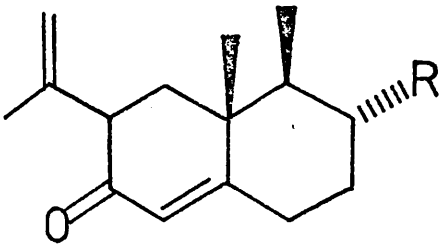
CXC

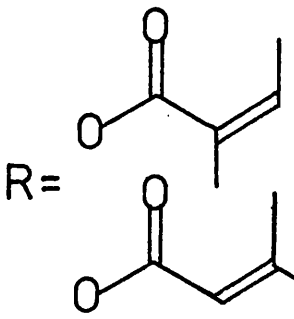


CXCIII



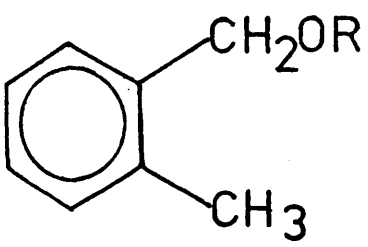
CXCII



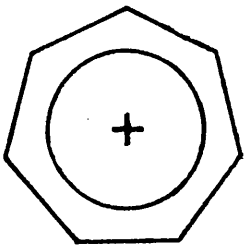


CLVII

CCXXXVII



CCXXXVIII



CCXIV

compound and that of VR6 is 200 index units, which supports the suggestion that the acyl group on the latter compound has one or two fewer carbons.

Compounds VR10 and 12 also have the mass spectra of petasin esters, but the molecular weights of these two compounds, both 316, imply that they are esters of unsaturated C_5 acids, such as angelic (CXC), tiglic (CXCIII) or dimethylacrylic acids (CXCII) (180). The retention indices of the compounds match those of the compounds in P.hybridus tentatively identified as the petasol ester of dimethylacrylic acid (CCXXXVII) and petasin (CLVII) respectively.

Between these two compounds on the GLC trace is a third with a retention index of 2280. This was not present in sufficient quantity, in this extract, to allow a scan of it to be made in the mass spectrometer.

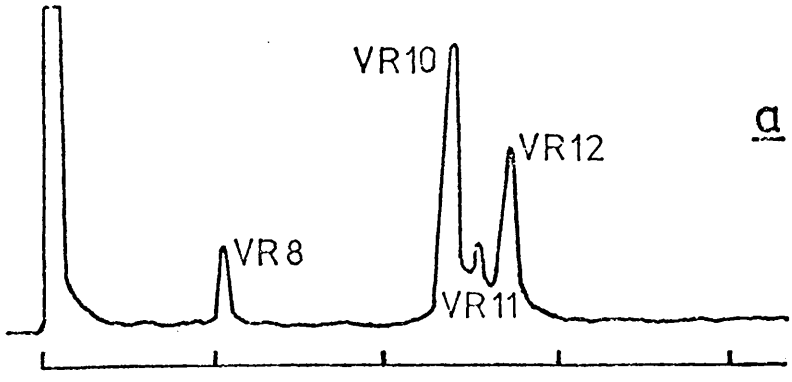
Compound VR13 matches compound GL4 which was found in the leaf extract from L.'Gregynog Gold'. This compound was assigned the general structure CCXXXVIII because of the base peak and the tropylium ion at m/e = 91 (CCXVII) (217a, b).

The molecular weight, the retention index and the pattern of peaks above m/e = 210 in the mass spectrum of compound VR14 match those of β-sitosterol (220).

Examination of the Petasin Esters

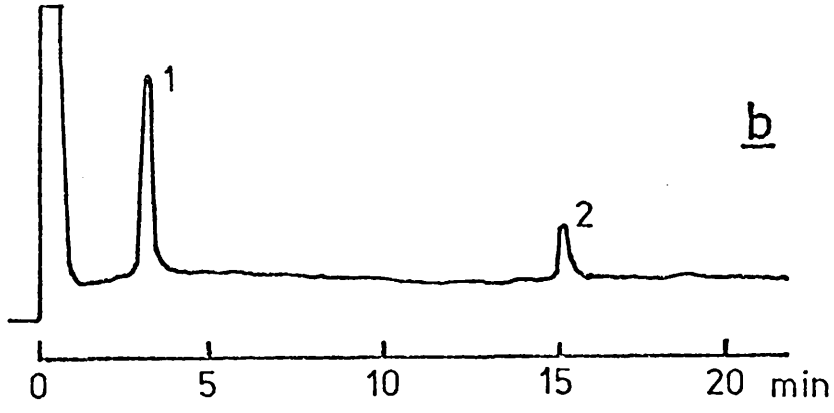
In order to obtain more information on the petasin esters found in the root material of this plant, a much larger sample was obtained from Logan Gardens, and extracted in the same way as before and examined by GLC. A comparison of this trace with that previously obtained, Fig. XXIX, shows differences in the intensities of a number of the peaks present and the absence in the second extract of compounds VR6, 7 and 8. The last two compounds were believed to be impurities, so their absence is not surprising. The other differences may be seasonal,

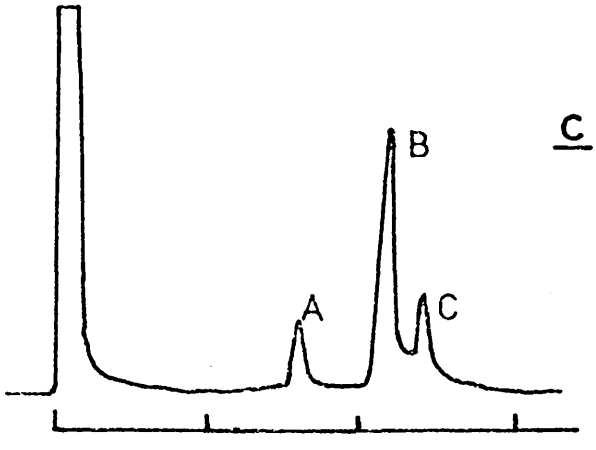
Fig. XXX



10ft., 1% SE-30,
145 C, N_2 30ml/min.

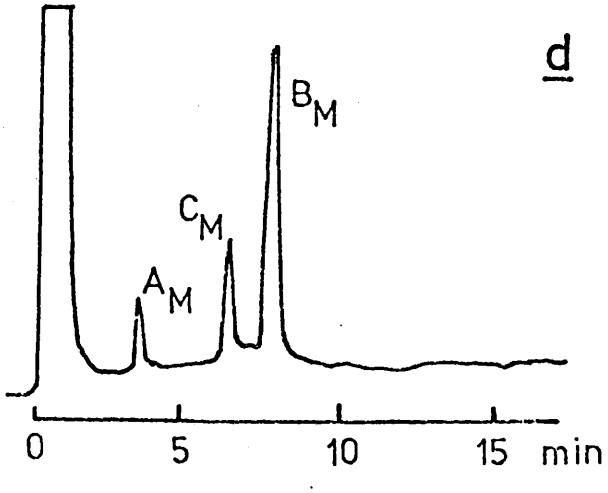
1 - isopetasol,
2 - isopetasin.





A - angelic acid,
B - dimethylacrylic acid,
C - tiglic acid.

10ft., 1% SE-30,
50 C, N_2 30ml/min.



A_M - methyl angelate,
B_M - methyl dimethylacrylate,
C_M - methyl tiglate.

The first batch of root material was obtained in January, 1973, while the second batch was collected in November of that year.

The extract obtained from the root material was then chromatographed on a reverse-phase liquid gel column (NH1114-60%-LH20, methanol/heptane 9:1) in order to isolate a fraction containing the petasin esters. These were eluted between SLV 110 and 190.

The esters were examined by GLC, and found to contain compounds VR8, 10, 11 and 12 (Fig. XXXa). This was verified by mass spectrometry, VR8, 10 and 12 giving the same mass spectra as before, and compound VR11 showing the base peak at m/e = 161 and the peak at m/e = 216 both characteristic of an ester of isopetasol (180). The molecular ion m/e = 316, suggests that the acid involved is one of the unsaturated C_5 acids, angelic, tiglic or dimethylacrylic. The retention index of the compound is too low for that of isopetasin itself, which would have a value of 2345, and from the tentative assignment of peaks in the extracts of P.hybridus and P.fragrans it is too low to be the ester of tiglic acid which has a retention index of 2330. This leaves only the possibility that it is the ester of dimethylacrylic acid, which was not found in either of the Petasites examined.

The esters were hydrolysed by treatment with base (ethanolic potassium hydroxide), and an ether extract was examined by GLC (Fig. XXXb). The two peaks present were identified as isopetasol and isopetasin from their retention indices which were 2010 and 2345 respectively, agreeing with those of standard samples obtained under the same conditions. Petasin is known to produce isopetasol on hydrolysis, due to a rearrangement under the reaction conditions (180) and so the isopetasin present presumably represents unreacted petasin which has also isomerised.

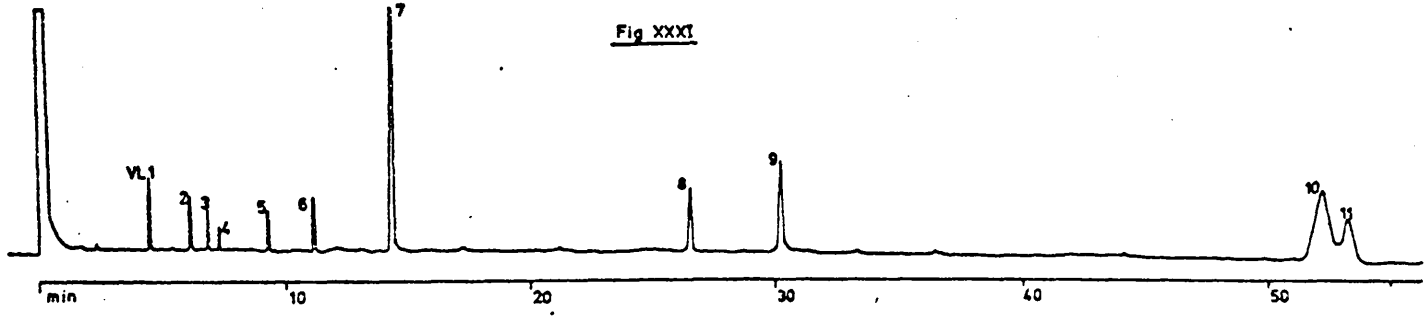
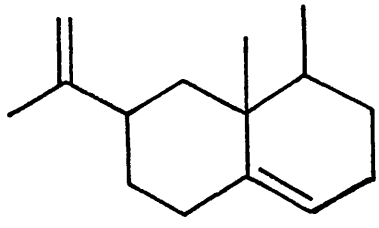
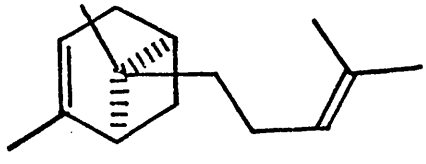


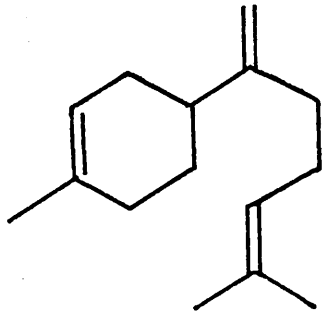
Fig XXXI



CCXIV



CCXVI



CCXXIII

The basic residue of the reaction mixture was acidified, extracted with ether and the dried ether solution was examined by GLC (Fig. XXXc). A portion of it was then treated with diazomethane and the methyl esters thus formed were also examined by GLC. The acids and their esters were identified by their retention indices and there was found to be a ratio of dimethylacrylic : tiglic : angelic acid of 6 : 2 : 1. Since the tiglic acid would arise from isomerisation of the angelic acid during the hydrolysis, this suggests an original ratio of dimethylacrylic : angelic acid of 2 : 1, which is consistent with the identification of compound VR12 as petasin and compound VR10 as the petasol ester of dimethylacrylic acid. The acid, with which petasol was combined to form compound VR8, and which was probably propionic acid, did not appear on the GLC trace under these conditions.

Examination of the Leaf Extract

The GLC and mass spectral data obtained for the leaf extract of L.veitchiana are summarised in Figure XXXI and Table 34.

Compounds VL1, 2, 3 and 4 have molecular weight of 204 and are, therefore, sesquiterpene hydrocarbons with the formula $C_{15}H_{24}$. VL1 shows a mass spectrum which matches that of one of the farnesenes (172), especially in the presence of the ion of m/e = 120, and its retention index is in the right region for one of these compounds, since β-farnesene is known to have a retention index of 1367 (158).

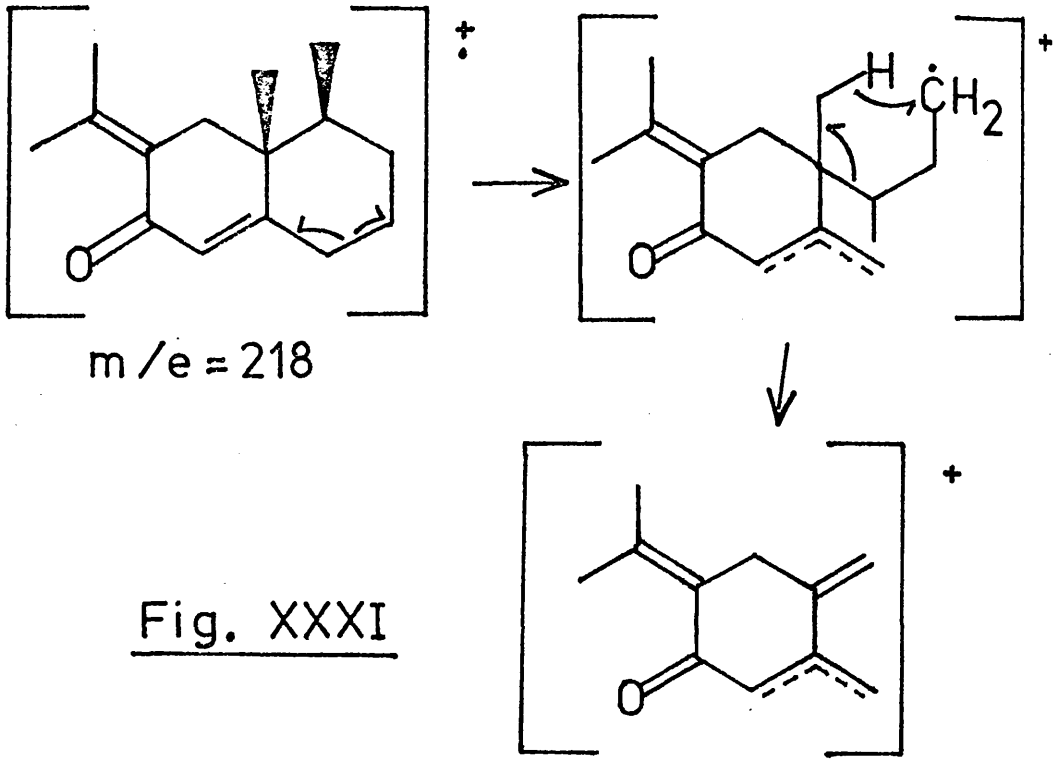
The retention index and mass spectrum of compound VL2 match those of the compound found in L.clivorum, L.'Gregynog Gold' and L.'Desdemona', which has been identified as eremophilene (CCXIV) (177, 180), while compound VL3 shows a close similarity to compound GL2, which was found in L.'Gregynog Gold' leaf material, and which has the mass spectral characteristics of α-bergamotene (CCXVI) (175). Lastly, compound VL4 can be identified as β-bisabolene (CCXIII) from both

TABLE 34

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
VL1	1390	204 (7)	41	69(85)	93(81)	79(53)
				91(52)	133(52)	55(49)
				100(29)		
VL2	1460	204 (22)	41	93(80)	79(70)	81(68)
				105(68)	107(67)	55(59)
				91(52)		
VL3	1485	204 (7)	93	41(98)	55(65)	119(64)
				69(59)	43(55)	79(48)
				81(48)		
VL4	1490	204 (13)	41	69(97)	93(79)	43(57)
				55(42)	44(36)	79(36)
				94(24)		
VL5	1605	236 (1)	43	41(69)	57(45)	55(44)
				59(44)	44(40)	91(31)
				161(27)		
VL6	1700	238 (7)	43	41(59)	55(54)	123(52)
				69(42)	29(39)	81(36)
				223(25)	205(21)	
VL7	1800	280 (3)	43	55(44)	205(40)	41(37)
				69(22)	81(21)	95(14)
				107(14)	105(14)	
VL8	2255	316 (11)	148	55(59)	43(36)	41(32)
				83(26)	105(25)	161(21)

TABLE 34 Contd.

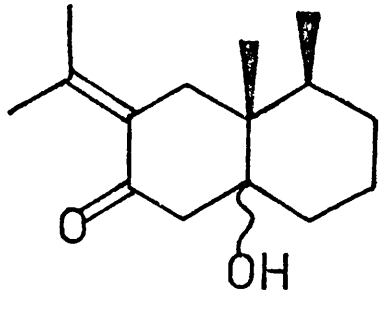
Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
VL9	2400	288 (51)	104	288(51)	184(49)	79(46)
				155(46)	80(42)	171(42)
VL10	3220	414	43	55(20)	41(60)	57(58)
				81(52)	69(50)	167(45)
VL11	3230	424	218	55(36)	43(34)	41(30)
				69(30)	81(25)	95(25)



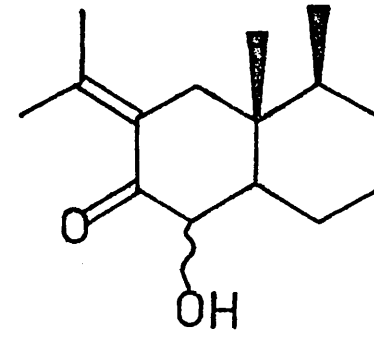
m/e = 218

m/e = 161

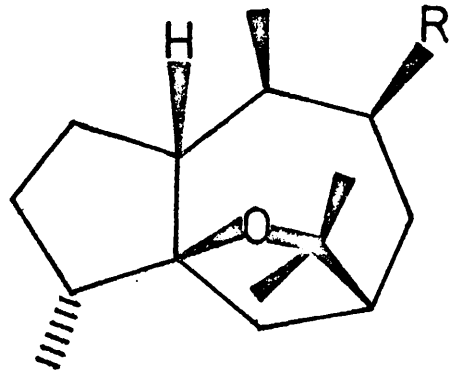
Fig. XXXI



CCXXXIX



CCXL



R = OH CXCVIII

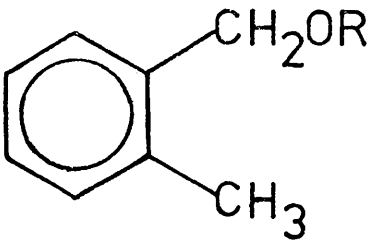
= OAc CXCIX

its retention index (158) and its mass spectrum (172, 175). This compound was also found in the extracts of L.'Desdemona' and L.tangutica.

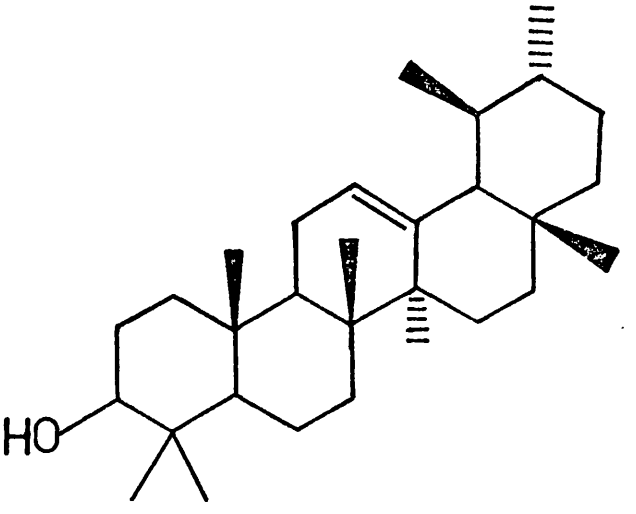
Compound VL5 forms a trimethylsilyl ether with a retention index of 1645. Its molecular weight suggests the formula $C_{15}H_{24}O_2$ which, in turn suggests an isomer of petasol or isopetasol which possesses two extra hydrogens. This is supported by the occurrence of the ion at m/e = 161, which could arise from an m/e = 218 ion also present in the spectrum by the process shown in Figure XXXI. In order to arrive at this m/e = 218 ion by elimination of a hydroxyl and hydrogen, the hydroxyl group would have to be situated on carbon - 8 (CCXXXIX) or carbon - 9 (CCXL). The ease of formation of the trimethylsilyl ether suggests the alcohol is not tertiary, supporting the 9-hydroxy isomer.

Compound VL6 also forms a trimethylsilyl ether, with a retention index of 1745. The compound has a molecular weight of 238, which suggests a formula of $C_{15}H_{24}O_2$. The strong M-15 peak, m/e = 223, is also seen in compound VL7, which is neither an alcohol nor a ketone, and in which it appears at m/e = 265, the molecular weight of this compound being 280. The strong ion in the spectrum of VL7 at m/e = 205 seems to arise by loss of 60 mass units from the M-15 ion, presumably due to the loss of the elements of acetic acid from this ion. If, then, this compound were an acetate it would be derived from an alcohol of molecular weight 238, such as VL6. The spectra of these compounds do show similarities, especially in the occurrence in each of the ion at m/e = 205, due in one to loss of CH_3 and acetic acid, and in the other to loss of CH_3 and H_2O. These compounds match the molecular weights of the compounds encountered in L.Fischeri, i.e. liguloxidol (CXCVIII) and liguloxidol acetate (CXCIX) (202, 203), but no further evidence for or against this possible correlation could be found.

Compound VL8 has the molecular weight and base peak of a petasol ester, and the retention index is that of the dimethylacrylic acid ester of petasol. Compound VL9 is that compound encountered in both the leaf extract of L.'Gregynog Gold' and the root extract of



CCXXXVIII



CCXXII

L.Veichiana and characterised as having the form CCXXXVIII.

Finally, compounds VL10 and 11 are respectively β-sitosterol, and α-amyrin (CCXXII) which was encountered in L.'Desdemona'.

The apparent absence of straight chain hydrocarbons or alcohols in the leaf extract of this plant, may be due to only a relatively small amount of these compounds being present. In the other plants examined, the peaks on the GLC trace due to these compounds were not very large when compared to those of the sesquiterpenoids present. In this plant, which is rich in sesquiterpenoid compounds of various types, any leaf waxes present might have given too minor a set of GLC peaks to allow their detection and examination.

The familial relationship between L.veitchiana and L.'Gregynog Gold', which is a daughter of it, is not as obvious from the chemistry of these plants as was the same relationship between L.'Gregynog Gold' and L.clivorum. It seems, therefore, that in the cross between L.veitchiana and L.clivorum, to produce L.'Gregynog Gold', L.clivorum is the dominant partner, at least from the point of view of the plant chemistry.

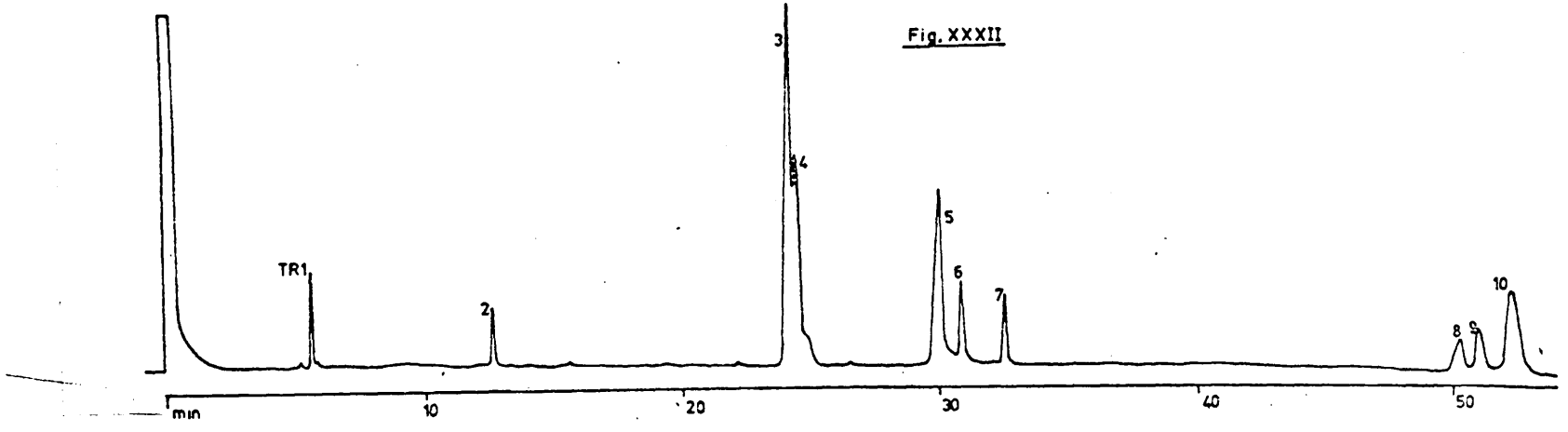
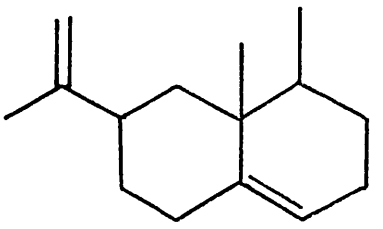
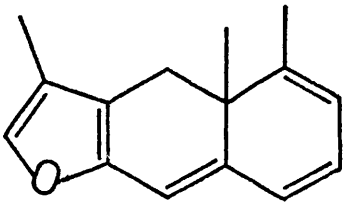


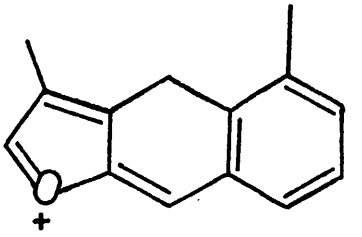
Fig. XXXII



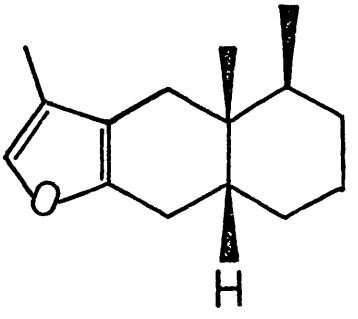
CCXIV



CCXLI



CCXLII



CLXVI

4 - 2 - 6 Ligularia tussilaginea

An extract of the root material of this plant was examined by GLC and GC-MS, and the results are summarised in Figure XXXII and Table 35.

Compound TR1 has the same retention index and mass spectrum as the compound previously identified as eremophilene (CCXIV).

Compound TR2 has a molecular weight of 212, which suggests a formula of $C_{15}H_{16}O$. The compound is inert to attempts to form either a methyl oxime or a trimethylsilyl ether. The base peak and the other major ions occur in the higher end of the mass spectrum, implying a fairly stable compound. Since furanoeremophilanes have been found in L.tussilaginea (198), it is possible that the compound has the structure CCXLI or a similar isomer. Loss of one of the $-CH_3$ groups would result in the ion of m/e = 197, which is the base peak. The stability of this ion would be accounted for by the isomerisation of its double bonds which would give an aromatic structure (CCXLII).

Compounds TR3 to 7 all contain the M-100 or M-x-100 ions characteristic of esters of unsaturated C_5 carboxylic acids such as angelic acid or dimethylacrylic acid. Their molecular weights range from 314 to 332, suggesting that they are in various stages of oxygenation. Since furanoid esters of angelic and dimethylacrylic acids have been shown to exist in this plant (198), it was assumed that these compounds were esters of such furanoids derived by different degrees of oxygenation from furanoeremophilane (CLXVI). In order to examine these compounds more closely it was decided that a larger sample of the root material would have to be extracted and the compounds separated by liquid gel chromatography.

Compounds TR8, 9 and 10 are all phytosterols, and have been identified from their retention indices and mass spectra as campesterol, stigmasterol and β-sitosterol respectively. These sterols have been assumed not to be the 24-epimers which would be indistinguishable by this method.

TABLE 35

Compound	$I_{1,SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
TR1	1460	209 (15)	41	93(80)	55(62)	91(55)
				43(54)	79(45)	107(40)
				105(40)	133(36)	
TR2	1770	212 (26)	197	169(38)	182(29)	212(26)
				154(30)	153(15)	43(13)
TR3	2215	316 (2)	83	159(14)	216(14)	55(13)
				41(8)	46(6)	109(6)
TR4	2220	314 (23)	159	83(68)	55(51)	214(40)
				199(34)	145(28)	43(15)
TR5	2370	332 (7)	109	105(72)	55(30)	124(27)
				83(18)	110(12)	43(10)
				214(8)		
TR6	2580	332 (6)	212	197(91)	124(81)	55(72)
				109(63)	83(51)	213(24)
				312(17)		
TR7	2410	312 (24)	197	212(71)	55(63)	83(56)
				312(24)	213(20)	187(18)
TR8	3140	400 (11)	43	83(88)	55(87)	57(70)
				41(58)	73(57)	
TR9	3165	412 (12)	69	55(87)	83(79)	43(57)
				41(52)	81(52)	
TR10	3220	414 (25)	43	55(81)	57(68)	41(58)
				69(46)	85(43)	

TABLE 36

Compound	$I_{1\%SE-30}$	M (% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
TR11	2590	330 (1)	83	55(31)	41(8)	84(7)
				230(3)		
TR12	2620	348 (1)	83	55(48)	228(30)	212(21)
				248(3)		
TR13	2805	362 (1)	83	55(47)	170(26)	110(26)
				262(2)		

Fig. XXXIII

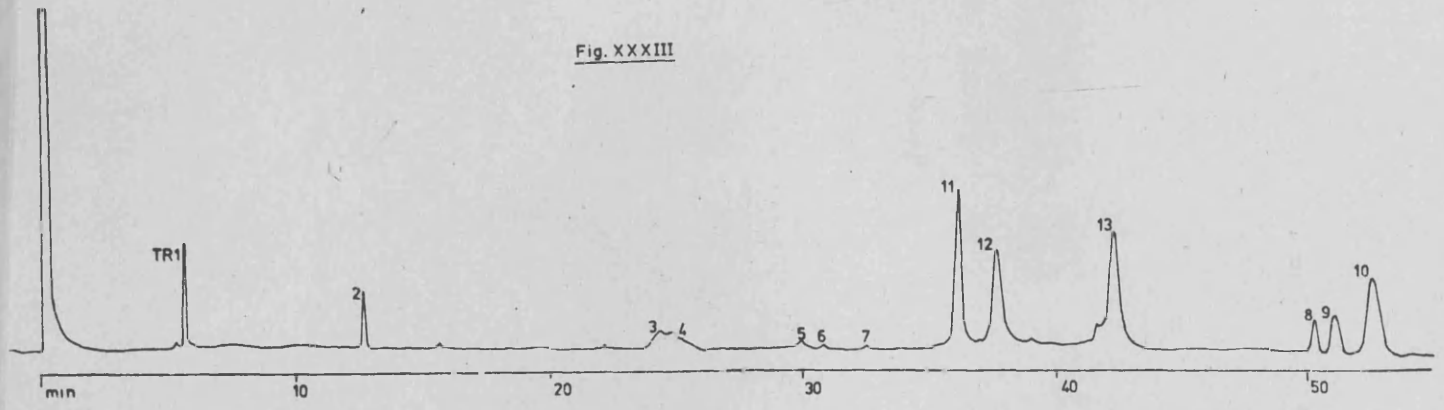
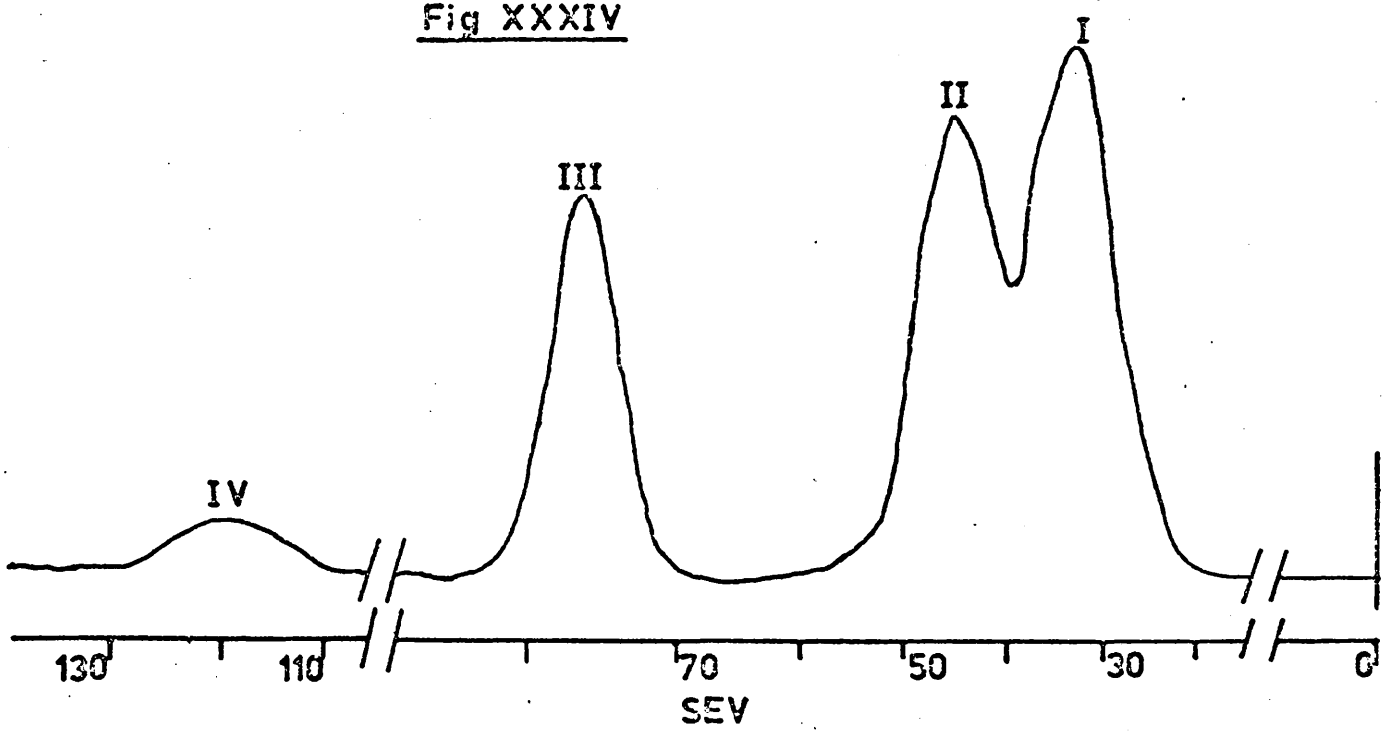


Fig XXXIV

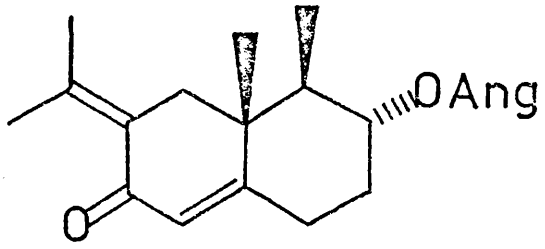


A re-examination of the extract, which had been kept under refrigeration for three weeks since the original trace was obtained, showed that the peaks due to the presumed furanoids were much smaller and that three new peaks were appearing at retention indices higher than those of the furanoids (Fig. XXXIII). The mass spectra and retention indices of these compounds are shown in Table 36. The molecular weights suggest that they are derived from the furanoids by oxidation, possibly of the furan ring, since each of them still shows the M-100 ion due to the loss of an unsaturated C_5 (acid) group. This tendency of the furanoids to oxidation, meant that great care had to be taken in the handling of both the extract and of any compounds isolated from it. After another two weeks in the refrigerator, the extract was found to contain none of the original furanoids, only the oxidation products derived from them.

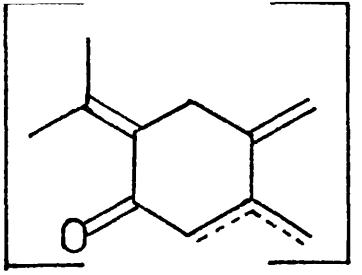
A fresh sample of the root material was extracted and examined by GLC. It was found to yield the same peaks as before, with the exception of TR2. The mass spectrum of that compound closely resembled that of compound TR7, so the former may have been an artefact, derived from the latter. The extract was chromatographed on a reverse phase liquid gel column (HH1518-60%-LH20, methanol/heptane 9:1), the eluate passing over the moving wire of a Pye Liquid Chromatograph Detector. The trace from the detector is shown in Figure XXXIV. Fractions from the column were collected at intervals corresponding to 1 SEV. These were combined to give the four larger fractions corresponding to the peaks on the detector trace.

Fraction II (SEV 42-52)

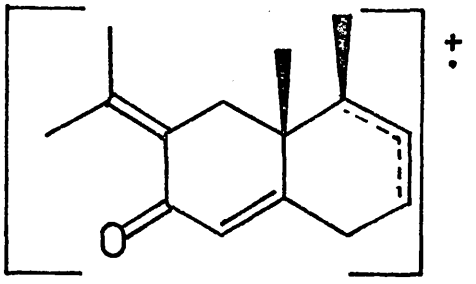
Compound TR4 was found in this fraction along with three other compounds which did not appear in the original extract. These three compounds were labelled TR4a, b and c. This mixture was chromatographed on a straight phase liquid gel column (HH1518-60%-LH20 benzene), and the fractions collected were analysed by GLC. Compound TR4 was found in the fractions of SEV = 66 - 68, and the other three compounds in the fractions of SEV = 70 - 74.



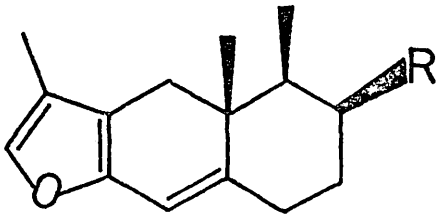
CLVIII



CCXXVIII

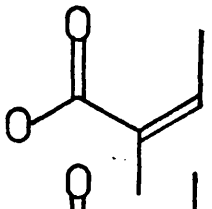


CCXXIX

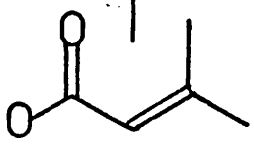


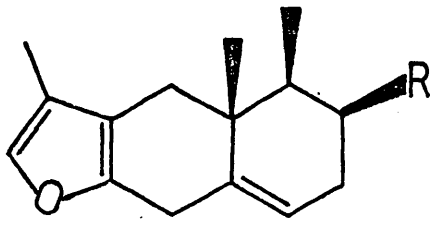
CCXLIII

a R=



b





=OAng a

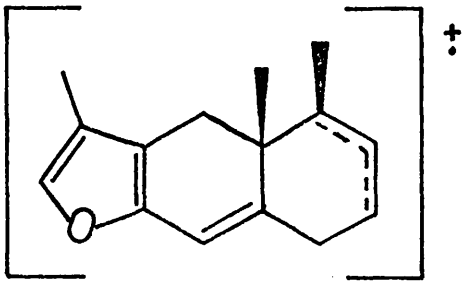
CCXLIV

b

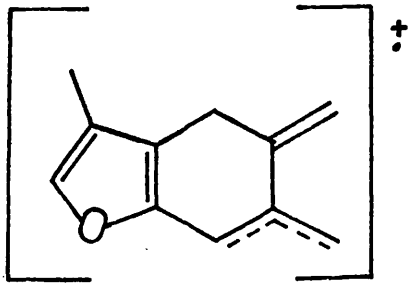
CCXLV

OH

CCXLVI



CCXLVII



CCXLVIII

The mass spectrum of TR4 showed a molecular ion at m/e = 314, and a base peak at m/e = 159, as well as an ion of m/e = 214, all of which suggest a parallel with isopetasin (CLVIII), which has a base peak of m/e = 161 (CCXXVIII) and a peak at m/e = 216 (CCXXIX). Since L.tussilaginea has been shown to contain furanosesquiterpenoids (198) it is probable that the compound is a furanoid analogue of isopetasin, and so the most probable structures are CCXLIII and CCXLIV. Neither of these compounds has previously been isolated from a plant, though the alcohol from which the latter would be derived, euryopsin (CCXLVI) was proposed as the precursor of a number of furanosesquiterpenes found in plants of the genus Euryops (222), while the alcohol CCXLV was prepared during investigation into the chemical constituents of L.tussilaginea (198).

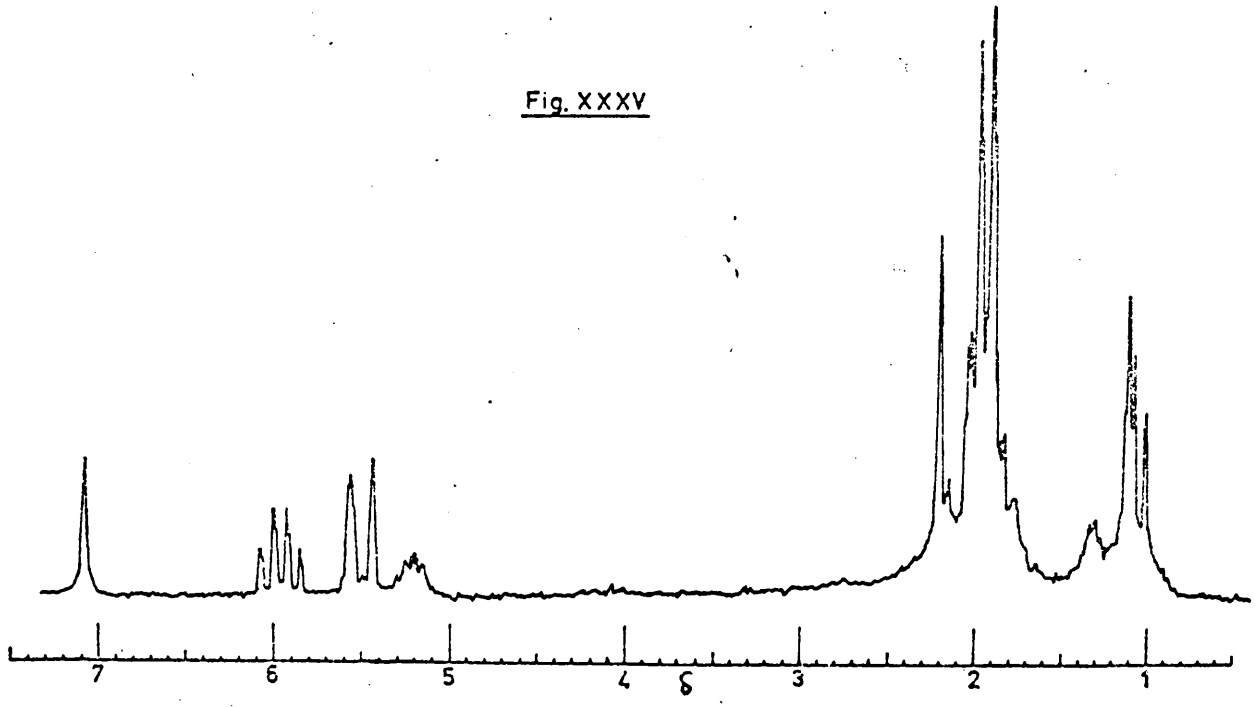
This last compound (CCXLV) gave a mass spectrum in which the base peak was m/e = 159, and the strongest ions were at m/e = 199 and 145 all three of which occur in the spectrum of compound TR4. Thus the most likely structure for compound TR4 is CCXLIII, where the acyl group is derived from an unsaturated C_5 acid. The peak at m/e = 214 would arise from loss of the acid group (CCXLVIII) of m/e = 159.

The identification of TR4 is strengthened by the UV spectrum, which shows an absorbance of $\lambda_{max.}$ = 290nm (ε = 9,000). Furanoeremophil-8-en-5β-ol (CCXLV) has $\lambda_{max.}$ = 292nm (ε = 10,000) (198) and since the change from an alcohol to an ester would not affect the major chromophore, which is due to the conjugation of the double bond with the furan system, this close similarity in UV spectra would be expected from the two compounds.

The NMR spectrum of TR4 (Fig. XXXV) was obtained and Table 37 shows a comparison of the chemical shifts observed with the corresponding values obtained for furanoeremophil-8-en-5β-ol (CCXLV) (198).

The similarities between the spectra again tend to confirm the

Fig. XXXV



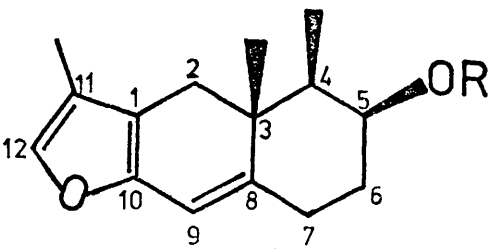
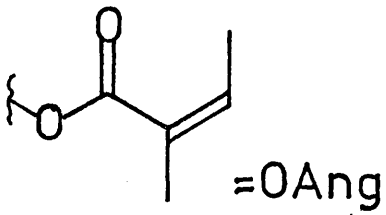


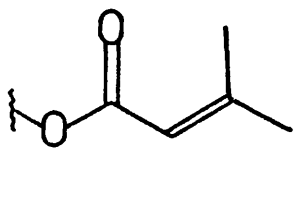
TABLE 37

Proton	δ (observed)	δ (R = H)
C_4 - CH_3	1.05 d (J = 4Hz)	1.10 d (J = 6Hz)
C_3 - CH_3	1.10	1.05
C_{11} - CH_3	2.0 d (J = 1Hz)	1.9 d (J = 1.5 Hz)
C_5 - H	5.2	3.84
C_9 - H	5.4	5.93 d
C_{12} - H	7.1 d (J = 1Hz)	6.86



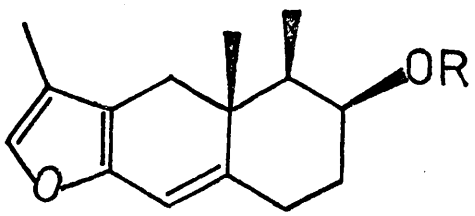
=OAng

CCXLIX



=OSen

CCL



R = Ang CCXLIIIa

Sen CCXLIIIb

H CCXLV

TABLE 38

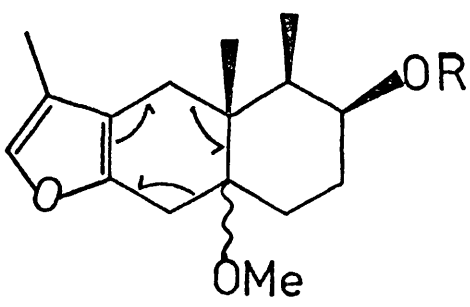
I (1% SE-30)

Acid	Methyl Ester	Benzyl Ester	Ratio	Identity
1015	950	1375	0.4	Angelic
1060	1015	1435	3	Dimethyla
1080	1010	1470	2	Tiglic
M 100	114	190		

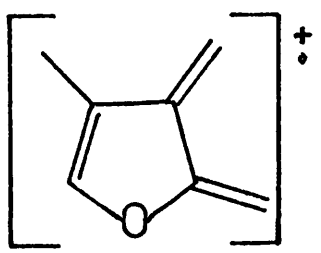
identification of compound TR4. The only major difference between the chemical shifts of corresponding protons is that observed for the C_3 hydrogen, caused by the deshielding effect of the ester group, which would be larger than the effect due to a hydroxyl. The methyl protons from the acid group would be expected to have chemical shifts of between 1.85 and 2.168 δ (198). There are, in fact, peaks in this region, but they form too complex a system to allow analysis. There are, however, peaks which correspond to the single proton present in each acid. That due to the angeloyloxy group (OCXLIX) would be a quartet centred around 5.9 δ, while that of dimethylacryloxy (CCL) group would be a multiplet centred around 5.55 δ (198). The spectrum of TR4 shows both of these signals, which suggests that the esters of both acids (CCXLIIIa, CCXLIIIb) are present as a mixture, of similar properties.

The mixture was hydrolysed by treatment with mild base, and the constituent alcohol and acid were extracted from the reaction mixture and examined by GLC and GC-MS. The alcohol was found to have a retention index of 1870 on 1% SE-30. The mass spectrum showed a molecular ion of m/e = 232, a base peak at m/e = 159 and major ions at m/e = 214, 199 and 145, matching the spectrum of furanoeremophil-8-en-5β-ol (CCXLV) (198).

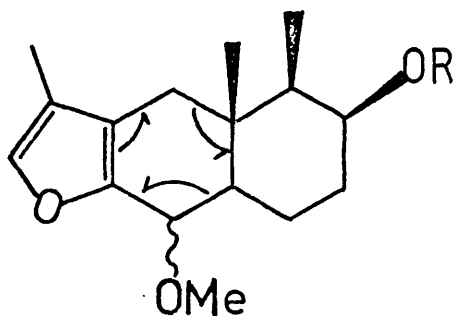
Portions of the acid solution were treated with diazomethane and with phenyldiazomethane to form the methyl and benzyl esters respectively. The retention indices of these esters and of the original acids are shown in Table 38. The mass spectra of the various methyl and phenyl esters were also obtained, but they proved no help in distinguishing the compounds, giving almost identical spectra for each type of compound. The molecular ions did confirm the molecular weights expected for the unsaturated C_5 acids and their derivatives. Comparison of the data with those of authentic samples shows that the acids are angelic, tiglic and dimethylacrylic in the ratio of 0.4 : 2 : 3, which, allowing for the isomerisation of angelic acid to tiglic acid, implies an original ratio of angelic to dimethylacrylic of 2.4 : 3 or 1 :1.25.



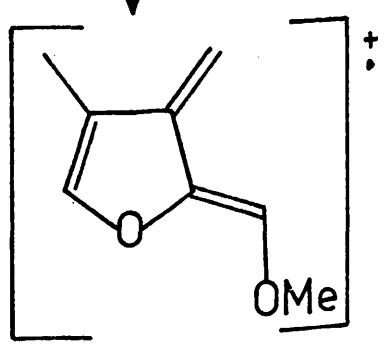
CCLI



CCLIII

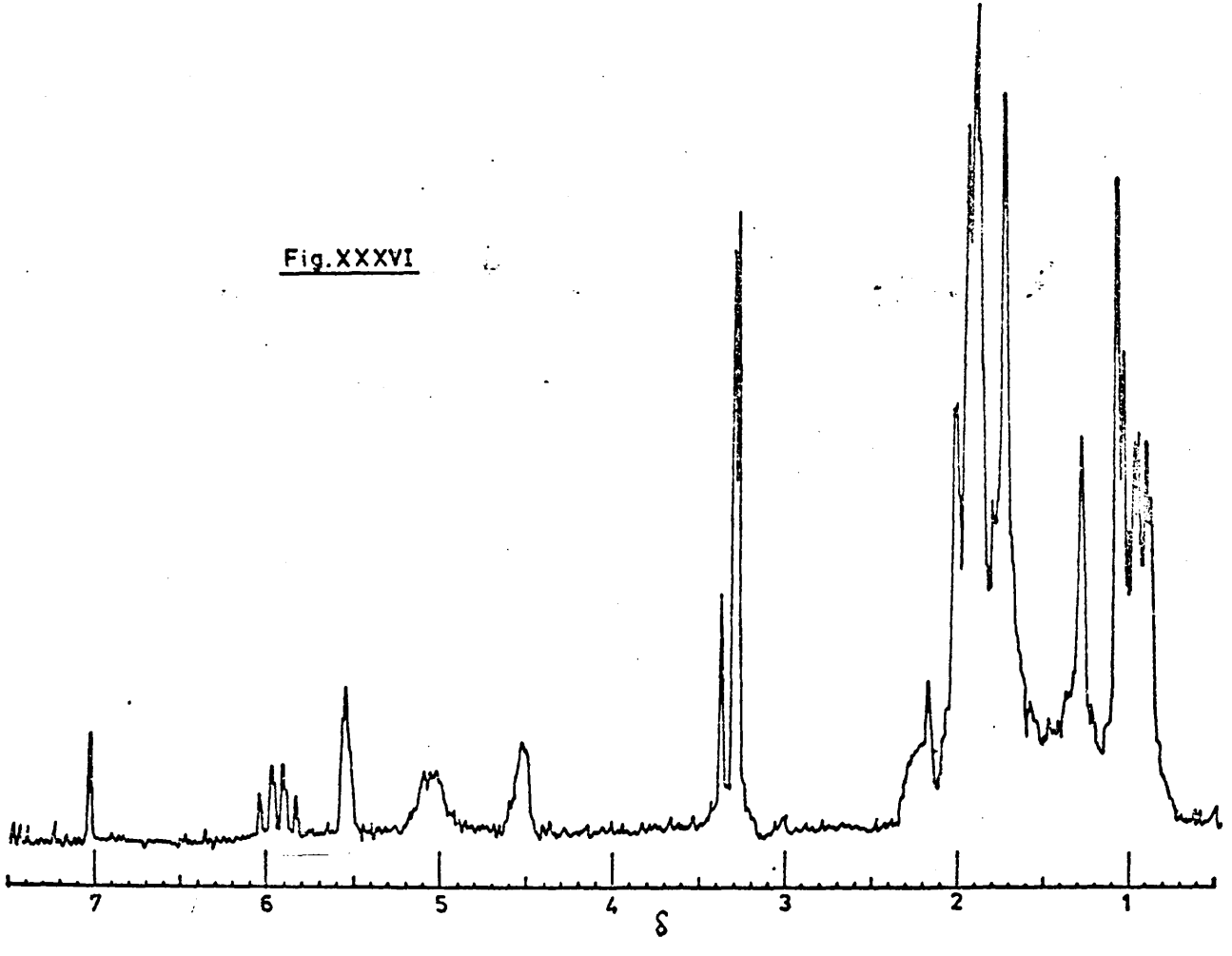


CCLII



CCLIV

Fig.XXXVI



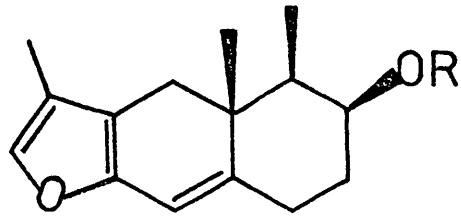
Thus the peak on the GLC trace attributed to TR4 is, in fact due to two isomeric esters, 5β-angeloyloxy-furanoeremophil-8-ene (CCXLIVa) and 5β-dimethylacrylyloxy-furanoeremophil -8-ene (CCXLIVb).

Compounds TR4a, b and c have the GLC and GC-MS properties summarised in Table 39. Their retention indices are different enough from those of compound TR4 and the amounts of each present with respect to TR4 are sufficient that they would not be obscured by it in the original GLC trace of the extract. They do not, however, appear in this trace, therefore they are artefacts, formed during the chromatographic procedures. This was confirmed by keeping a sample of TR4 in methanol overnight at room temperature. The products were compounds TR4a, b and c. The molecular weight, 346, and the easy elimination of 32 mass units to give the ion of m/e = 314, as well as the ions of m/e = 199 and 159, suggest that these compounds have been derived from TR4 by the addition of methanol across the double bond. This is confirmed by the NMR spectrum (Fig. XXXVI) of the mixture of compounds. This is identical to that of compound TR4, except for the absence of the signal due to the C_9 proton at 5.45δ, and the presence of a large doublet (J = 2Hz) integrating for three protons at 3.3δ where one would expect to see a methoxy signal (139). The UV spectrum also supports this contention, showing an absorption at $\lambda_{max.}$ = 218 (ε = 14,000) which is the expected value (198).

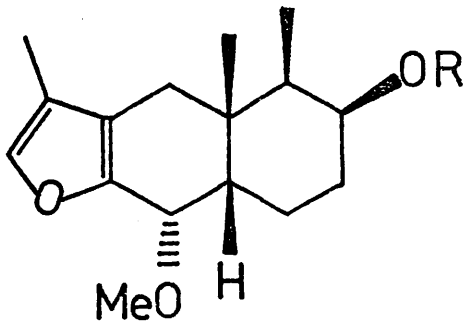
Of the two possible structures CCLI and CCLII, the latter is the more likely from the mass spectral evidence. A Retro Diels-Alder fragmentation of the type shown (198) would result in the ions of m/e = 108 (CCLIII) and m/e = 138 (CCLIV) from compounds CCLI and CCLII respectively. The ion of m/e = 138 (CCLIV) is the base peak in the spectrum of TR4a, and occurs, to a lesser extent, in the spectra of TR4b and c, while the ion of m/e = 108 (CCLIII) does not occur in any of the spectra. Thus the compounds have the general formula CCLII.

TABLE 39

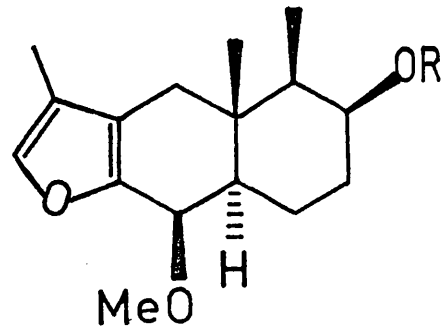
Compound	$I_{(1\%SE-30)}$	Ratio	M	Base Peak	Strongest Ions						
TR4a	2230	1	346	138	55	83	159	41	43	199	314
TR4b	2235	5	346	83	55	159	263	43	314	199	138
TR4c	2250	6	346	83	55	159	43	213	314	199	138



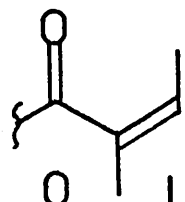
CCXLIII



CCLV



CCLVI

a R = 

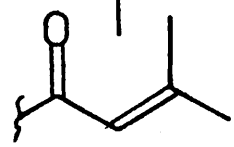
b 

TABLE 40

Compound	$I_{1\%SE-30}$	Ratio	M	Base Peak	Strongest Ions			
TR3	2215	2	316	159	83	145		
TR3a	2220	1	316	108	83	55	41	43
TR3b	2230	9	316	108	83	55	43	41

The similarity of the mass spectra of compounds TR4b and c, coupled to the ratio of one to another of 5:6, which matches the ratio of the dimethylacrylic ester to the angelic ester, which were their parent compounds, suggests that these two compounds are respectively the angelic and dimethylacrylic esters of the same alcohol. Compound TR4a and possibly a second compound masked by b and c, would, therefore, be an ester of the complementary alcohol in which the methoxyl group had the opposite configuration.

The addition of methanol to a double bond is a stereoselective process, the methoxyl and hydrogen adding trans- to each other, so from TR4 (CCXLIII) the expected products would be either the α-methoxy isomer in which the rings are cis-fused (CCLV) or the β-methoxy isomer in which the rings are trans-fused (CCLVI). Retro Diels-Alder fragmentations in systems like these have been shown to be more favoured when the rings are cis-fused than when they are trans-fused (223, 224), thus compound TR4a, which under electron impact gives a Retro Diels-Alder ion as the most stable ion, probably has the structure CCLV, while TR4b and c, have the structure CCLVI.

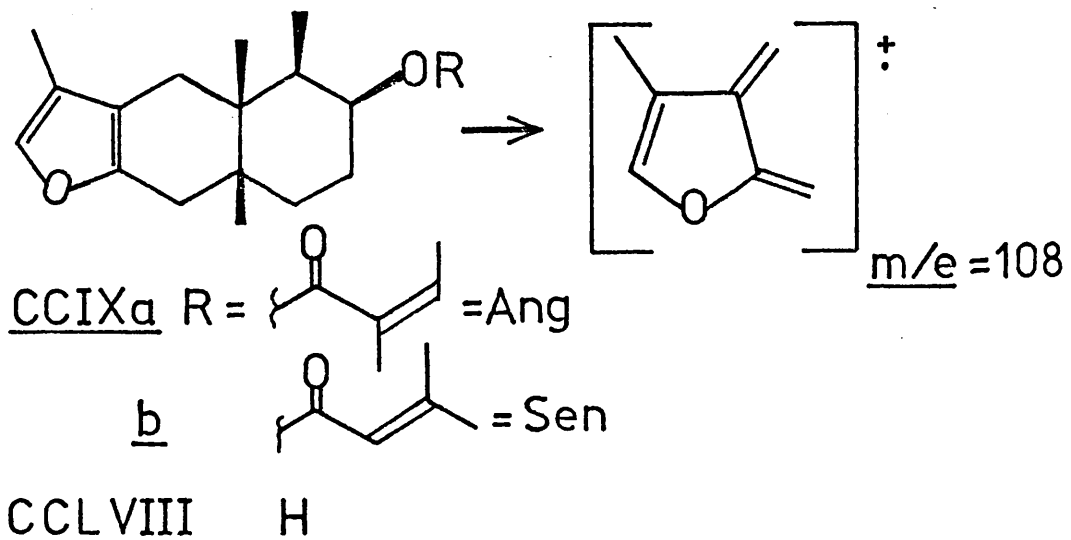
Hydrolysis of the compounds gave two alcohols, retention indices 1810 and 1820 in the ratio 5:1, and with the expected molecular weights of 264. The acid fraction gave the same results as that of compound TR4, with a ratio of angelic to tiglic to dimethylacrylic acid of 0.4 : 2 : 3, implying an original ratio of angelic to dimethylacrylic of 5:6. This ratio is the same as that of compounds TR4b to TR4c, thus TR4b is probably the ester of angelic acid (CCLVIa) and TR4c of dimethylacrylic acid (CCLVIb).

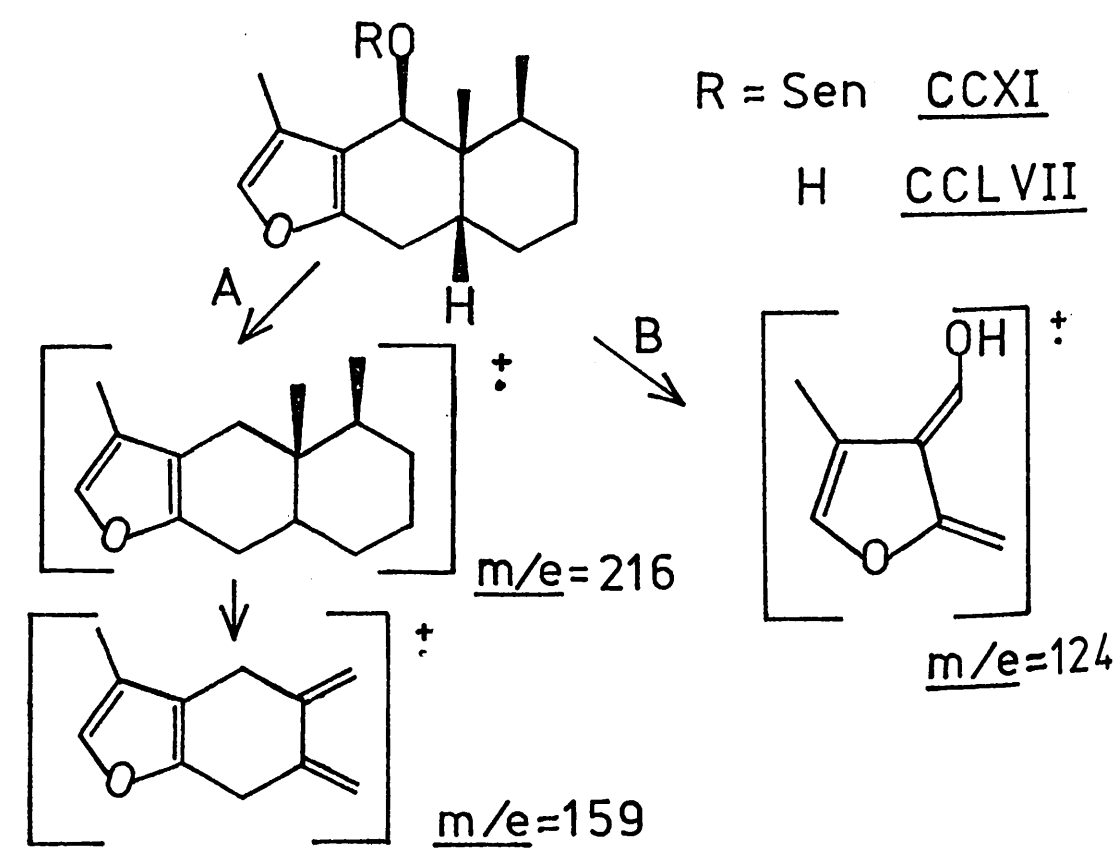
Fraction III (SLV 65 - 80)

Compound TR3 was found in this fraction along with two other compounds of the same molecular weight and of retention indices 2220 and 2230 (Table 40). These compounds labelled TR3a and b, could easily have been masked in the GLC trace of the original extract by the large peak at 2220 index units. The mass spectrum of compound TR3 matches

TABLE 41

Compound	$I_{(1\%SE-30)}$	Ratio	M	Base Peak	Strongest Ions	
CCLVIII	1700	1	234	124	159	216
CCLVII	1760	5	234	108	216	





that of 2 β-dimethylacryloyloxy-furanoeremophilane (CCXI) while the very similar spectra of TR3a and b match that of 5β-angeloyloxy-furanoeremophilane (CCIXa), both of which have been previously found in L.tussilaginea (198). The presence of two compounds with similar mass spectra again suggests that TR3a and b are the isomeric esters of angelic and dimethylacrylic acids. A sample of the mixture of all three compounds was hydrolysed and the fraction containing the acids was examined by GLC. Both angelic acid and dimethylacrylic acid were present, in the ratio of 3:1. From the ratios of the esters (Table 40), this could only arise if both TR3 and TR3a were the dimethylacrylic acid esters (CCXI, CCIXb), and TR3b was the angelic acid ester (CCIXa).

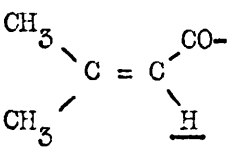
The alcohol-containing extract of the hydrolysis was found by GLC to be a complex mixture of peaks, implying that the alcohols had decomposed under the reaction conditions. Treatment of a mixture of the esters with lithium aluminium hydride, however, gave the alcohols derived from the esters (Table 41). The alcohol from compounds TR3a and b (CCLVIII) shows a mass spectrum similar to those of the esters, with the base peak due to the m/e = 108 ion derived from a Retro Diels-Alder fragmentation. In the other alcohol (CCXLVII) there are two major fragmentation processes, loss of the elements of water to give the ion of m/e = 159 as the base peak (Path A) or the Retro Diels-Alder which gives the ion of m/e = 124 as the base peak (Path B). When R is an ester group (CCXI), path A is preferred and the base peak is m/e = 159. When, however, R is a hydrogen (CCXLVII), loss of H_2O is not so easy, so path B is preferred, although path A still contributes to the spectrum.

Fraction I (SEV 36-40)

This fraction was found to contain compounds TR5, 6 and 7, as well as two minor compounds of retention indices 2217 and 2220, which would be contained in the GLC trace of the total extract, in the large peak of retention index 2220. These compounds were labelled TR4d and e. The fractions were then chromatographed on a straight phase liquid gel

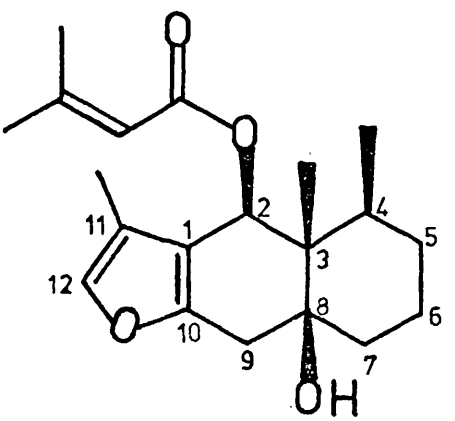
TABLE 42

NMR Data

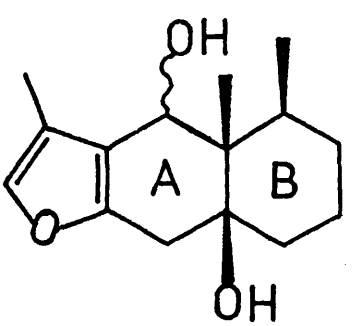
	TR4d and e	Compound CCX
C_4 - CH_3	0.96	0.95m
C_3 - CH_3	1.04	0.98s
C_{11} - CH_3	1.90	1.89
C_2 - H	6.10	6.07s
C_9 - H	2.60, 3.12 — AB quartet J=17Hz	2.55, 3.08 — AB quartet J= 19Hz
C_{12} - H	6.98	6.96
	5.58m	5.58m

s - singlet

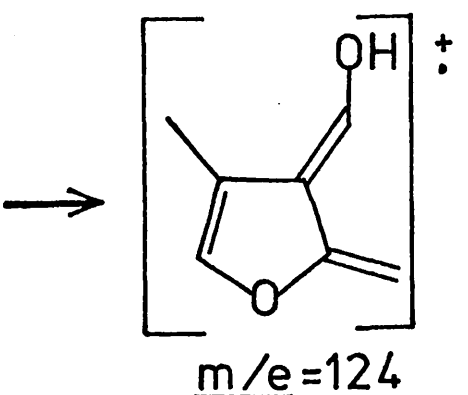
m - multiplet



CCX



CCLIX



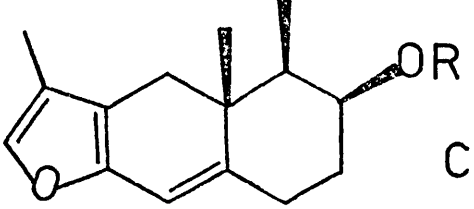
m/e=124

column (N1518-60%-LH20, benzene) to separate the compounds.

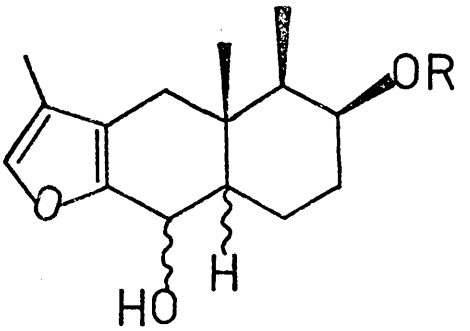
Compounds TR4d and e were eluted at SEV = 49-52. They show almost identical mass spectra, with molecular ions at m/e = 332, base peaks at m/e = 83 and strong ions at m/e = 55, 159, 199, 232 and 214. These spectra match that of 2β-dimethylacrylyloxy, 8β-hydroxy-furanoeremophilane (CCX), as does the NMR spectrum of the mixture of TR4d and e (Table 42) (198). The NMR spectrum shows a signal due to the proton of the dimethylacrylyloxy group, but none from the proton of an angelyloxy group, suggesting that both compounds are esters of dimethylacrylic acid. This was confirmed by hydrolysis of the compounds, which produced one acid. The retention indices of this acid and its methyl ester were those of dimethylacrylic acid. The alcohol-containing extract from the hydrolysis was shown by GLC to contain a complex mixture of peaks, but reduction of compounds TR4d and e, with lithium aluminium hydride resulted in two alcohols.

These alcohols had retention indices of 1865 and 1880, and were present in the ratio of 2:1, hence the former is derived from compound TR4e and the latter from compound TR4d. The mass spectra are extremely similar, both having a molecular ion of m/e = 250 and a base peak of m/e = 124, which would be expected to arise from compounds of the structure CCLIX by a Retro Diels-Alder process. Since this is the base peak of both spectra the rings A and B are probably cis-fused in both compounds (223, 224,), so the alcohols and the original esters are most likely isomeric in the configuration of the hydroxyl group on carbon-2.

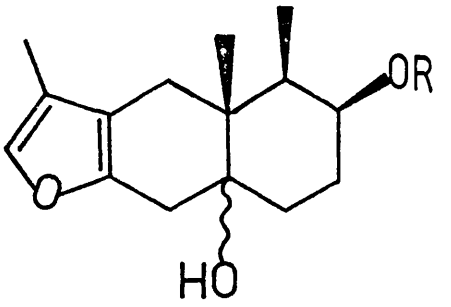
Compound TR5 was found in fractions, from the straight phase column, of SEV = 61-67. The mass spectrum of this compound has a molecular ion of m/e = 332 and shows peaks at m/e = 314, 214, 199 and 159. These suggest that TR5 has a structure which can lose a hydroxyl and hydrogen under electron impact to give the pattern of ions observed in the spectrum of TR4 (CCXLIII) consistent with the structures CCLX



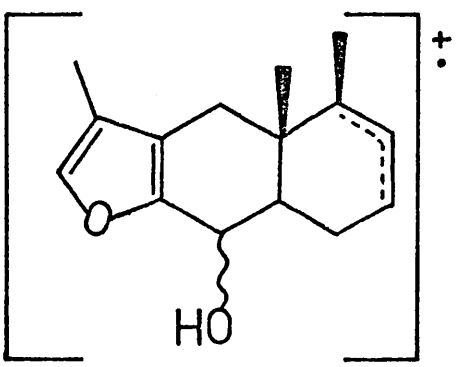
CCXLIII



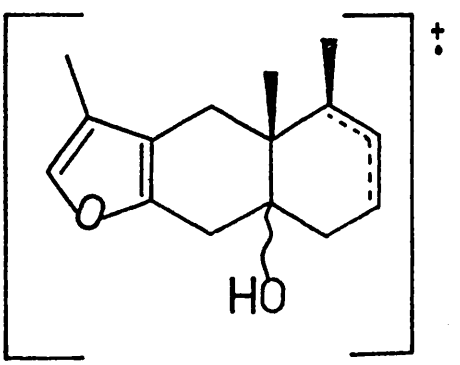
CCLX

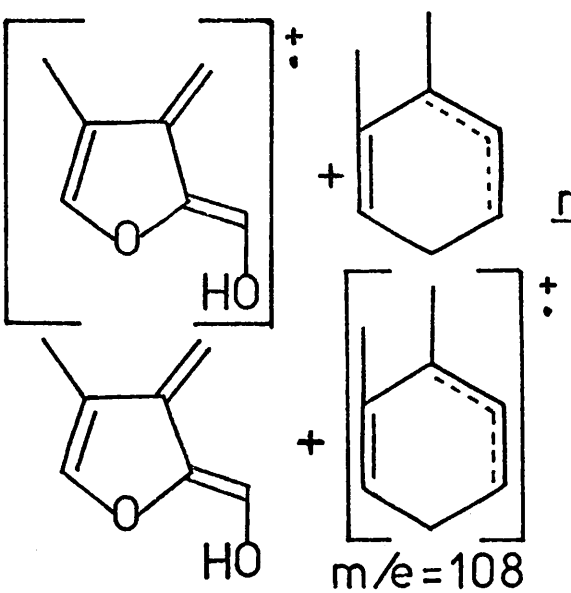


CCLXI



m/e= 232



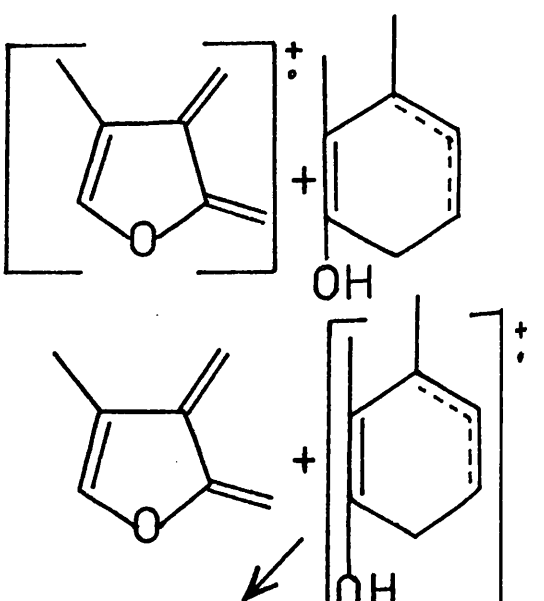


m/e= 124

or

m/e=108

Fig. XXXVIII



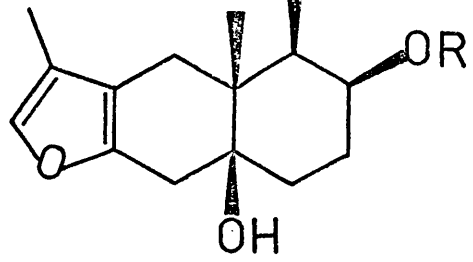
m/e=108

or

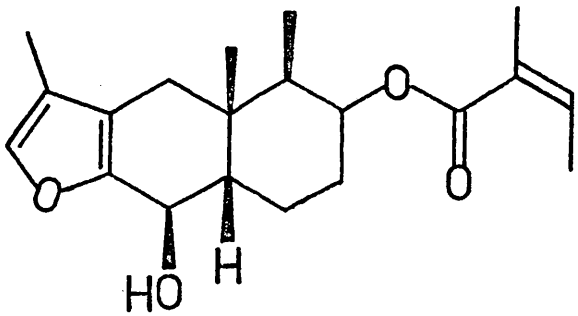
m/e=124

m/e =109

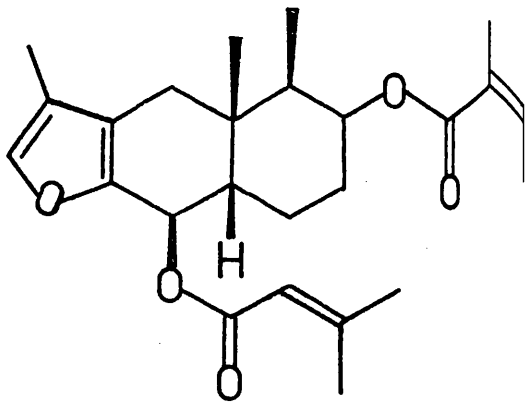
Fig. XXXIX



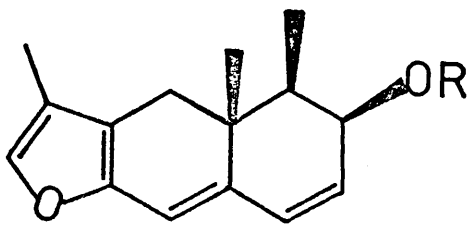
CCLXII



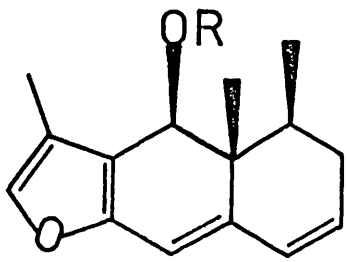
CCXII



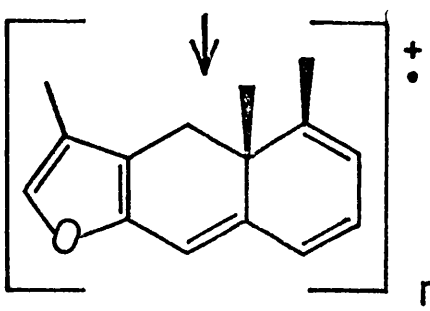
CCVIII



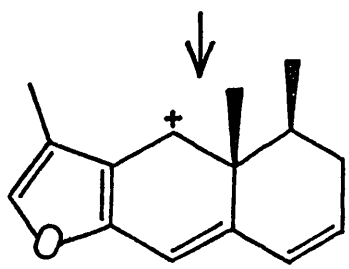
CCLXIII



CCLXIV



m/e = 212

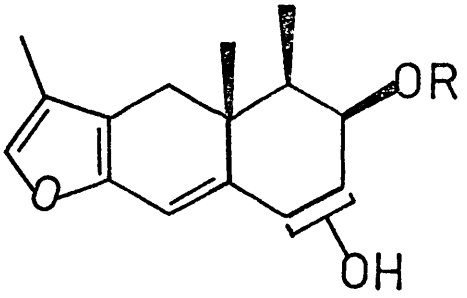


or CCLXI. Fragmentation of both compounds could result in the Retro Diels-Alder peaks at m/e = 108 and 124 found in the spectrum (Fig. XXXVIII, XXXIX), but only in the fragmentation of CCLXI could the base peak, of m/e = 109 be so easily derived (FIG. XXXIX). The spectrum of compound CCLX would also be expected to show the m/e = 124 ion as the base peak (198). Thus the structure of compound TR5 is probably CCLXI. Since the Retro Diels-Alder peaks have such a high intensity in the spectrum, rings A and B are probably cis-fused (223, 224) making the structure CCLXII or an isomer where the acyl group is positioned on either carbon 6 or 7. A compound of this type has been reported as occurring in plants of the genus Euryops (222).

Compound TR6 was eluted from the straight phase column with an SEV = 67-73. The mass spectrum of this compound also has a molecular ion at m/e = 332, but here the base peak is at m/e = 124, suggesting a furanosesquiterpenoid with a hydroxyl at C-9. This and the rest of the spectrum matches the data obtained for 5β-angeloyloxy-9β-hydroxy-furanoeremophilane (CCXII) which has been found in L.tussilaginea (198). The previous workers state that this compound is present as an artefact arising from the hydrolysis, during column chromatography on silica gel, of the compound CCVIII, and that it is not present in the original extract. In this case, however, it was found in the original extract of the root material which suggests that it is an actual component of the plant, or that the di-ester (CCVIII) is being totally hydrolysed during the extraction and gel-filtration of the plant material, since the di-ester was not encountered in this extract of the plant.

The last compound to be eluted from the straight phase column was compound TR7, at SEV = 74-76. The highest ion in the mass spectrum of this compound was at m/e = 312, which, along with the ion at m/e = 212, suggests a more unsaturated form of compound TR4 with a structure of the type shown (CCLXIII, CCLXIV), or some isomer of these. The comparatively high intensity of both the m/e = 312 and 212 ions (Table 34) suggests that the structure CCLXIII is the more

CCLXV



CCLXVI

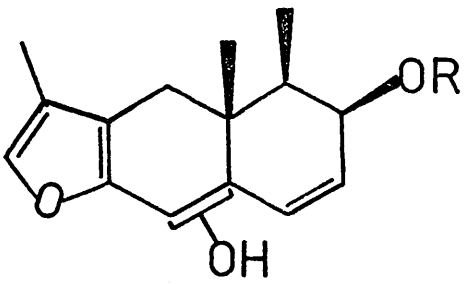
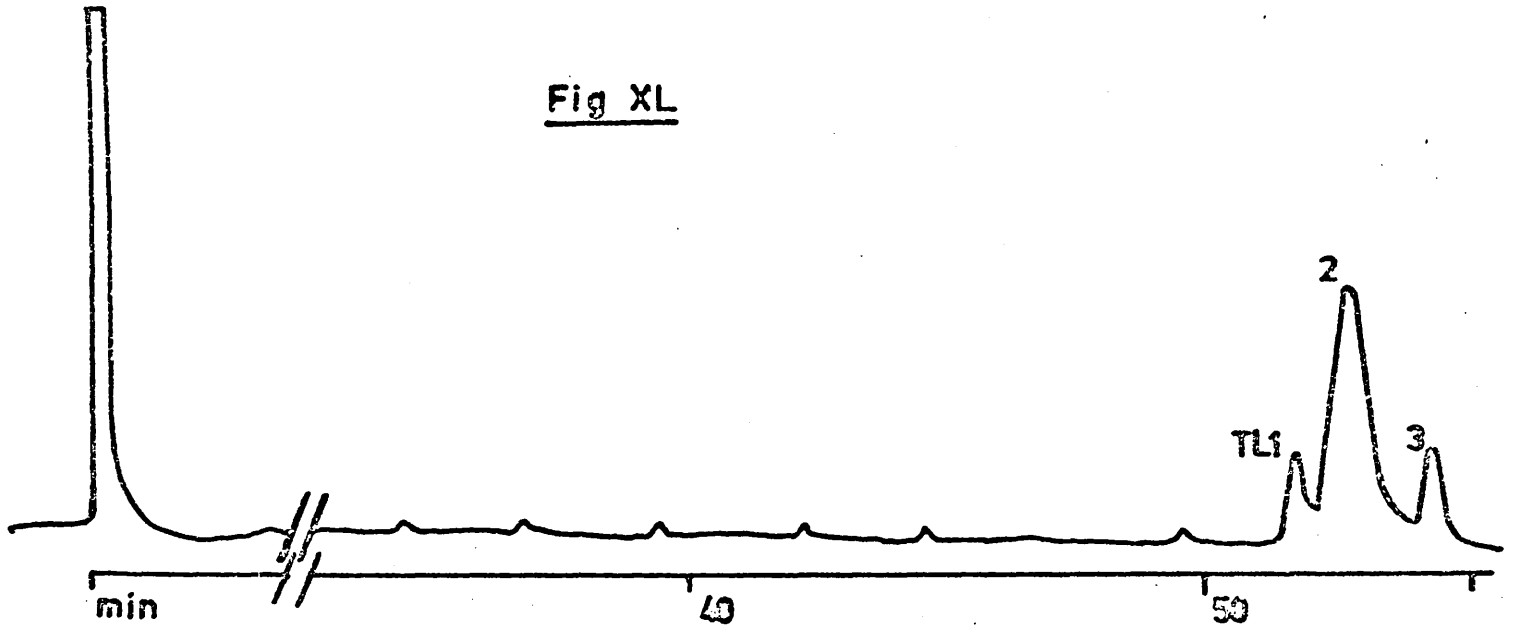


Fig XL



likely because the m/e = 212 ion derived from it contains three double bonds, all of which are conjugated with the furan ring, increasing the stability of the ion. The SIV and retention index values of this compound, however, suggest that it is a polar compound since its nearest analogues in both GLC and liquid gel chromatography, are the hydroxy-furanoids TR5 and TR6. Thus the compound is more likely to have the structure CCLXV, from which the hydroxyl is easily eliminated, under electron impact, to give the ion of m/e = 312, which is stabilised by the conjugation of the double bonds. Compound CCLXVI is less likely, since it would be expected to undergo a Retro Diels-Alder fragmentation, giving an ion of m/e = 108 for the 8-hydroxy isomer, and 124 for the 9-hydroxy isomer (198).

Fraction IV (SIV 110-120)

This remaining fraction was found to contain the sesquiterpene hydrocarbons and the sterols TR8, 9 and 10. The two classes of compound were separated by liquid gel chromatography on the straight phase column, the hydrocarbons being eluted at SIV = 30-34, the sterols at SIV = 35-40. The column was not sufficiently efficient to separate individual members of each type.

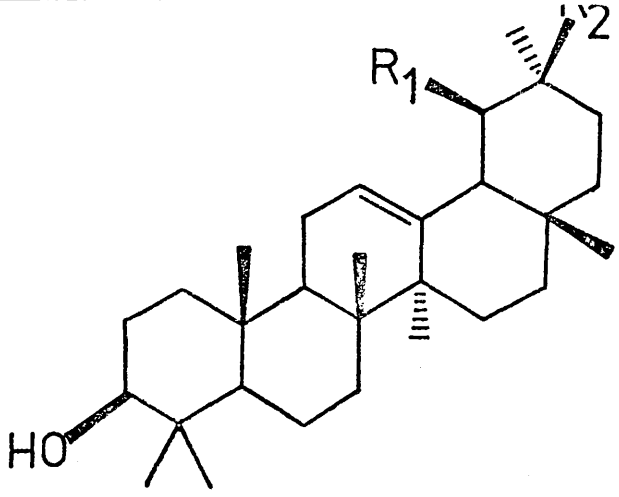
The mixtures were examined by GLC at constant temperature, and the retention indices confirmed as those stated in Table 34.

Examination of the Leaf Material

The extract of leaf material from L.tussilaginea was found to contain three major compounds **(Fig. XL)**(Table 43). Compounds TL2 and 3 were identified as β-sitosterol (220) and α-amyrin which was also encountered in the leaf extract of L.'Desdemona' and L.veitchiana. The mass spectrum of compound TL1 contains ions due to compound TL2, because of their similar retention times, and the greater quantity of compound TL2 present. If these ions are neglected the base peak of the spectrum of compound TL1 becomes m/e = 218, characteristic of the

TABLE 43

Compound	$I_{(1\%SE-30)}$	M(% of Base Peak)	Base Peak	Strongest Ions (% of Base Peak)		
TL1	3215	424 (5)	55	218(89)	69(83)	135(82)
				43(74)	188(79)	
TL2	3220	414 (21)	43	55(76)	41(63)	69(61)
				57(58)	95(54)	81(53)
TL3	3230	424 (4)	218	43(36)	55(36)	41(32)
				95(26)	81(25)	



$R_1 = CH_3$ $R_2 = H$ CCXXII

H CH_3 CCXXV

amyrins (218, 219). The molecular ion m/e = 424 also suggests that compound TL1 is an amyrin. Thus TL1 and TL3 are β-(CCXXV) and α-amyrin (CCXXI) respectively.

There is also a series of minor peaks at retention indices of 2500, 2600, 2700, 2800, 2900 and 3100 which were too small to allow their examination by GC-MS. They are presumably straight chain hydrocarbons or alcohols from the leaf wax.

A comparison of the compounds found in these six plants of the genus Ligularia (Table 44) shows a chemotaxonomical relationship between various members of the set examined. The parent-daughter relationship of L.clivorum and L.'Gregynog Gold' for example, is demonstrated by the occurrence, in each of them, of eremophilene as the major component. L.'Desdemona', which is a cultivated variety of L.clivorum, was also found to have eremophilene as the major constituent of its root material.

The relationship between all six as a group however, is best shown by the occurrence, in each of the plants examined, of oxygenated eremophilanes, ranging from the eremophilene in L.clivorum, L.'Desdemona', L.'Gregynog Gold' and L.veitchiana to esters of petasol and isopetasol in L.tangutica and L.veitchiana and esters of furanoeremophilanes in L.tussilaginea (Table 45). The occurrence of these compounds in the plants confirms chemotaxonomically the membership of the plants in the tribe Senecioneae of the family Compositae which has been ascribed to them taxonomically. Of all the thirteen tribes numbered among the the Compositae, the tribe Senecioneae, is unique in containing oxygenated eremophilanes of these types (221).

In view of the proposal that eremophilene is a precursor of the petasins and furanoids in plants of this tribe (225), the results may also suggest L.clivorum, its daughter L.'Gregynog Gold' and its cultivar

TABLE 44

	L. clivorum Root		L. 'Gregynog Gold' Root	Leaf	L. 'Desdemona' Root	Leaf	L. tangutica Root		L. veitchiana Root	Leaf	L. tussilaginea Root	Leaf
α-bergamotene				+								
β-bisabolene					+		+		+	+		
eremophilene	+++		+++		+++					+	+	
farnesene										+		
β-humulene				+								
Petasins and oxygenated eremophilanes							+++			+		
oxygenated furanoeremophilanes											+++	
α-amyrin				+		+				+		+
β-amyrin												+
β-sitosterol						+				+	+	+
campesterol											+	
stigmasterol											+	

TABLE 45

COMPOUND	L.clivorum	L.'Gregynog Gold'	L.'Desdemona'	L.tangutica	L.veitchiana	L.tussilagine	*Identification	Reference
eremophilene	+	+	+		+	+	P	173, 177, 180
petasin				+	+		P	180
isopetasin				+			P	180
petasyl propionate					+		T	-
petasyl dimethylacrylate					+		P	180
5-acyloxy-eremophilo-6, 8, 11-trien-10-one					+		T	-
eremophila-9, 11-dien-10-one					+		P	180
eremophilo-1(11)-en-[illegible]-hydroxy-10-one					+		T	-
furanoeremophil-[illegible], 6, 8-triene						+	T	-
5β-dimethylacryloyloxy-furanoeremophil-8-ene						+	E	-
5β-angeloyloxy-furanoeremophil-8-ene						+	E	-
5β-angeloyloxy-9α-methoxy-furanoeremophilane						+	E	-
5β-angeloyloxy-9β-methoxy-furanoeremophilane						+	E	-
5β-dimethylacryloyloxy-9α-methoxy-furanoeremophilane						+	E	-
2β-dimethylacryloyloxy-furanoeremophilane						+	P	198
5β-dimethylacryloyloxy-furanoeremophilane						+	P	198
5β-angeloyloxy-furanoeremophilane						+	P	198
2α-dimethylacryloyloxy-3β-hydroxy-furanoeremophilane						+	P	198
2β-dimethylacryloyloxy-3β-hydroxy-furanoeremophilane						+	P	198
5-acyloxy-3α-hydroxy-furanoeremophilane						+	T	222
5β-angeloyloxy-3β-hydroxy-furanoeremophilane						+	P	198
5β-acyloxy-6 or 7-hydroxy-furanoeremophil-8-ene						+	T	198

* E - Structure established

P - Structure Probable

T - Structure tentative

L.'Desdemona' (199) which contain only eremophilene, are lower on the evolutionary scale than the other Ligularias examined.

The occurrence in L.tangutica and L.veitchiana of petasins but no furanoids, and the reverse situation in L.tussilaginea where only furanoids were found, suggests that like some members of the genus Petasites (191, 194) species of the genus Ligularia e.g. L.sibirica (20 , 201, 210) are split into two sub-species, one containing petasins only, the other furanoids and sometimes eremophilenolide derivatives.

From these results it can also be seen that the techniques of gas liquid chromatography and combined gas liquid chromatography and mass spectrometry can be usefully applied to the systematic study of the chemotaxonomy of a series of plants. Even when as in this study, only the major peaks of the GLC trace are examined in the mass spectrometer, the results obtained can reveal a good deal about the constituents and relationships of the plants. The techniques are especially valuable when used in parallel with larger scale separation and analytical techniques to demonstrate whether or not the compounds under examination have survived the analytical procedures. This last advantage was especially useful when dealing with, for example, compound TR4, which was susceptible, not only to air oxidation, even when dissolved in a degassed solvent and kept under refrigeration, but also to addition reactions with solvents such as methanol, with which it formed a methoxy adduct. This compound would have been very difficult to separate and characterise by classical means.

4 - 3 Experimental

4 - 3 - 1 Extraction and General Chromatographic Techniques

The procedures followed for the extraction of the plant material are those detailed in the experimental section of chapter 3 (3 - 3 - 1), using a mixture of benzene and isopropanol in the ratio of 3:1 as the solvent. Both of these compounds form azeotropes with water, so the evaporation of the solvents resulted in the removal of most of the water present in the extracts.

Gel filtration of the extract, and its subsequent examination by GLC and GC-MS, was also performed as described in chapter 3, as was the treatment of portions of the extract to form the trimethylsilyl ethers of any alcohols present, and the methyl oximes of any ketones or aldehydes.

The NMR spectra were recorded at 100 MHz on a Varian HA100 NMR spectrometer. The UV spectra were recorded on a Unicam SP800 spectrophotometer.

Chromatography of the extracts was performed on three liquid gel columns:-

Column 1 - *NH1114-60%-LH20; methanol/n-heptane, 9/1; bed volume 65ml; cross sectional area $1cm^2$ (Reverse Phase)

Column 2 - NH1518-60%-LH20; methanol/n-heptane, 9/1; bed volume 67ml; cross sectional area $1cm^2$ (Reverse Phase).

Column 3 - NH1518-60%-LH20; benzene; bed volume 74ml: cross sectional area, $1cm^2$ (Straight Phase).

*NH1114-60%-LH20, means Nedox LH20 gel which is a cross-linked polysaccharide polymer, in which the remaining free hydroxyls are substituted to 60% of dry weight with C_{11} to C_{14} straight chain hydroxyalkyl groups.

The effluent from the liquid gel columns was passed over the moving wire of a Pye Liquid Chromatography Detector, then into the fraction collector. The detector operates by passing the wire, coated with column effluent into a low temperature oven where the solvent is removed by evaporation, and then into a high temperature oven where any remaining organic material is pyrolysed, the pyrolysis products being passed to a hydrogen flame detector connected, through an amplifier, to a chart recorder.

Two fraction collectors were used, the BTL Chromatronix Fraction Collector and the Central Fraction Collector. The former uses an electrically operated time switch to change from one collection vessel to the next, while in the latter the fraction is first collected in a syphon located at the end of a counterbalanced arm. The weight of solvent in the syphon when it is half full causes the arm to tilt triggering the change of collecting vessels. When the effluent reaches the top of the syphon it flows out into the vessel and the arm tilts back, resetting the changeover trigger. The syphons available for this fraction collector allow the collection of from 1 to 25ml. When in use both machines were arranged to collect 1ml fractions.

4 - 3 - 2 Plant Extracts

Table 46 shows, for the preliminary extracts, the weights of the tissue extracted, the total extract and the insoluble residue. From these last two terms the weight of soluble material present can be calculated, and this is shown in the next column. After the gel filtration of the soluble portion the weights of material in fractions A and B were also obtained, and these are shown in the last two columns. From these figures it can be seen that the yields from the gel filtration column are quantitative, within the limits of errors of weighing.

Large Scale Extract of L.veitchiana

950g of root material from this plant was extracted as described above and yielded 13.672g of total extract. A sample of this extract (2.367g) was purified for GLC by the techniques described, and the weights taken at each stage of the procedure were:-

insoluble portion	-	1.636g	soluble portion	-	0.367g
Fraction A	-	0.249g	Fraction B	-	0.115g

Extraction of "Petasins"

Fraction B was chromatographed on column 1 (NH1114-60/-LH20, methanol/n-heptane, 9/1) using the Pye Liquid Chromatograph Detector. Fractions which were shown to contain organic material were examined by GLC.

The petasin-containing fractions were of SEV = 110-190. These were combined and the solvent removed at room temperature, under vacuum on a Büchi Rotary Evaporator. The petasins were weighed (0.983g).

TABLE 46

WEIGHT III g

Root Material

PLANT	TISSUE	EXTRACT	INSOLUBLE PORTION	SOLUBLE PORTION	FR. A	FR. B
L.clivorum	20.12	.382	.282	.105	.084	.019
L.'Gregynog Gold'	20.03	.207	.159	.048	.039	.009
L.'Desdemona'	20.20	.590	.421	.169	.141	.021
L.tangutica	18.83	.034	.002	.032	.011	.021
L.veitchiana	20.04	.282	.217	.065	.045	.021
L.tussilaginea	20.09	.355	.320	.035	.028	.006

Leaf Material

L.'Gregynog Gold'	12.55	0.312	.210	.102	.089	.011
L.'Desdemona'	9.45	0.566	.377	.189	.170	.015
L.veitchiana	10.25	0.412	.310	.102	.043	.055
L.tussilaginea	16.40	0.565	.433	.132	.070	.040

Hydrolysis of "Petasins"

50mg of the petasins were dissolved in a minimum of benzene (0.2ml) and treated with 2ml of a mixture of 5 parts ethanol, by volume, to two parts, by volume, of 33⅓% aqueous potassium hydroxide (w/v). The reaction mixture was heated at 40°C for one hour. The resultant solution was extracted with ether (3 x 1ml) then the residue was acidified to pH 6 with 10% hydrochloric acid (v/v). This was then extracted with ether (3 x 1ml). The ethereal solutions were dried over anhydrous magnesium sulphate, filtered, their volume made up to 5ml and examined by GLC and GC-MS.

Methylation of Acids

A sample of the extract containing the acids (100µl ≙ 100 µg) was treated with an ethereal solution of diazomethane until the yellow colouration of the reagent just persisted. Excess reagent was removed with the solvent by evaporation at room temperature under a stream of nitrogen. The residue was then dissolved in 100µl of ethyl acetate and examined by GLC.

4 - 3 - 3 Large Scale Extract of L.tussilaginea

102g of root material were extracted and gave 2.316g. This was subsequently separated into 2.013g of insoluble material and 0.313 g of soluble material, which on gel filtration gave 0.193g of fraction A and 0.110g of fraction B.

Fraction B was then chromatographed on Column 2 and the fractions from this were re-chromatographed on column 3 (4 - 2 - 6), to separate as many of the compounds as possible. The compounds were left in solution because of the possibility of oxidation.

Hydrolysis of the Furanosesquiterpenoids

Each set of compounds to be hydrolysed was treated in the same way. An aliquot (100μl) of solution was treated with 1ml of a mixture of 5 parts, by volume, of ethanol to 1 part, by volume, of 33% (w/v) aqueous potassium hydroxide. The reaction mixture was heated at 35°C for 2 hours, then extracted as described above (4 - 3 - 2).

Esterification of the Acids

An aliquot of the ethereal solution, amounting to a quarter of the total volume, was treated with an ethereal solution of diazomethane as described above (4 - 3 - 2). A further quarter was treated with an ethereal solution of phenyldiazomethane (226-228) till the pink colour of the reagent persisted. The excess reagent was destroyed by adding 1% ethereal phosphoric acid till the pink colour just disappeared (228). The solution was then ready for GLC and GC-MS.

Reduction of the Sesquiterpenoids

An aliquot of the solution containing the compounds (100μl) was evaporated to dryness at room temperature under a stream of nitrogen.

The residue was immediately dissolved in 200μl of ether. To this was added excess lithium aluminium hydride ($\sim$4mg) and the mixture was heated for 30 minutes at 40°C. The excess reagent was destroyed by the addition of 0.2ml of distilled water, and the reaction mixture was then extracted with ether (3 x 1ml). The ether extracts were dried over anhydrous magnesium sulphate, filtered and examined by GLC and GC-MS.

4 - 3 - 4 Materials

Preparation of Diazomethane

The reaction was performed in smooth-walled, round-bottom flasks, with no glass joints. The reaction vessel and receiving vessel were fitted with corks through which passed the ends of a U-tube. The receiving vessel contained ~10ml of ether, sufficient to cover the end of the U-tube arm. The arm in the reaction vessel reached to just below the level of the cork, leaving a head space above the reaction mixture.

A mixture of 6ml of 33% (w/v) aqueous potassium hydroxide, 30ml of ethyl digol and 30ml of ether were mixed in the reaction vessel, and heated to 30°C. To this was added 1g of bis-(N-methyl-N-nitroso) terephthalamide (Nitrosan). An effervescence was observed and an azeotrope of ether and diazomethane distilled over the receiving flask which was cooled in an ice bath. After the effervescence ceased, more Nitrosan was added, and the process was continued until the solution in the receiving vessel was a brilliant yellow.

The solution was stored under refrigeration (~4°C) in a smooth-walled flask fitted with a cork.

Preparation of Phenyldiazomethane (226-223)

2.1g of N-benzyl-p-toluenesulphonamide were dissolved in 10ml of glacial acetic acid and 40ml of acetic anhydride in a flask cooled to 5°C. 12g of sodium nitrite were added with stirring, keeping the temperature below 10°C. The mixture was stirred overnight, then poured into excess ice water with vigorous stirring and cooled for one hour in an ice bath. The precipitate was filtered, washed thoroughly with water and dried overnight under vacuum. Recrystallisation from ethanol yielded 1.98g (80%) of N-nitroso-N-benzyl-p-toluensulphonamide. This was added to 0.45g of sodium methylate, 2.1ml of

methanol and 13ml of ether which were being stirred vigorously at 0°C. This was then refluxed for 20 minutes, with a drying tube fixed to the top of the condenser to exclude water. The solvent was evaporated at 25°C in a Büchi Rotary Evaporator. The residue was dissolved in 15ml of n-pentane and filtered.

The filtrate was cooled to -20°C whereupon a liquid phase separated out and then solidified. The supernatant liquid was decanted off and reduced to about 0.3ml. This was distilled under vacuum (0.1mm Hg) at room temperature with constant stirring, into a low temperature condenser. The resultant product, phenyldiazomethane, was dissolved in ether (20ml) and kept under refrigeration (4°C) in a smooth surfaced flask with a polythene stopper.

REFERENCES

1. Anderle, D., Petrakova, M., & Kovac, P., ... J. Chromatog, 55, 209, (1971).

2. Anderson, D.M.W., Hirst, E., & Stoddart, J.F., .. J. Chem. Soc. C., 1959, (1966).

3. Anderson, D.M.W., Hirst, E., & Stoddart, J.F., .. J. Chem. Soc. C., 1476, (1967).

4. Bayer, E., & Widder, P., ... Ann. Chem., 686, 181, (1965).

5. McInnes, A.G., Ball, D.H., Cooper, E.P., & Bishop, C.T., ... J. Chromatog, 1, 556, (1958).

6. Ovoduv, Yu.S., & Evtushenko, E.V., ... J. Chromatog, 31, 527, (1967).

7. Shaw, D.H., & Stephen, A.M., ... Carbohydrate Res. 1, 400, (1966).

8. Shaw, D.H., & Stephen, A.M., ... Carbohydrate Res. 1, 414, (1966).

9. Jones, H.G., ... Meth. Carbohydrate, Chem., 6, 25, (1972).

10. Geyer, H.V., ... Stärke, 17, 307, (1965).

11. Jones, H.G., Jones, J.K.N., & Perry, M.B., .. Can. J. Chem., 40, 1559, (1962).

12. Albersheim, P., Nevins, D.J., English, P.D., & Karr, A., ... Carbohydrate Res., 5, 340, (1967).

13. Davison P.K., & Young, R., ... J. Chromatog, 41, 12, (1969).

14. Gunner, S.W., Jones, J.K.N., & Perry, M.B., .. Chem. Ind. (London), 255, (1961).

15. Hause, J.A., Hubicki, J.A., & Hazer, G.G. .. Analyt. Chem., 34, 1569, (1962).

16. Sawardeker, J.G., Sloneker, J.H., & Jeanes, A., Analyt. Chem. 37, 1603, (1965).

17. Holligan, P.M., ... New Phytol., 70, 239, (1971).

18. Holligan, P.M., & Drew, E.A., ... New Phytol., 70, 271, (1971).

19. Clamp, J.R., Bhatti, T., & Chambers, R.E., .. Methods of Biochemical Analysis, (ed. Glick, D.) 19, 229, (1971).

20. Horii, Z., Makita, M., & Tamura, Y., ... Chem. Ind., (London), 1494, (1965).

21. Horning, M.G., Boucher, F.A., Moss, A.M., & Horning, E.C., ... Analyt. Lett., 1, 713, (1968).

22. Petersson, G., ... Carbohydrate Res., 33, 97, (1974).

23. Laine, R.A., & Sweeley, C.C., ... Analyt. Biochem., 43, 533, (1971).

24. Heyns, K., Scharmann, H., ... Ann., 667, 183, (1963).

25. Biemann, K., DeJongh, D.C., & Schnoes, K.K., .. J. Amer. Chem. Soc., 88, 1763, (1963).

26. DeJongh, D.C., ... J. Org. Chem., 30, 453, (1965).

27. Young, R., & Adams, G.A., ... Can. J. Chem., 43, 2929, (1965).

28. Dutton, G.G.S., & Unrau, A.M., ... J. Chromatog., 20, 78, (1965).

29. Kochetkov, N.K., Mulfson, N.S., Chizov, O.S., & Zolotarev, B.M., ... Tetrahedron, 19, 2209, (1963).

30. Kochetkov, N.K., & Chizov, O.S., ... Tetrahedron, 21, 2029, (1965).

31. Heyns, K., & Müller, D., ... Tetrahedron, 21, 55, (1965).

32. Heyns, K., & Scharmann, H., ... Tetrahedron, 21, 507, (1965).

33. Kochetkov, N.K., Chizov, O.S., & Zolotarev, B.M., ... Carbohydrate Res., 2, 89, (1966).

34. Kochetkov, N.K., Chizov, O.S., & Zolotarev, B.M., ... Dokl. Nauk. SSSR, 165, 569, (1965): Chem. Abs., 64, 6738, (1966).

35. Hedgley, E.J., & Overend, W.G., ... Chem. Ind., 378, (1960).

36. Sweeley, C.C., Bentley R., Makita, M., & Wells, W.W., ... J. Amer. Chem. Soc., 85, 2497, (1963).

37. Sweeley, C.C., Wells, W.W., & Bentley, R., .. Methods in Enzymology, (ed. Colowick, S.P., & Nathan, N.O.), VIII, 95, (New York, 1963).

38. Rowland, M., & Riegelman, S., ... Analyt. Biochem., 20, 463, (1967).

39. Perry, M.B., & Hulyakar, R.K., ... Can. J. Biochem., 43, 573, (1965).

40. Vecchi, M., & Kaiser, K., ... J. Chromatog., 26, 22, (1967).

41. Alexander, R.J., & Garbutt, J.T., ... Analyt. Chem., 37, 303, (1965).

42. Brower, H.E., Jeffery, J.W., & Folsom, M.W., Analyt. Chem., 38, 362, (1966).

43. Dizparoglu, M., Henneberg, D., & Von Sonntag, C., ... Org. Mass Spectrom., 8, 335, (1974).

44. Laver, M.L., Root, D.F., Shafizadeh, F., & Lowe, J.C., ... TAPPI, 50, 618, (1967).

45. Marinelli, L., & Whitney, D., ... J. Inst. Brewing, 72, 35, (1967).

46. Clamp, J.R., Dawson, G., & Hough, L., .. Biochem. Biophys. Acta., 148, 342, (1967).

47. Bolton, C.H., Clamp, J.R., & Hough, L., .. Biochim. J., 96, 5C, (1965).

48. Tomada, M., ... Yakugaku Zasshi, 87, 1057, (1967).

49. Loewus, F., ... Carbohydrate Res., 3, 130, (1967).

50. Yamakawa, T., Ueta, N., & Ishizuki, T., .. Jap. J. Exp. Med., 34, 231, (1964).

51. Sweeley, C.C., & Walker, B., ... Analyt. Chem., 36., 1461, (1964).

52. Cayle, T., Viebrock, F., & Schafferio, J., Cereal Chem., 45, 154, (1968).

53. Wulff, G., ... J. Chromatog., 18, 285, (1965).

54. Raunhardt, O., Schmidt, H.W.H., & Neukom, H., ... Helv. Chim. Acta., 50, 1267, (1967).

55. Roelfs, R.W., Gibbs, G.B., & Griffin, G.D., ... Amer. J. Dis. Child., 113, 419, (1967).

56. Percival, E., ... Carbohydrate Res., 7, 272, (1968).

57. Kim, S.H., Bentley, R., & Sweeley, C.C. Carbohydrate Res., 5, 373, (1967).

58. Kimura, M., Hattori, Y., Yoshizawa, I., & Tohma, K., ... Chem. Pharm. Bull., (Japan), 16, 613, (1968).

59. Bentley, R., Sweeley, C.C., Makita, M., & Wells, W.W., ... Biochem. Biophys. Res. Commun., 11, 14, (1963).

60. Cheminat, A., & Brine, M., ... Bull. Soc. Chim. (France), 80, (1966).

61. Sawardeker, J.S., & Sloneker, J.H., ... Analyt. Chem., 37, 945, (1965).

62. Gunner, S.W., Jones, J.K.N., & Perry, M.B., ... Chem. Ind. (London), 255, (1961).

63. Gunner, S.W., Jones, J.K.N. & Perry, M.B., ... Can. J. Chem., 39, 1892, (1961).

64. Kotchetkov, N.K., & Chizov, O.S., ... Meth. Carbohydrate Chem., 6, 590, (1972).

65. Ellis, W.C., ... J. Chromatog., 41, 335, (1969).

66. Curtius, H-Ch., Muller, M., & Völlmin, J.A., ... J. Chromatog., 37, 216, (1968).

67. Oates, K.D.G., & Schrager, J., ... J. Chromatog. 28, 232, (1967).

68. Reid, P.M., Donaldson, B., Secret, D.M., & Bradford, B., ... J. Chromatog. 47, 199, (1970).

69. Buchanan, J.G., & Clode, D.M., ... J. Chem. Soc. Perkin I, 3, 385, (1974).

70. Koenig, W.A., Bauer, H., Voelter, W., & Rayer, E., ... Chem. Ber., 106, 1905, (1973).

71. DeJongh, D.C., Radford, T., Hribar, J.D., Hanessian, S., Bieber, M., Dawson, G., & Sweeley, C.C., ... J. Amer. Chem. Soc., 91, 1728, (1969).

72. Sharkey, A.G., Friedel, R.A., & Langer, S.H., ... Analyt. Chem., 29, 770, (1957).

73. Richter, W.J., & Hunnemann, D.H., ... Helv. Chim. Acta., 57, 1131, (1974).

74. Beynon, J.H., Saunders, R.A., & Williams, A.E., ... The Mass Spectra of Organic Molecules, p.424 Elsevier, Amsterdam (1968).

75. Zinbo, M., & Sherman, W.R., ... J. Amer. Chem. Soc., 92, 2105, (1970).

76. Draffan, G.H., Stillwell, R.N., & McCloskey, J.A. .. .. Org. Mass Spectrom., 1 669, (1968)

77. McLafferty, F.W., ... Analyt. Chem. 31, 82, (1959).

78. Kingston, D.G.I., Bursey, J.T., & Bursey, M.M., ... Chem. Rev., 74, 215, (1974).

79. Meyerson, S., & Weitkamp, A.W., ... Org. Mass Spectrom., 1, 659, (1968).

80. Natalis, P., ... Mass Spectrometry, NATO Advanced Study Inst., (Glasgow) 379, (1964).

81. Petersson, G., & Samuelson, O., ... Svensk. Papperstidn., 71, 731, (1968).

82. Kochetkov, N.K., & Chizkov, O.S., ... Advan. Carbohydrate Chem., 21, 39, (1966).

83. Hanessian, S., ... Methods of Biochemical Analysis (ed. Glick, D), 1 105, (1971).

84. Cooks, R.G., & Johnson, G.S., ... Specialist Periodical Reports. Mass Spectrometry Chem. Soc., (London) 1, 164.

85. Finan, P.A., Reed, R.I., & Sneddon, W., .. Chem. Ind., (London). 1722, (1958).

86. Finan, P.A., Reed, R.J., & Wilson, J.M., .. J. Chem. Soc., 5945, (1963).

87. Campbell, I.R., & Bentley, R., ... Adv. Chem. Ser., 117, 1, (1973).

88. Kochetkov, N.K., Chizov, O.S., & Molodtsov, M.V., ... Tetrahedron, 24, 5587, (1968).

89. Havlicek, S.C., Brennan, M.R., & Scheuer, P.J., ... Org. Mass Spectrom., 5, 1273, (1971).

90. Gustafsson, J.A., Ryhage, R., Sjövall, J., Moriarty, R.M., ... J. Amer. Chem. Soc., 91, 1234, (1969).

91. Sloan, S., Harvey, D.J., & Vouros, P., .. Org. Mass Spectrom, 5, 748, (1971).

92. Harvey, D.J., Vouros, P., & Horning, M.G., ... Appl. Spectroscopy, 25, 139, (1971).

93. Harvey, D.J., & Horning, M.G., ... J. Chromatog., 76, 51, (1973).

94. Petersson, G., ... Tetrahedron, 25, 4437, (1969).

95. Petersson, G., Samuelson, G., Anjou, K., & Sydow, E., von., ... Acta. Chem. Scand., 21, 1251, (1967).

96. Petersson, G., ... Carbohydrate Res., 33, 47, (1974).

97. deWilt, H.G.J., & Tsuchiya, T., ... Mass Spectroscopy, 18, 1274, (1970).

98. deWilt, H.G.J., ... J. Chromatog, 63, 379, (1971).

99. Kammerling, J.P., Vliegenthart, J.F.G., Vink, J., & deRidder, J.J., ... Tetrahedron, 27, 4275, (1971).

100. Kamerling, J.P., Vliegenthart, J.F.G., Vink, J., & deRidder, J.J., ... Tetrahedron Letts., 26, 2367, (1971).

101. Kamerling, J.P., Vliegenthart, J.F.G., Vink, J., & deRidder, J.J., ... Tetrahedron, 27, 4749, (1971).

102. Hayashi, A., Matsubara, T., ... Paper given at International Symposium on Mass Spec. in Biochem. and Med. (Pfeiffer Int. House), 1973.

103. Wolfrom, M.L., & Solms, J., ... J. Org. Chem., 21, 815, (1956).

104. Sugihara, J.M., & Bowman, C.M., ... J. Amer. Chem. Soc., 80, 2443, (1958).

105. Letsinger, R.L., & Hamilton, S.B., ... J. Org. Chem., 25, 592, (1960).

106. Ferrier, R.J. .. .. J. Chem. Soc., 2325, (1961).

107. Aroney, M.J., Le Fevre, R.J.W., Murthy, D.S.N., & Saxby, J.D., ... J. Chem. Soc., B., 1066, (1966).

108. Ferrier, R.J., Prasad, D., Rudowski, A., & Sangster, I., ... J. Chem. Soc., 3330, (1964).

109. Ferrier, R.J., Prasad, D., & Rudowski, A., ... J. Chem. Soc., 858, (1963).

110. Ferrier, R.J., Hanneford, A.J., Overend, W.G., & Smith, B.C., ... Carbohydrate Res., 1, 38, (1965).

111. Ferrier, R.J., & Prasad, D., ... J. Chem. Soc., 3798, (1965).

112. Ferrier, R.J., & Prasad, D., ... J. Chem. Soc., 7425, (1965).

113. Bourne, E.J., McKinley, I.R., and Weigel, H., ... Carbohydrate Res., 25, 516, (1972).

114. Brimacombe, J.S., & Portsmouth, D., ... J. Chem. Soc., C, 499, (1966).

115. Veronikina, S.G., Yurkevitch, A.M., & Preobrazhensku, N.R., ... Zh. Obsch. Khim., 37, 2181, (1967). via. Chem. Abs. 68, 96091n, (1968).

116. Bowie, R.A., & Musgrave, O.C., ... J. Chem. Soc., 3945, (1963).

117. McKinley, I.R., & Weigel, H., ... Carbohydrate Res., 31, 17, (1973).

118. Shimanouchi, H., Saito, N., & Sasadu, Y., ... Bull. Chem. Soc., Japan, 42, 1239, (1969).

119. Baillie, T.A., ... Ph.D. Thesis, (1973). U. GLASGOW

120. Robinson, D.S., Bagles, J., and Self, R., ... Carbohydrate Res., 26, 204, (1973).

121. Brimacombe, J.S., Hunedy, F., and Husain, A., ... Carbohydrate Res., 10, 141, (1969).

122. Brooks, C.J.W., & Watson, J., ... Chem. Comm., 952, (1967).

123. Brooks, C.J.W., & Watson, J., ... Gas Chromatography, (Harbourn, C.L.A., ed), Proceedings of The 7th International Symposium of Gas Chromatography, Copenhagen, Institute of Petroleum (London), 129, (1969).

124. Anthony, G.M., Brooks, C.J.W., MacLean, I., & Sangster, I., ... J. Chromatog. Sci., 8, 623, (1969).

125. Brooks, C.J.W., & Harvey, D.J., ... Biochem. J., 114, 15P, (1969).

126. Brooks, C.J.W., & Middleditch, B.S., & Anthony, G.M., ... Org. Mass Spectrom., 2, 1023, (1969).

127. Brooks, C.J.W., Harvey, D.J., & Middleditch, B.S., ... Org. Mass Spectrom., 3, 231, (1970).

128. Brooks, C.J.W., Anthony, G.M., & Middleditch, B.S., ... J. Pharm. Parmacol., 22, 204, (1971).

129. Brooks, C.J.W., & Harvey, D.J., ... J. Chromatog, 54, 193, (1971).

130. Brooks, C.J.W., & MacLean, I., ... J. Chromatog. Sci., 2, 18, (1971).

131. Brooks, C.J.W., Middleditch, B.S., & Harvey, D.J., ... Org. Mass Spectrom., 5, 1429, (1971).

132. Wood, P.J., & Siddiqui., ... Carbohydrate Res., 19, 283, (1971).

133. Greenhalgh, R., & Wood, R.J., ... J. Chromatog, 82, 410, (1973).

134. Eisenberg, F., ... Carbohydrate Res., 19, 135, (1971).

135. Eisenberg, F., ... Analyt. Methods, 11, 168, (1972).

136. McKinley, I.R., & Weigel, H., ... Chem. Comm., 1051, (1972).

137. Wood, P.J., & Siddiqui, I.R., ... Carbohydrate Res., 32, 97, (1974).

138. Bourne, E.J., et al., ... Carbohydrate, Res., 35, 141, (1974).

139. Williams, D.H., & Fleming, I., ... Spectroscopic Methods in Organic Chemistry 105, McGraw Hill, London, (1966).

140. Von Rudloff, E., ... Recent Advances in Phytochemistry, 2, 127, (1969).

141. Narayuran, C.S., Kulkarni, K.S., Vardya, A.S., Kanthamani, S., Lakshmi Kumari, G., Bupat, B.V., Paknikar, S.K., Kulkarni, S.N., Kelkar, G.R., & Bhattacharyya, S.C. Tetrahedron, 20, 936, (1964).

142. Lee, K.H., & Geissman, T.A., ... Phytochemistry, 9, 403, (1970).

143. Sánchez-Viesca, F., & Romo, J., ... Tetrahedron, 19, 1285, (1963).

144. DeVivar, A.R., Guerrero, C., Diaz, E., & Ortega, A. .. .. Tetrahedron, 26, 1657, (1970).

145. Holub, M., Popu, D.P., Sumek, Z., Herout, V., & Sǒrm, F., ... Coll. Czech. Chem. Comm. 35, 3296, (1970).

146. Somasekar Rao, A., Paul, A., Bhattacharyya, S.C., ... Tetrahedron, 13, 319, (1961).

147. Kingston, D.G.I., Rao, M.M., Spittler, T.D. Tetrahedron Letts., 1613, (1971).

148. Shaligram, A.M., Rao, A.S., & Bhattacharyya, S.C., ... Tetrahedron, 18, 969, (1962).

149. Kulkarni, G.H., Kelkar, G.R., & Bhattacharyya, S.C., ... Tetrahedron, 20, 2639, (1969).

150. Ashima, H., Kato, H., & Fukawa, H., ... Ger. Offen. 2.019, 835 12 Nov. 1970 (Japan) via. Chem. Abs. 74 28733t. (1971).

151. Aebi, A., & Waaler, T., ... Über die Inhaltsstoffe von Petasites hybridus (L7 Fl. Wetl.) Verlag Helbing and Lichtenhahn, Basil, 1959.

152. Morikaw, K., & Hirose, Y., ... Tetrahedron Letts. 1799 1969.

153. Borges del Castillo, J., Brooks, C.J.W., & Campbell, M.M. .. .. Tetrahedron Letts., 3731, (1966).

154. Kubelka, V., Mitero, J., & Zachař, P., .. J. Chromatog, 74. 195, (1972).

155. Vanden Heuvel, W.J.A., & Horning, E.C., .. Biochim. Biophys. Acta. 64, 616, (1962).

156. Connel, D.W., ... J. Chromatog, 45, 129, (1969).

157. Andersen, M.H., & Falcone, M.S., ... J. Chromatog, 44, 52, (1969).

158. McDonald, K.L., & Cartlidge, D.M., ... J. Chromatog, Sci., 9, 440, (1971).

159. Juvonen, S., Huovinen, K., ... Farm. Aikak, 81, 80, (1972).

160. Von Schantz, M., Widen, K.G., & Granvist, L., ... Rev. Ital. Essenze, Profumi, Piante Off. Aromi, Saponi Cosmet., Aerosol, 55, 565, (1973). via. Chem. Abs. 80, 124571h (1974).

161. Hodges, R., White, E.P., Shannon, J.S. .. Tetrahedron Letts., 371, (1964).

162. Boocock, D.G.B., & Waight, E.S., ... Chem. Commun., 90, (1966)

163. Kupchan, S., Cassady, J.M., Kelsey, J.E., Schnoes, H.K., Smith, D.H., & Burlingame, A.L., ... J. Amer. Chem. Soc., 88. 5292, (1966).

164. Hayashi, S., Sato, H., Hayashi, N., Okuda, T., & Matsuura, T., ... J. Sci. Hiroshima Univ., Ser. A-Z 31, 217, (1967).

165. Wasadua, N., Tsuchiya, T., Yoshi, E., & Watanabe, E., ... Tetrahedron, 23, 4223, (1967).

166. Sathe, R.M., Kulkarni, G.H., Kelkar, G.R., & Das, K.G., ... Org. Mass Spectrom, 2, 935, (1969).

167. Shirahita, K., Kato, T., & Kitahara, Y., .. Tetrahedron, 25, 3179, (1969).

168. Tsai, L., Highet, R.J., Herz, W., ... J. Org. Chem., 34, 945, (1969).

169. Irie, T., Suzuki, M., Kurosawa, E., & Masamune, T., ... Tetrahedron, 26, 32, 71, (1970).

170. Das, K.G., Nayar, N.S.B., Joshi, B.S., .. Org. Mass Spectrom, 5, 187, (1971).

171. Matsuo, A., ... Tetrahedron, 27, 2757, (1971).

172. Hirose, Y., ... Mass Spectroscopy, (Shitsuryo Bunseki), 15, 162, (1967).

173. Moshonas, M.G., & Lund, E.D., ... The Flavour Industry, June, 1970.

174. Stenhagen, E., Abrahamsson, S., & McLafferty, F.W., ... Atlas of Mass Spectral Data, 2, 1448, (1969).

175. Von Sydow, E., Anjou, K., Karlsson, G., .. Archives of Mass Spectral Data 1, 499, (1970).

176. Mass Spectrometry Data Centre, Aldermaston, Reading, RG7 4PR., U.K.

177. Keates, R.A.B., Ph.D. Thesis, Appendix A. (Ref. 80)

178. MacKintosh, A.G.M., Ph.D. Thesis, Univ. of Glasgow, 1971.

179. Juvonen, S., ... Farm. Aikak, 79, 137, (1970). via. Chem. Abs., 74, 67611w (1971).

180. Keates, R.A.B., Ph.D. Thesis, University of Glasgow, 1971.

181. Hutchinson, J., ... The Genera of Flowering Plants, Vol. 1. 62. Oxford, (1962).

182. Appel, H.H., Brooks, C.J.W., & Overton, K.H., ... J. Chem. Soc., 3322, (1959).

183. Appel, H.H., Connolly, J.D., Overton, K.H., & Bond, R.P.M., ... J. Chem. Soc., 4658, (1960).

184. Bond, R.P.M., & Overton, K.H., ... Tetrahedron, 19, 635, (1963).

185. Poyer, P.M., Ritchie, E., & Taylor, W.C. Aust. J. Chem., 21, 1379, (1968).

186. Aebi, A., Büchi, J., Waaler, T., Eichenberger, E., & Schmutz, J., .. Pharm. Acta. Helv., 29, 277, (1955).

187. Aebi, A., & Djerassi, C., ... Helv. Chim. Acta., 42, 1785, (1959).

188. Herbst, D., & Djerassi, C., ... J. Amer. Chem. Soc., 82, 4337, (1960).

189. Novotný, L., Jizba, J., Herout, V., & Šorm, F., ... Colln. Czech. Chem. Commun. 27. 1393, (1962).

190. Novotný, L., Jizba, J., Herout, V., Šorm, F., Zalkow, L.H., Hu, S., & Djerassi, C., ... Tetrahedron, 19, 1101, 1963.

191. Novotný, L., Tabačíková - Wlotzka, Ch., Herout, V., & Šorm, F., ... Colln. Czech. Chem. Comm. 29, 1922, (1964).

192. Novotný, L., Herout, V., & Šorm, F. .. .. Colln. Czech. Chem. Commun., 27, 1400, (1962).

193. Novotný, L., Kotva, K., Toman, J., & Herout, V., ... Phytochem, 11, 2795, (1972).

194. Novotný, L., Toman, J., Starý, F., Marquez, A.D., Herout, V., & Sorm, F., .. Phytochem, 5, 1281, (1966).

195. Hegi, G., ... Illutrierte Flora Von Mittel - Europa. Band VI: 2 Teil, 680, Carl Hanser Verlag, Munchen, (Second ed.) 1954.

196. Otham, G., & Stenhagen, E., ... Biochemical Applications of Mass Spectrometry (Waller, G.R. ed) 216, Wiley - Interscience, London, (1972).

197. Gilbert, J.D., Ph.D. Thesis, Appendix II, University of Glasgow, 1972.

198. Nagano, N., Tanahashi, Y., Moriyama, Y., & Takahashi, T., ... Bull Chem. Soc. Japan 46, 2840, (1973).

199. Dress, W.J., ... Baileya, 10, 63, (1962).

200. Ishii, H., Tozyo, T., & Minato, H., ... Tetrahedron, 21, 2605, (1965).

201. ibid., ... J. Chem. Soc., C., 17, 1545, (1966).

202. Ishii, H., Tozyo, T., Nakamura, M., & Minato, H., ... Chem. Commun., 106, (1968).

203. ibid., ... Tetrahedron, 26, 2911, (1970).

204. Ishizaki, Y., Tanahashi, Y., Moriyama, Y., Takahashi, T., & Koyama, H., ... Phytochem., 13, 674, (1974).

205. Ishizaki, Y., Tanahashi, Y., & Takahashi, T., Chem. Commun, 551, (1969).

206. Mariyana, Y., Tsuyuki, T., Takahashi, T., & Koyama, H., ... Phytochem., 13, 288, (1974).

207. Tada, M., Moriyama, Y., Tanahashi, Y., & Takahashi, T., ... Tetrahedron Lett., 43, 4007, (1971).

208. Ishizaki, Y., Tanahashi, Y., Takahashi, T., & Tori, K., ... Tetrahedron, 26, 5387, (1970).

209. Nagano, H., Moriyama, Y., Tanahashi, Y., Takahashi, T., Fukuyama, M., & Sato, K. Chem. Lett. 13. (1972).

210. Ohwi, J., ... Flora of Japan, (in English) 881, Smithsonian Institution, Washington, D.C., (1965).

211. Tanahashi, Y., Takeyoshi, T., & Hiroshige, K., ... Bull. Nat. Science Mus. (Tokyo), 12, 633, (1969). via. Biol. Abs. 52, 41490 (1971).

212. Klásek, A., Vrublovský, P., & Šantavý, F., . Collect. Czech. Chem. Commun. 32. 2512, (1967).

213. Klásek, A., Sedmera, P., & Šantavý, F., .. Collect. Czech. Chem. Commun. 35. 956, (1970).

214. Birnbaum, K.B., Klasek, A., Sedmera, P., Snatzke, G., Johnson, L.F., Šantavý, F., ... Tetrahedron Lett., 3421, (1971).

215. Klásek, A., Sedmera, P., Šantavý, F., ... Collect. Czech. Chem. Commun., 36., 2205, (1971).

216. Eglinton, G., & Hamilton, R.J., ... Chemical Plant Taxonomy, 137, (ed. Swain, T.,) Academic Press, London. (1963).

217. Budzikiewicz, H., Djerassi, C., & Williams, D.H., ... Interpretation of Mass Spectra of Organic Compounds (a) 198, (b) 163, Holden Day Inc., San Francisco, 1964.

218. Budzikiewicz, H., Djerassi, C., & Williams, D.H., ... Structure Elucidation of Natural Products by Mass Spectrometry, Vol. II. Holden-Day Inc., London, (1964).

219. Budzikiewicz, H., Wilson, J.M., & Djerassi, C., ... J. Amer. Chem. Soc., 85, 3688, (1963).

220. Knights B.A., ... J. of Gas Chromatog., 273, (1967).

221. Herout, V., & Šorm, F., ... Perspectives in Phytochemistry, 139, (ed. Swain, T.) Proceedings of the Phytochemical Soc. Symposium, Cambridge, (April, 1968).

222. Bohlmann, F., Zdero, C., & Rao, N., .. Chem. Ber. 105, 3523, (1979).

223. Karpati, A., Rave, A., Deutsch, J., & Mandelbaum, A., ... J. Amer. Chem. Soc., 95, 4244, (1973).

224. Hammerum, S., & Djerassi, C., ... J. Amer. Chem. Soc., 95, 5806, (1973).

225. Brooks, C.J.W., & Keates, R.A.B., ... Phytochem., 11, 3235, (1972).

226. Overberger, C.G., & Anselme, J.P., .. J. Org. Chem. 28. 592, (1963).

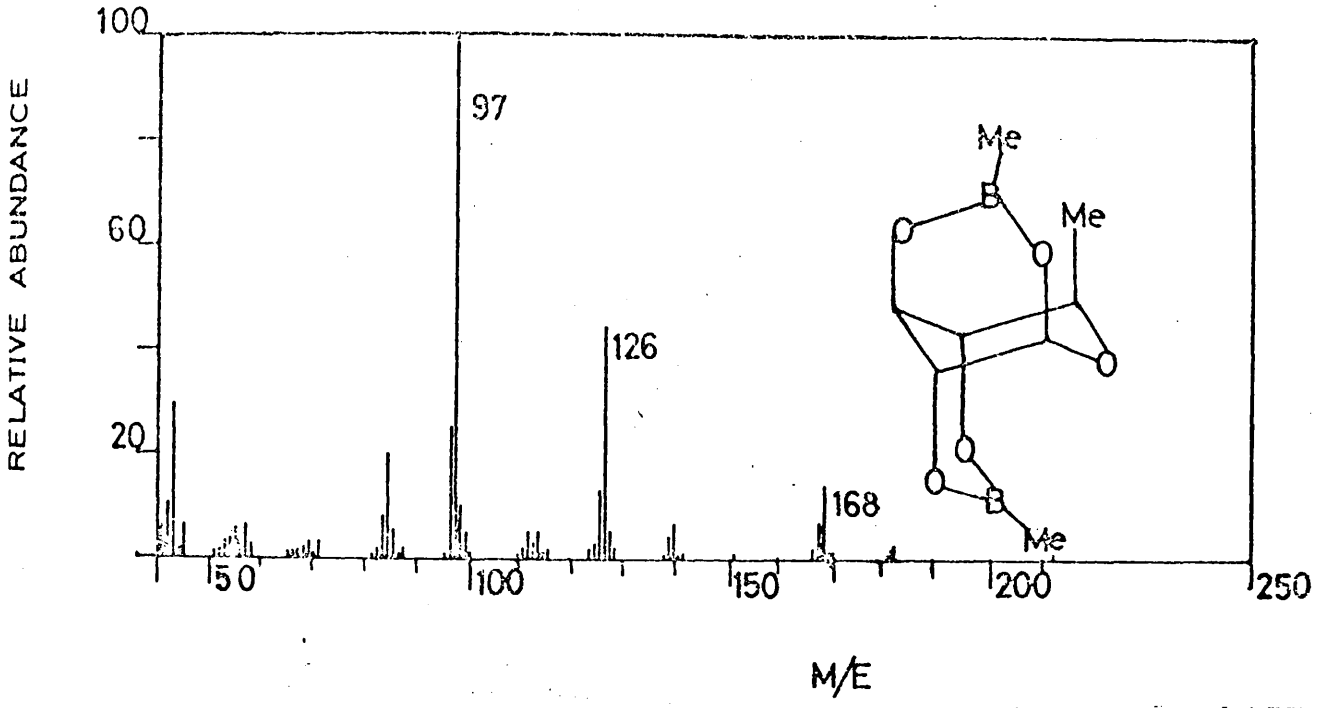
227. Corina, D.L., ... Analyt-Biochem., 53, 571, (1973).

228. Klemm, H.P., Hintze, U., & Gercken, G., . J. Chromatog, 75. 19, (1973).

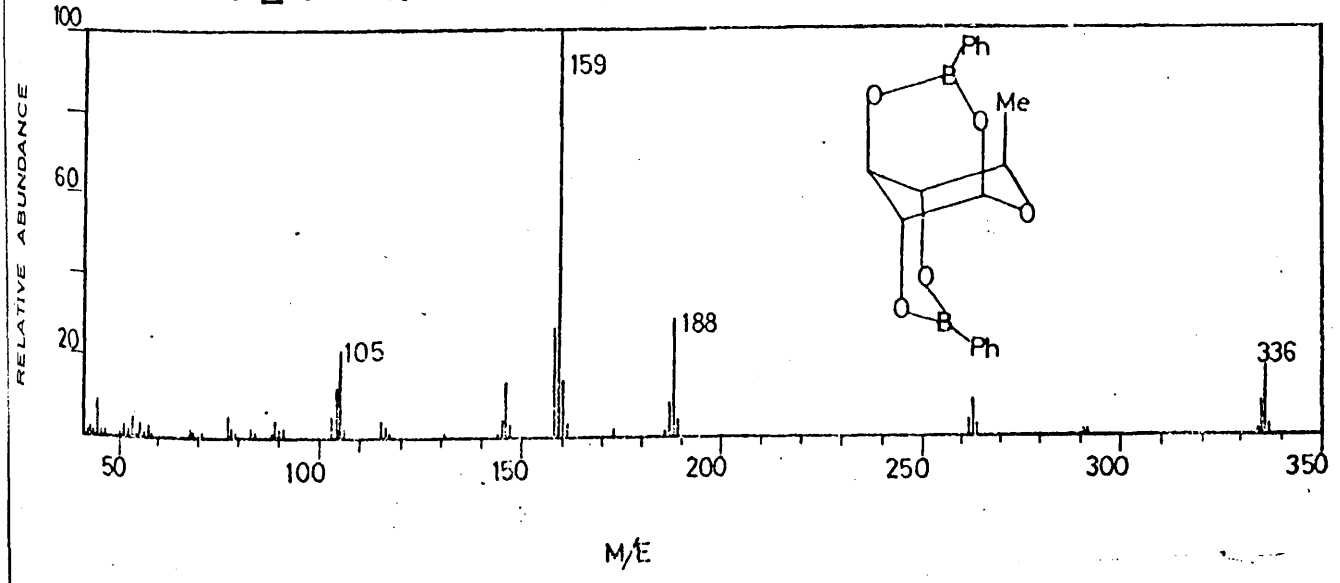
APPENDIX I

Mass Spectral Line Diagrams.

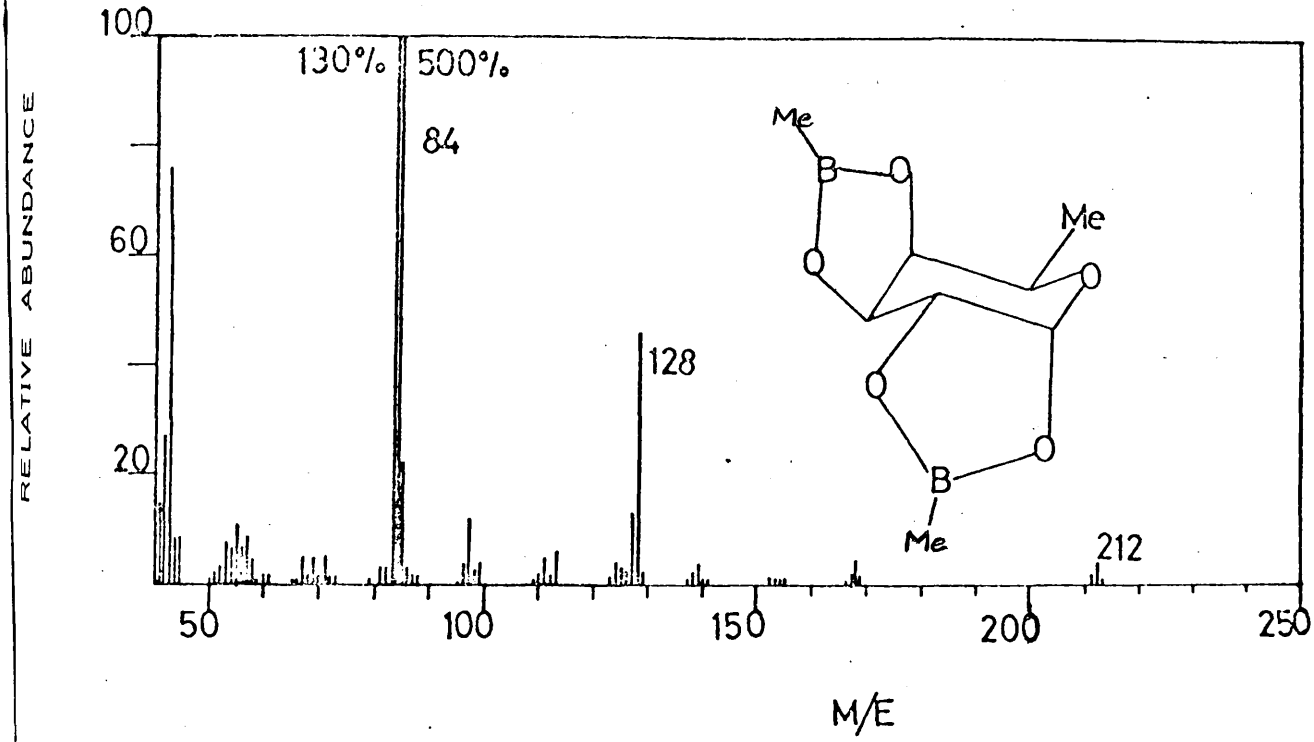
1. 6-deoxy-D-glucopyranose methyl diboronate.



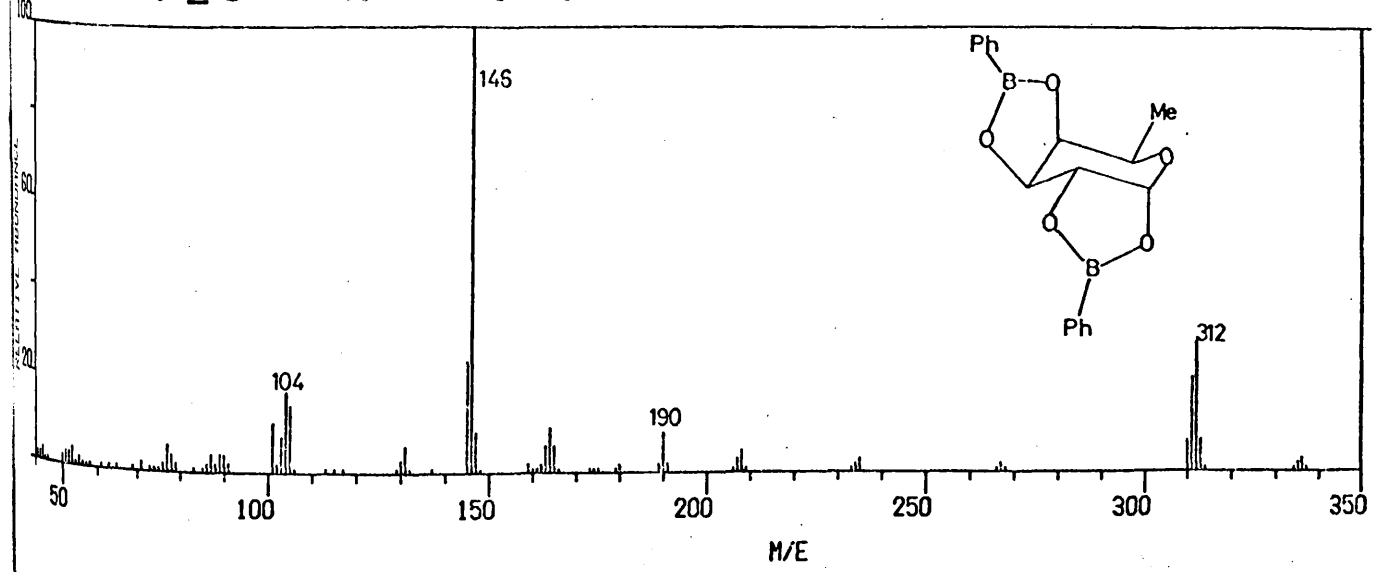
2. 6-deoxy-D-glucopyranose phenyl diboronate.



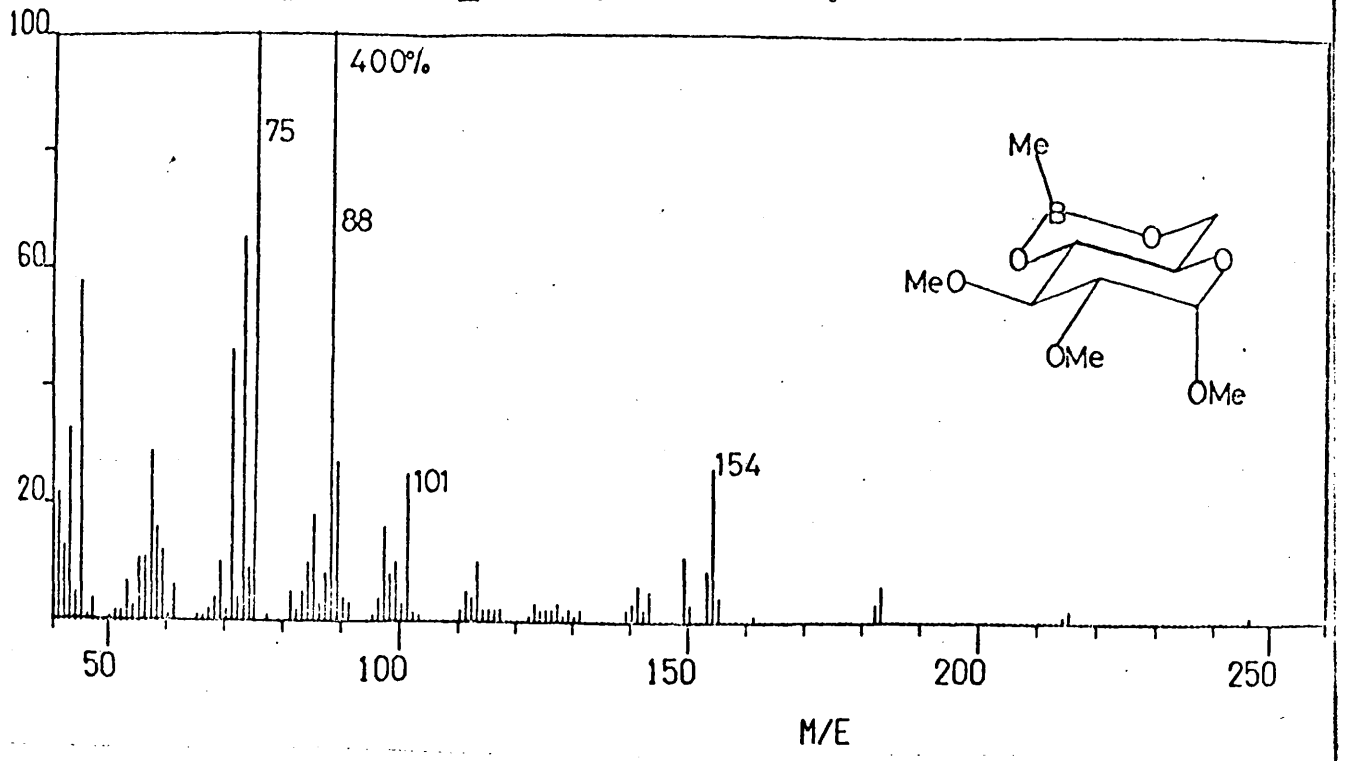
3. 6-deoxy-D-galactopyranose methyl diboronate.



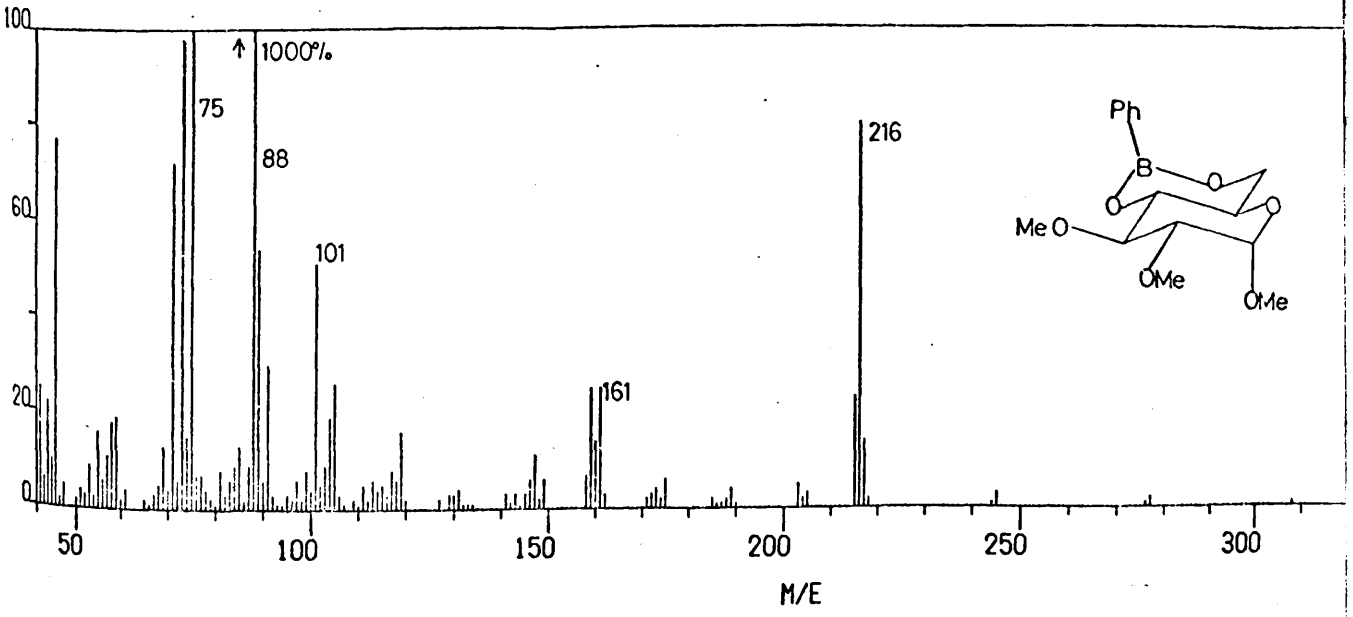
4. 6-deoxy-D-galactopyranose phenyl diboronate.



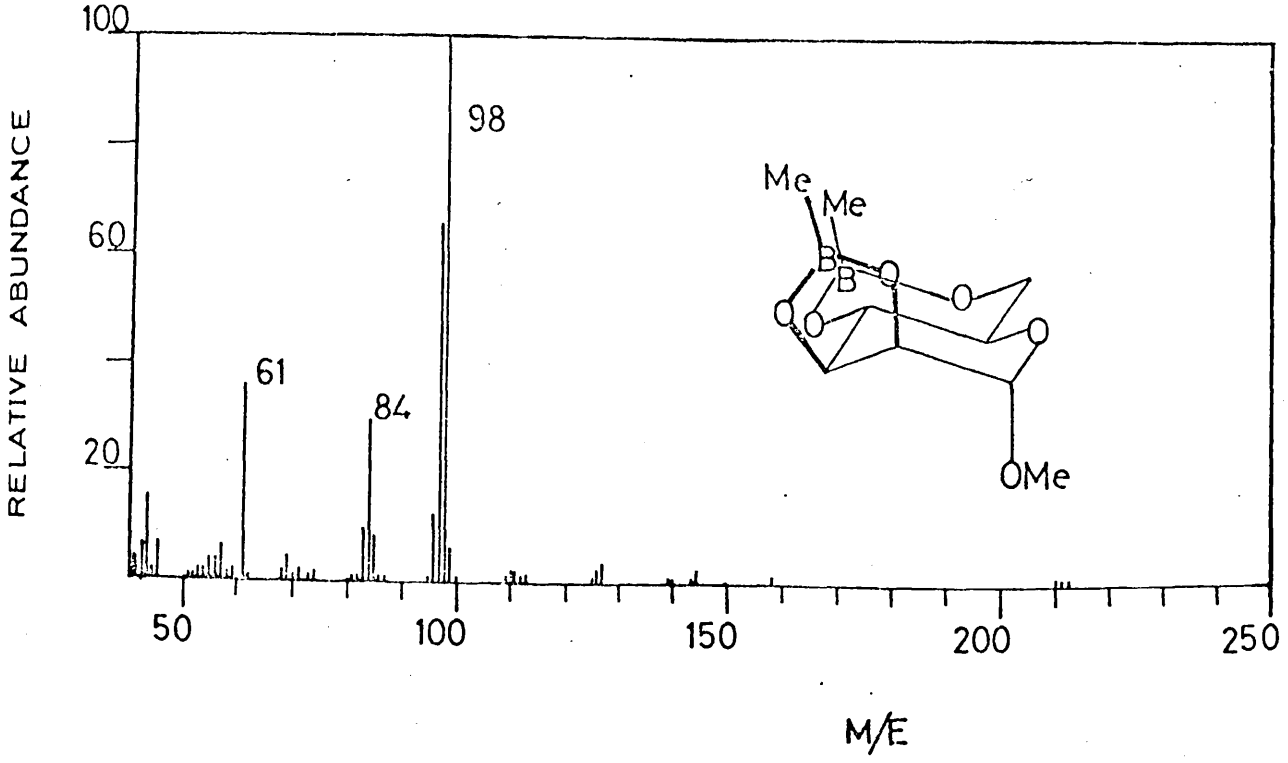
5. Me 2,3-di-O-methyl α-D-glucopyranoside methylboronate



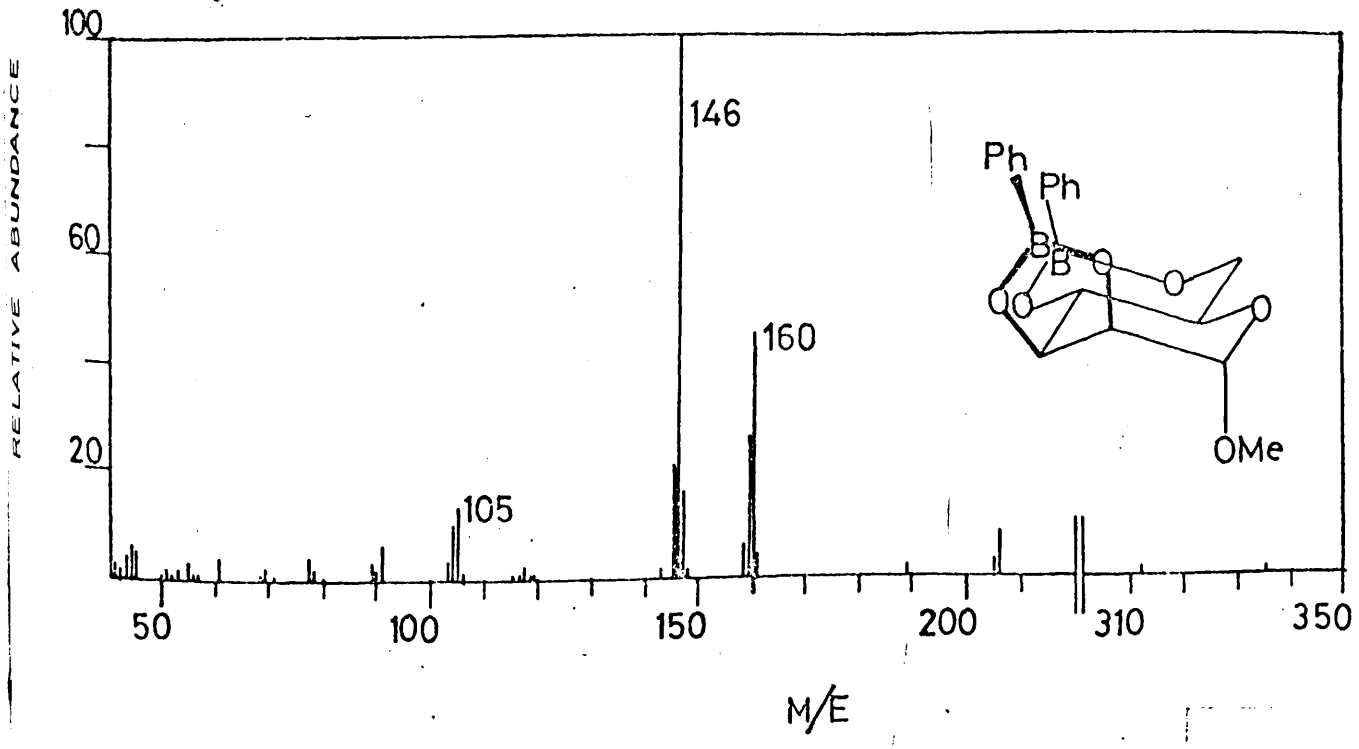
6. Me 2,3-di-O-methyl α-D-glucopyranoside phenylboronate



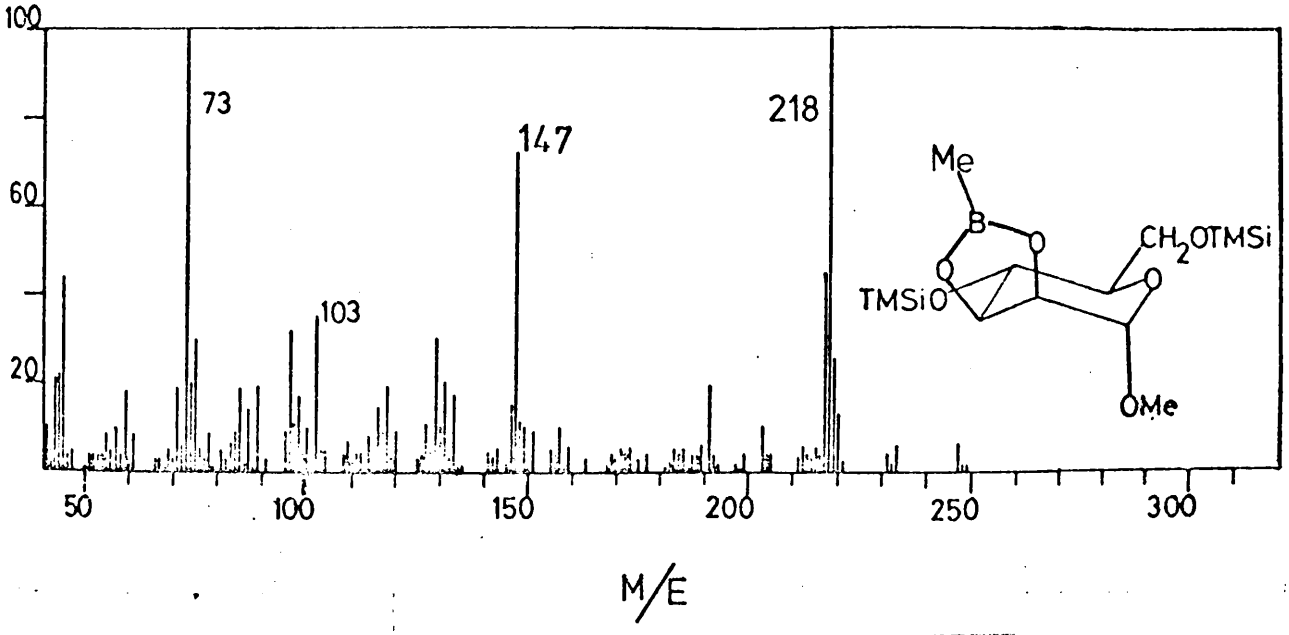
7. Me α-D-mannopyranoside methyl diboronate.



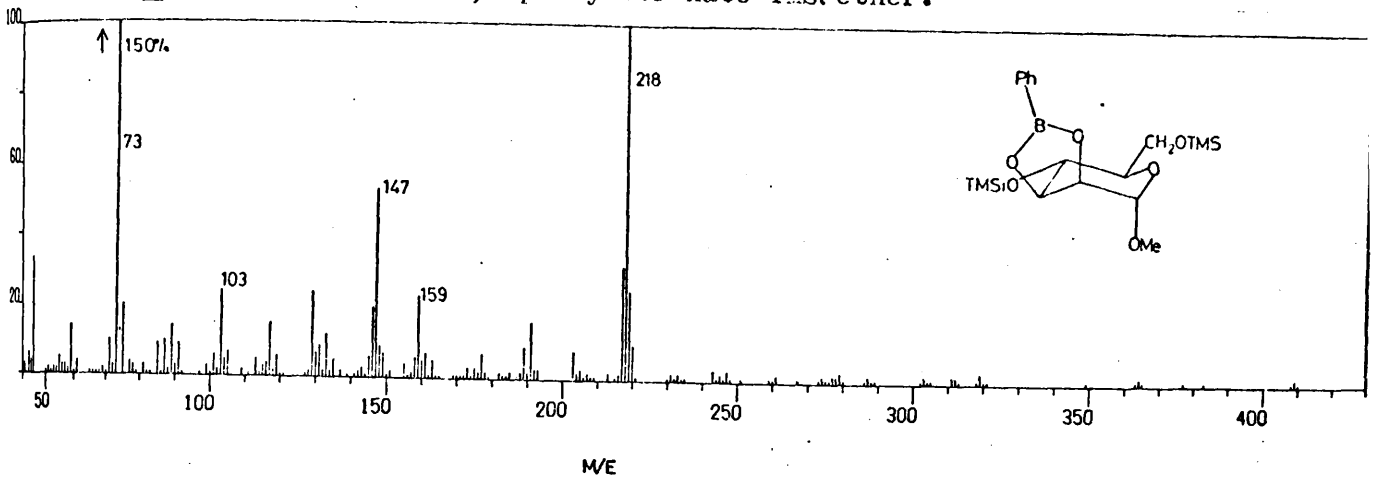
8. Me α-D-mannopyranoside phenyl diboronate.



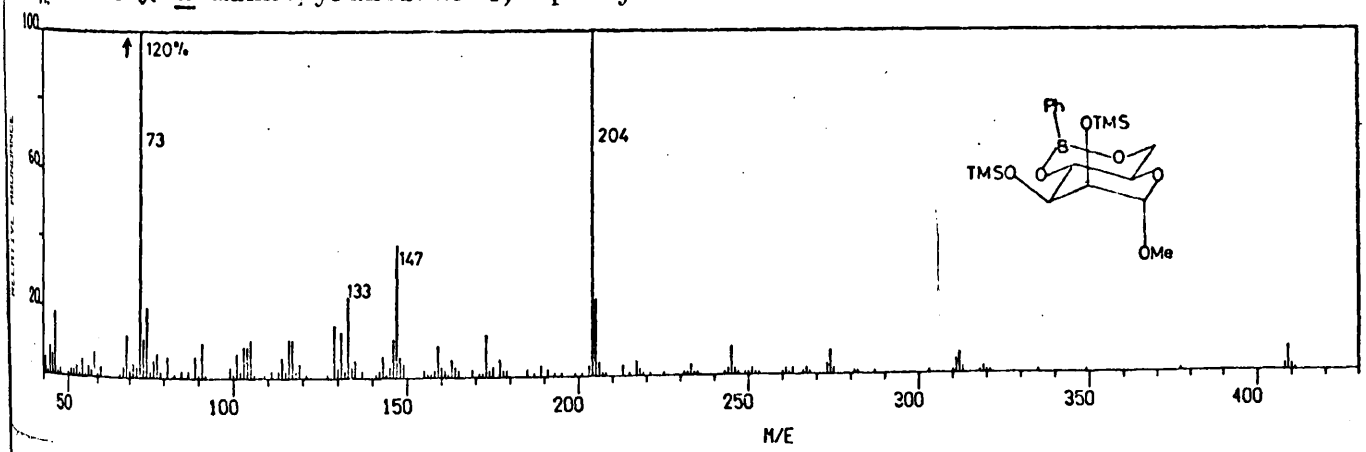
9. Me α-D-mannopyranoside 2,3-methylboronate TMSi ether.



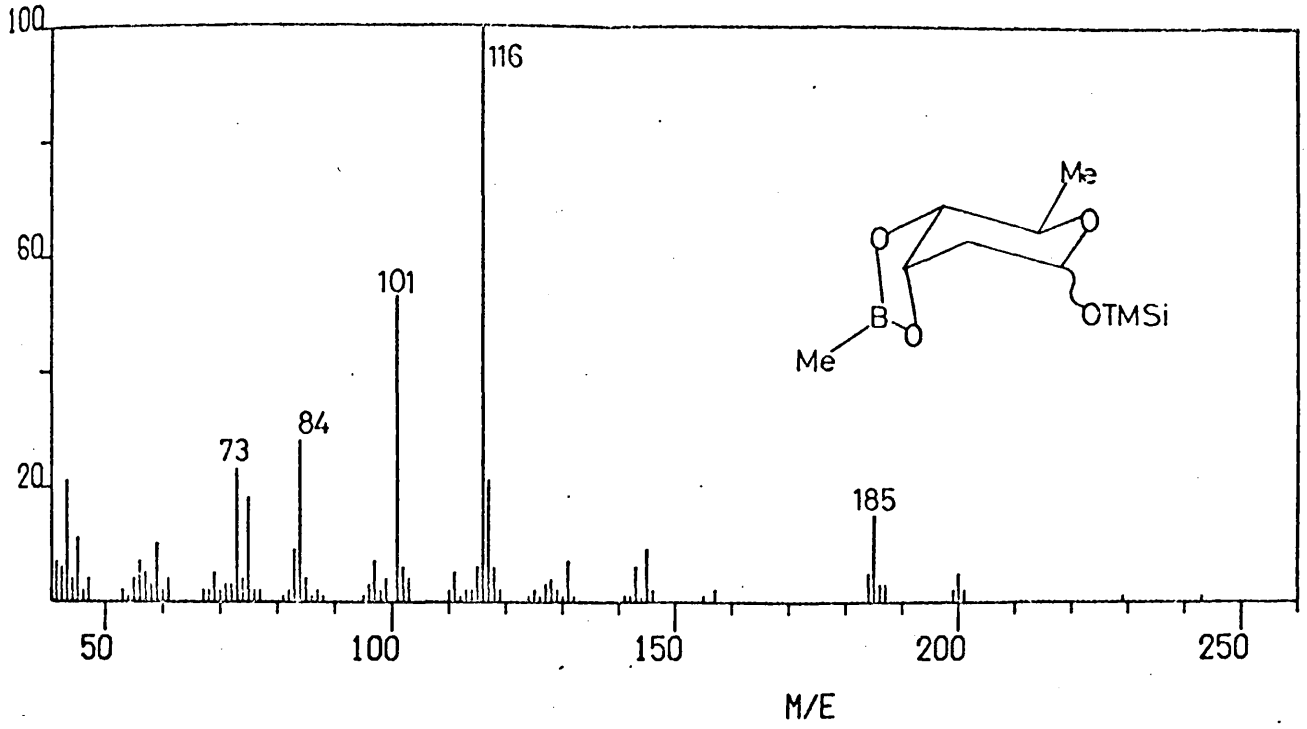
10. Me α-D-mannopyranoside 2,3-phenylboronate TMSi ether.



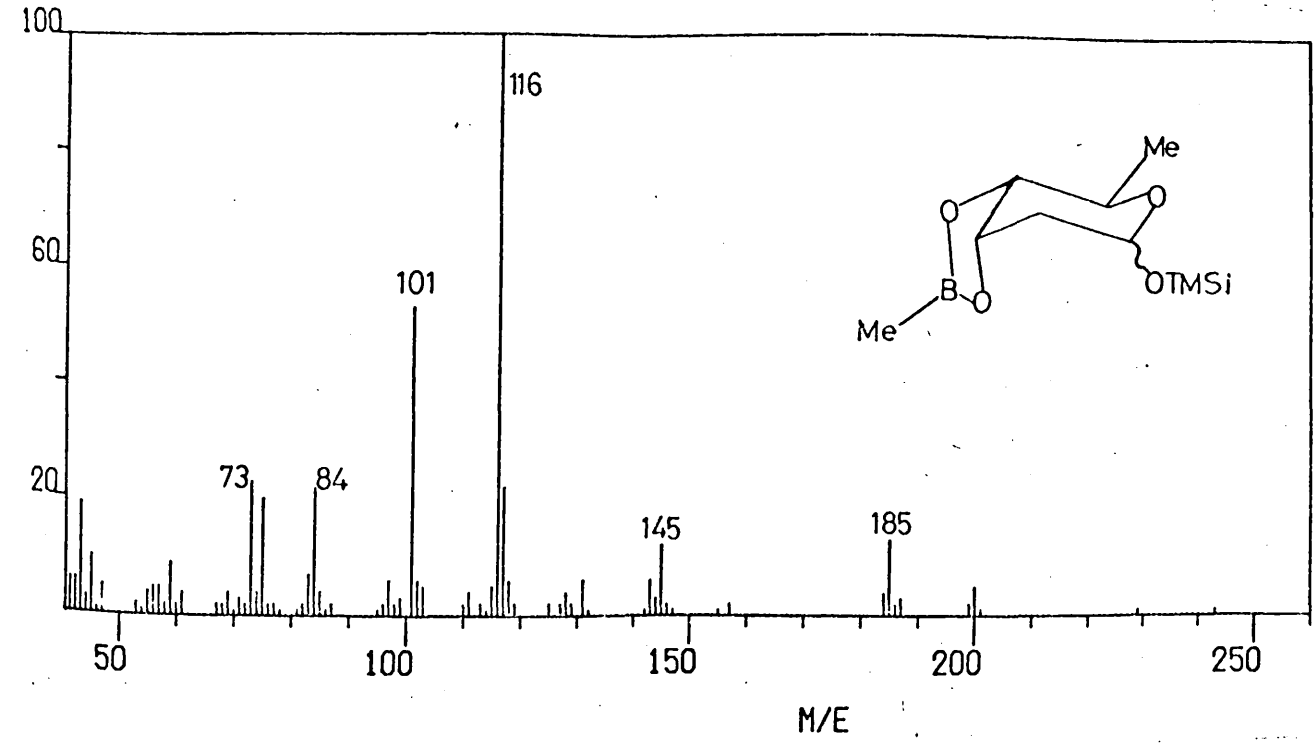
11. Me α-D-mannopyranoside 4,6-phenylboronate TMSi ether.



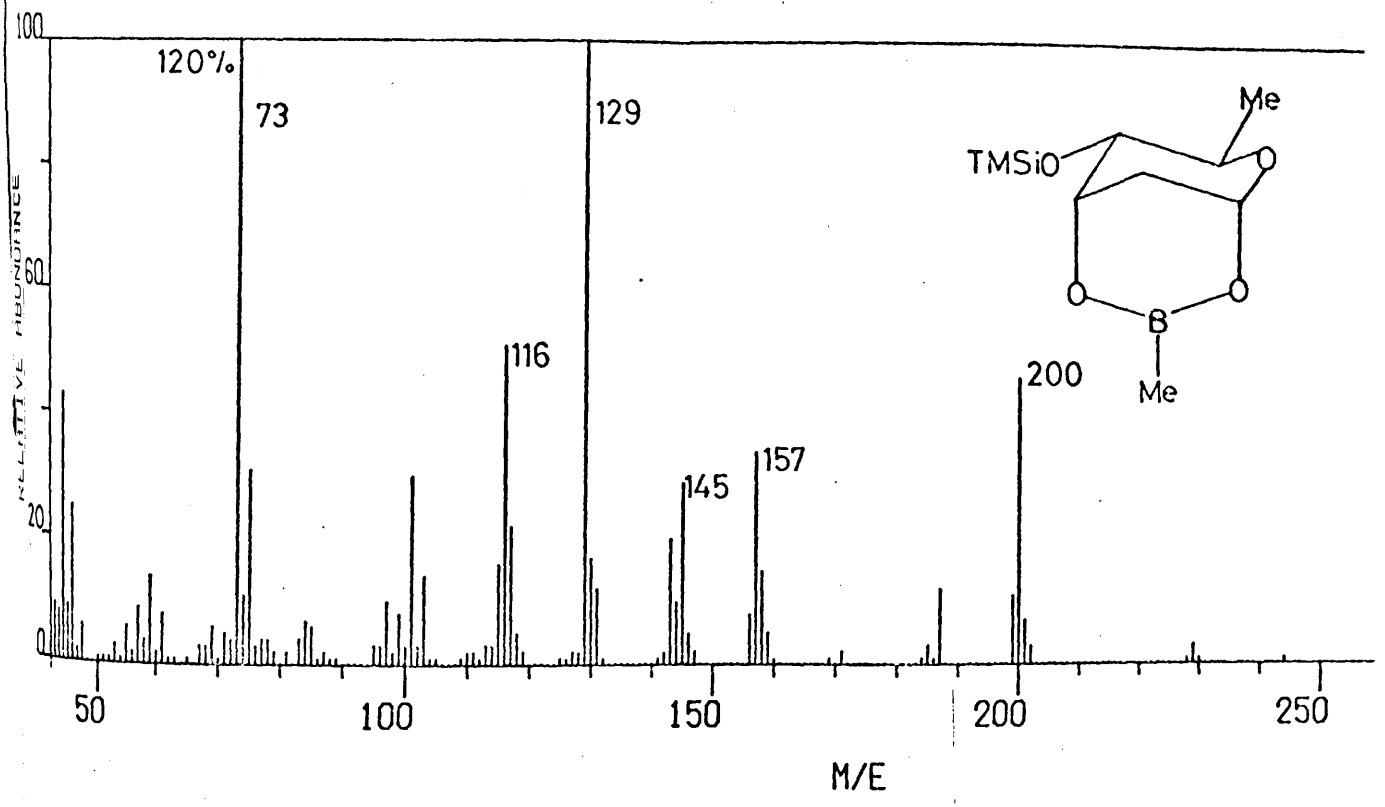
12. D-digitoxose 3,4-methylboronate TMSi ether.



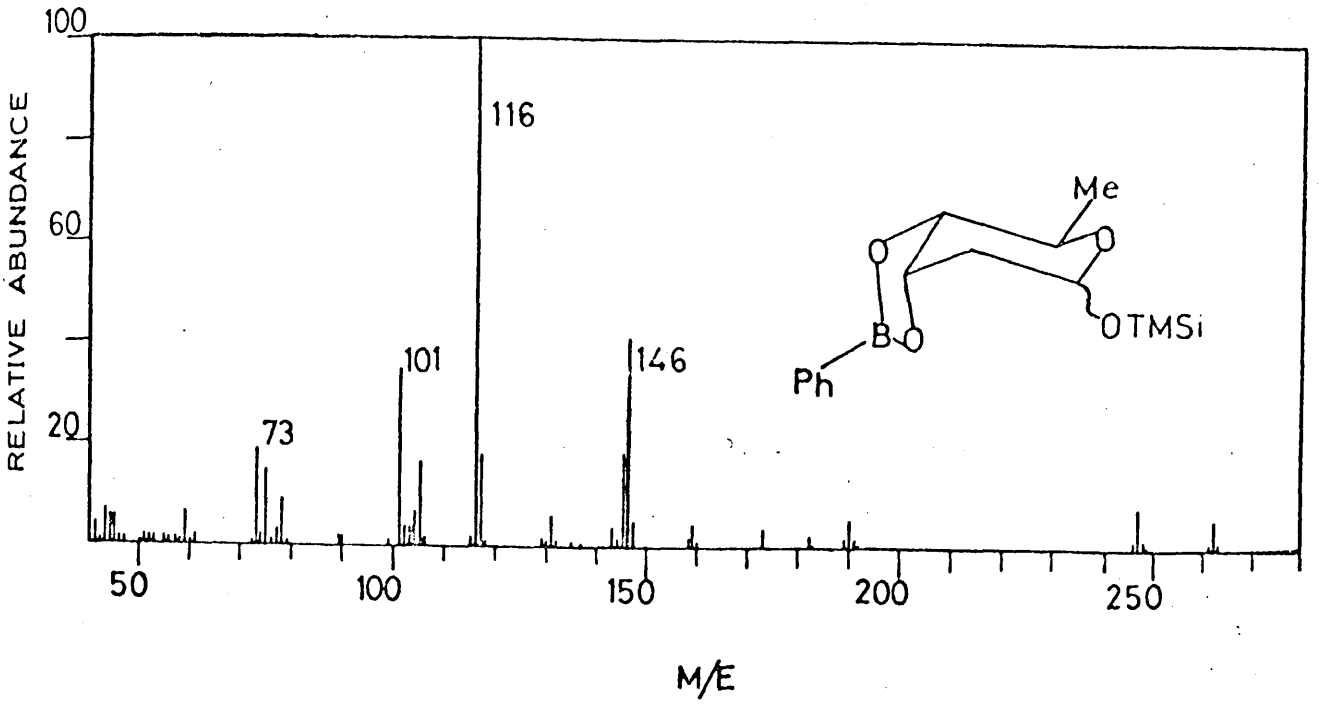
13. D-digitoxose 3,4-methylboronate TMSi ether.



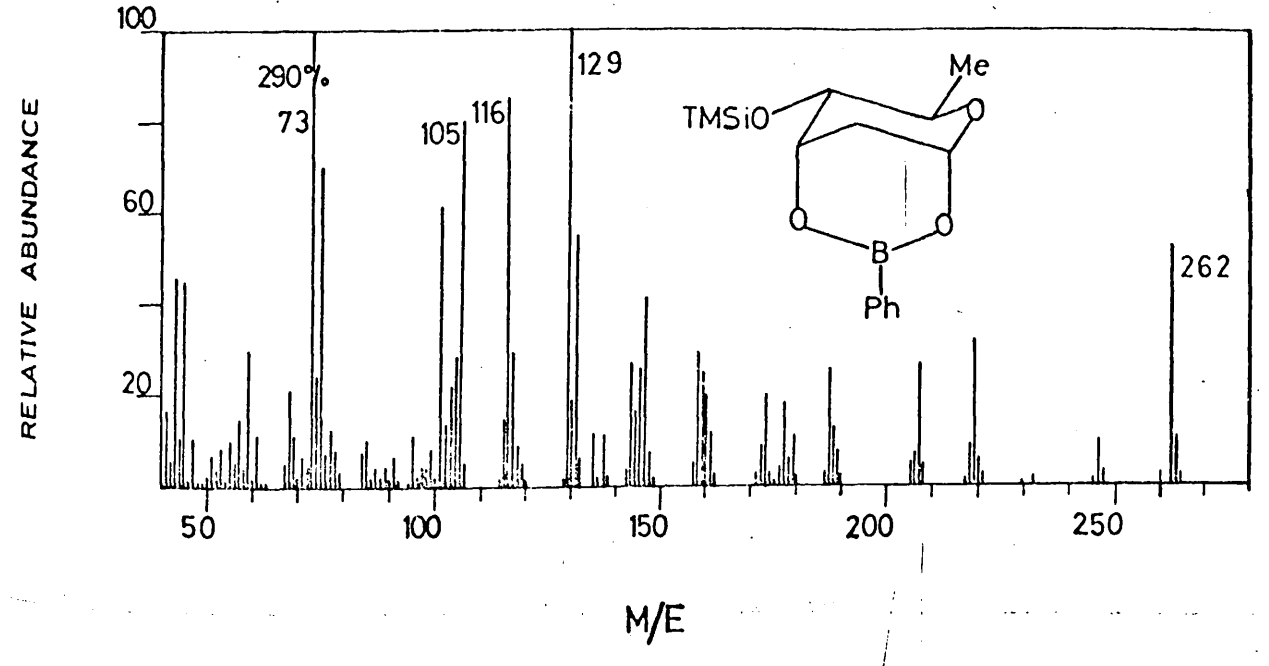
14. D-digitoxose 1,3-methylboronate TMSi ether.

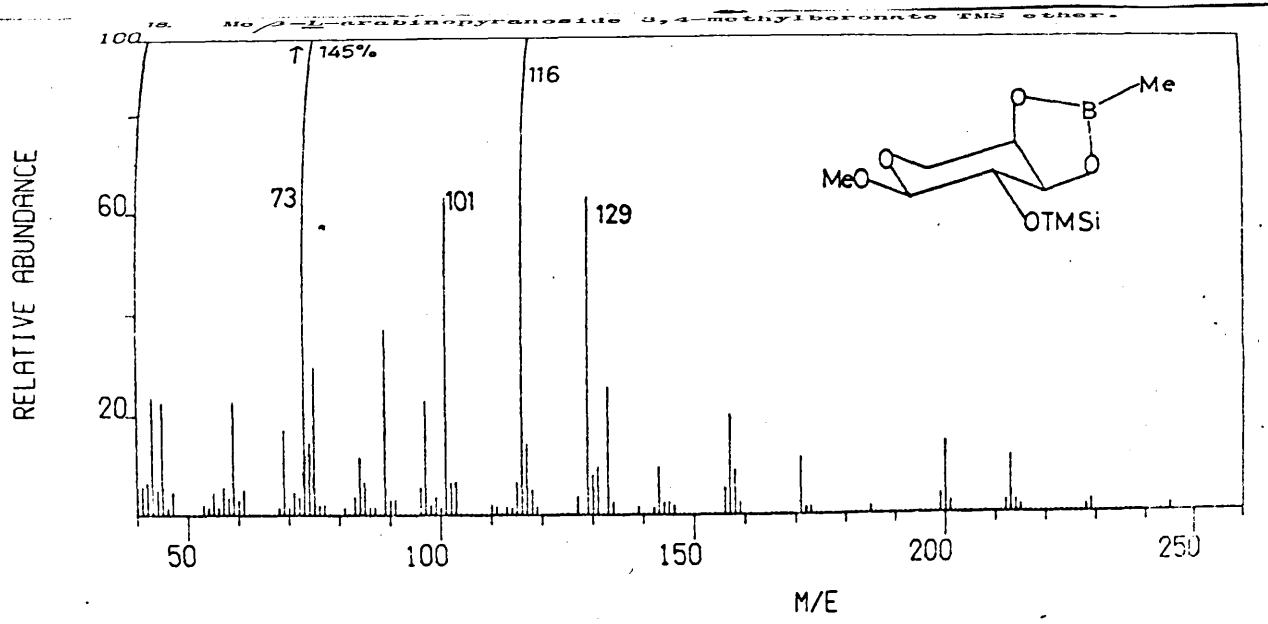


15/16. D-digitoxose 3,4-phenylboronate TMSi ether.

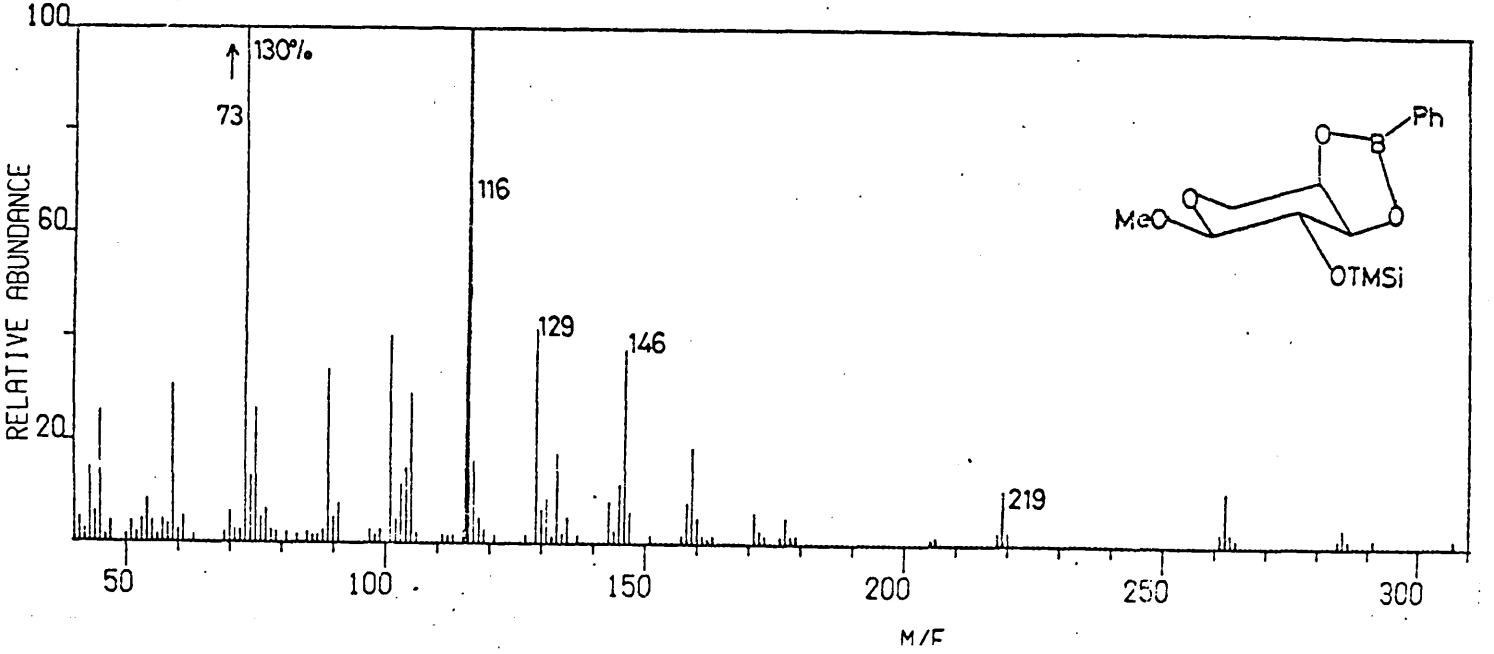


17. D-digitoxose 1,3-phenylboronate TMSi ether.

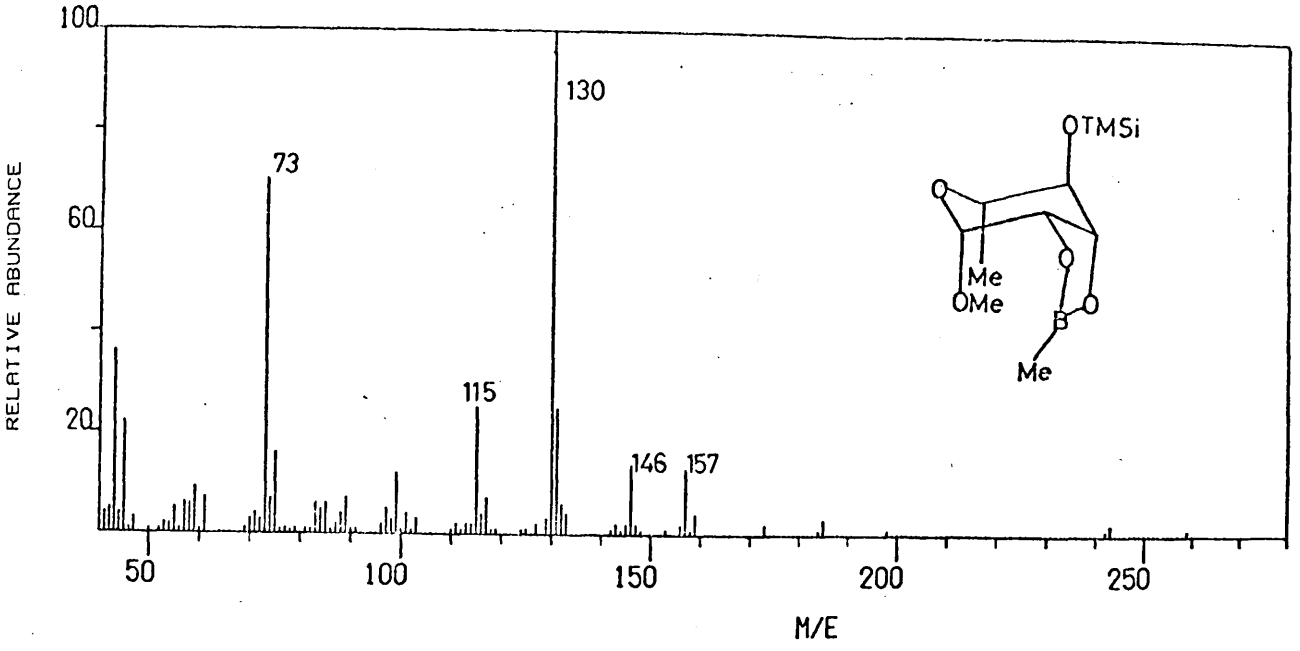




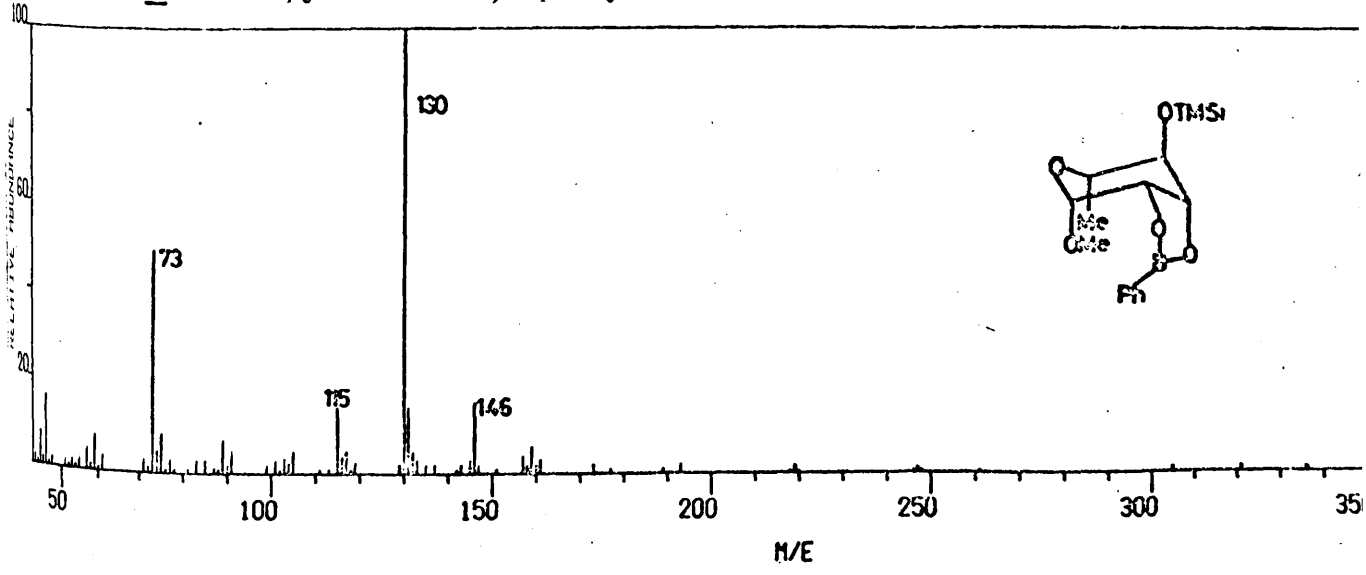
19. Me β-L-arabinopyranoside 3,4-phenylboronate TMSi ether.



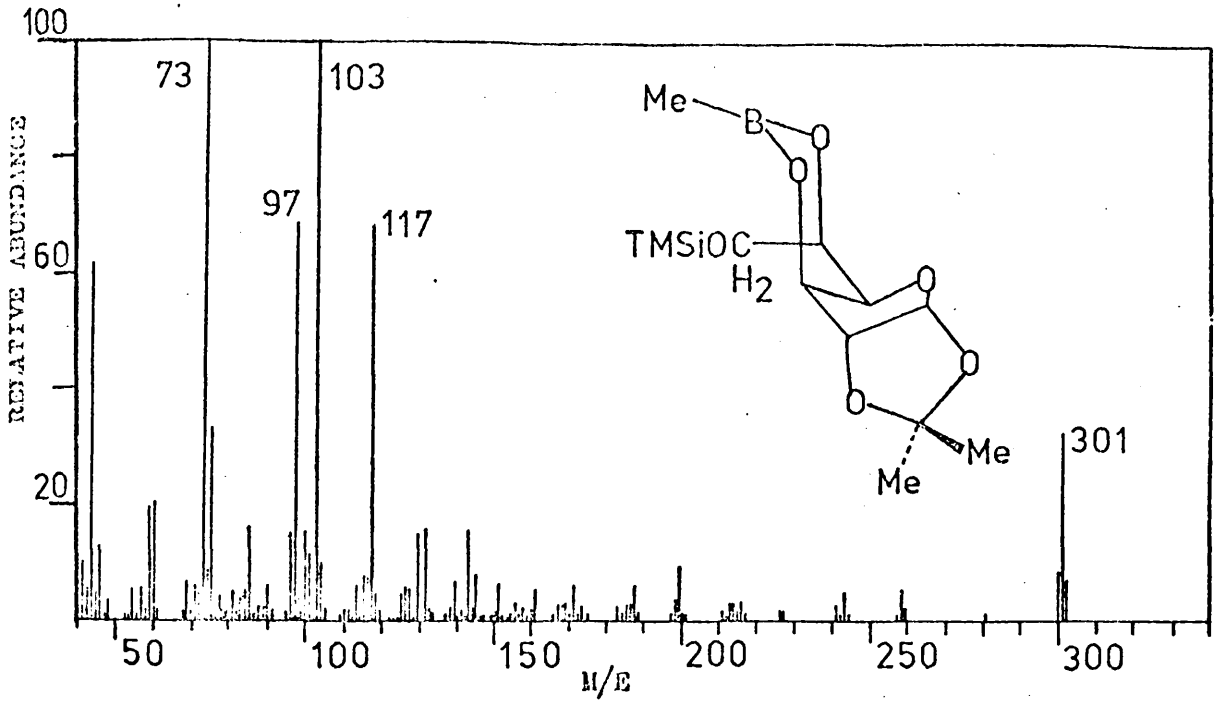
20. Me α-L-rhamnopyranoside 2,3-methylboronate TMSi ether.



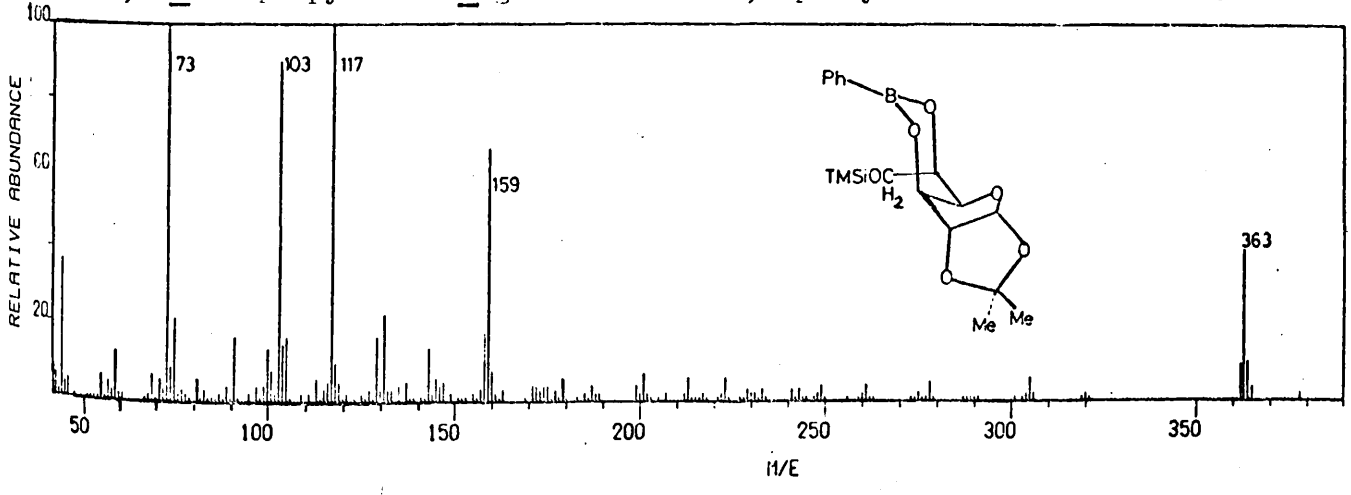
21. Me α-L-rhamnopyranoside 2,3-phenylboronate TMSi ether.



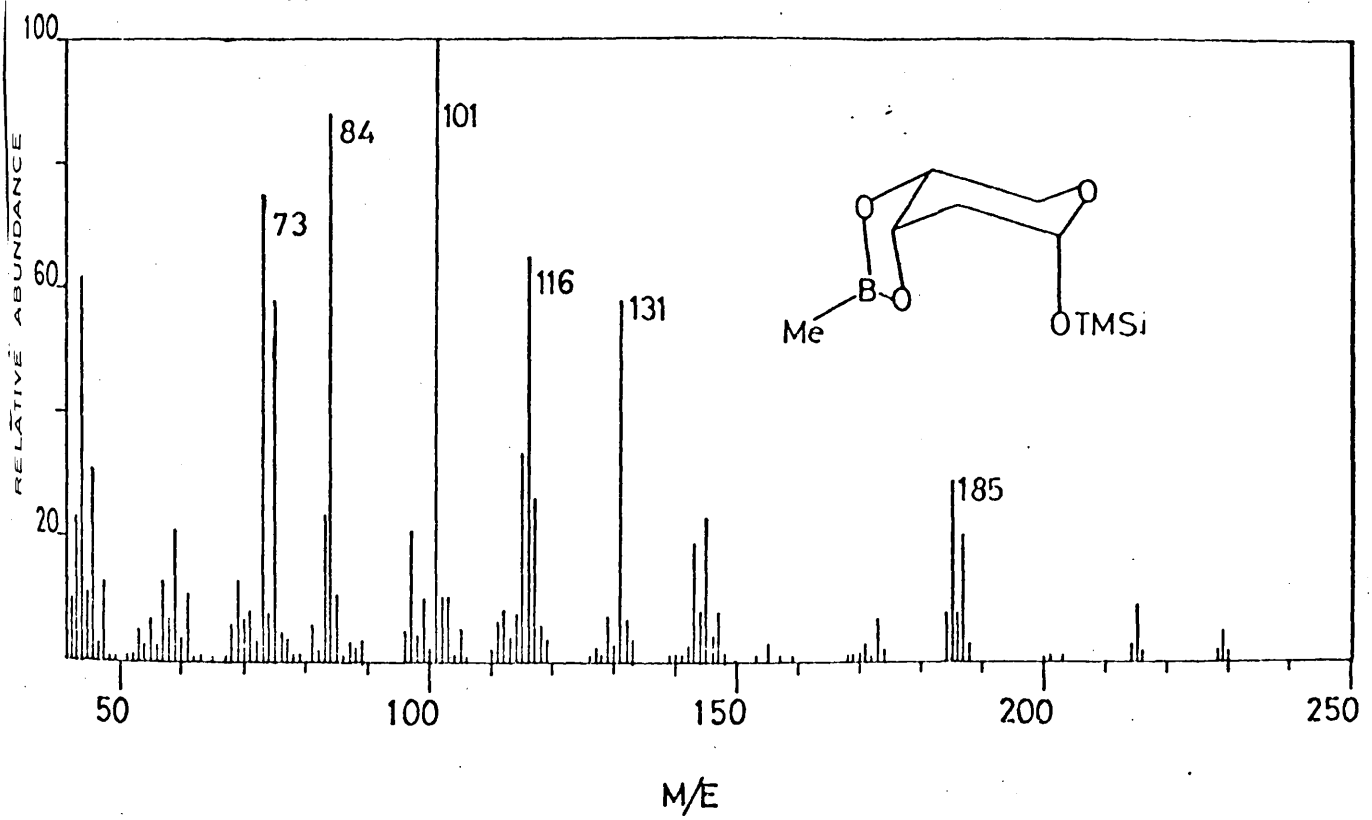
22. 1,2-O-isopropylidene-D-glucofuranose 3,5-methylboronate TMSi ether.



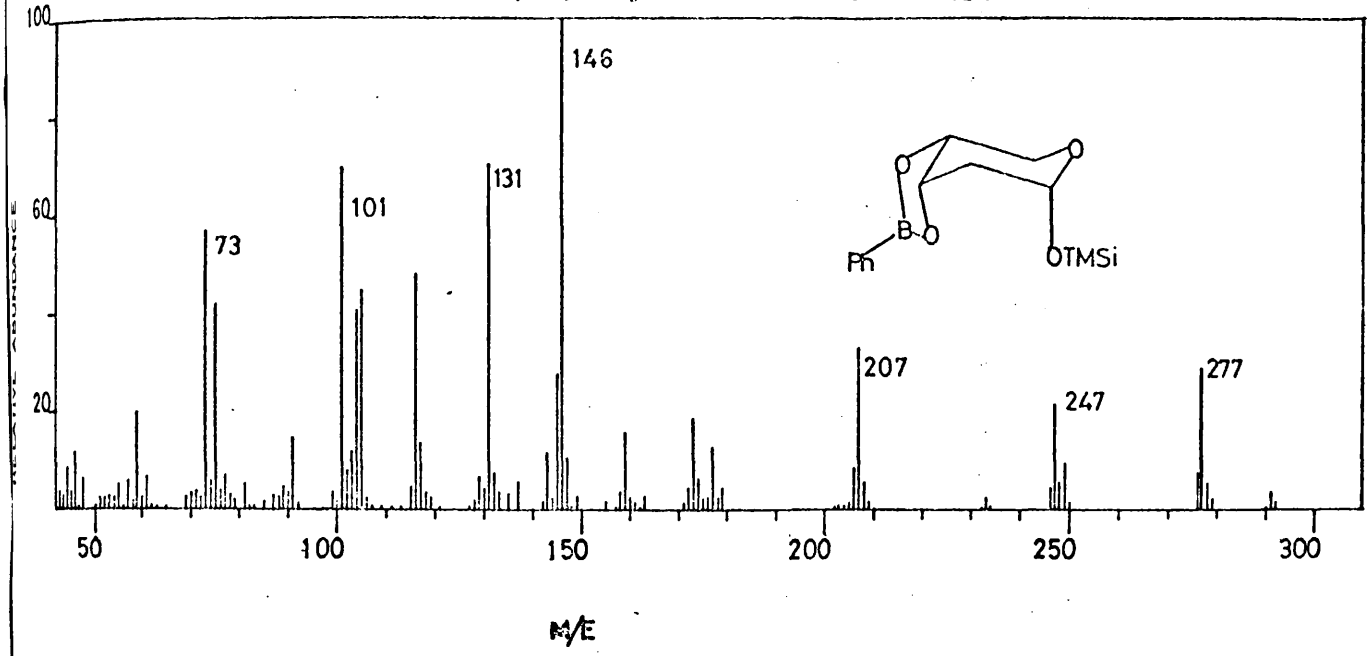
23. 1,2-O-isopropylidene-D-glucofuranose 3,5-phenylboronate TMSi ether.



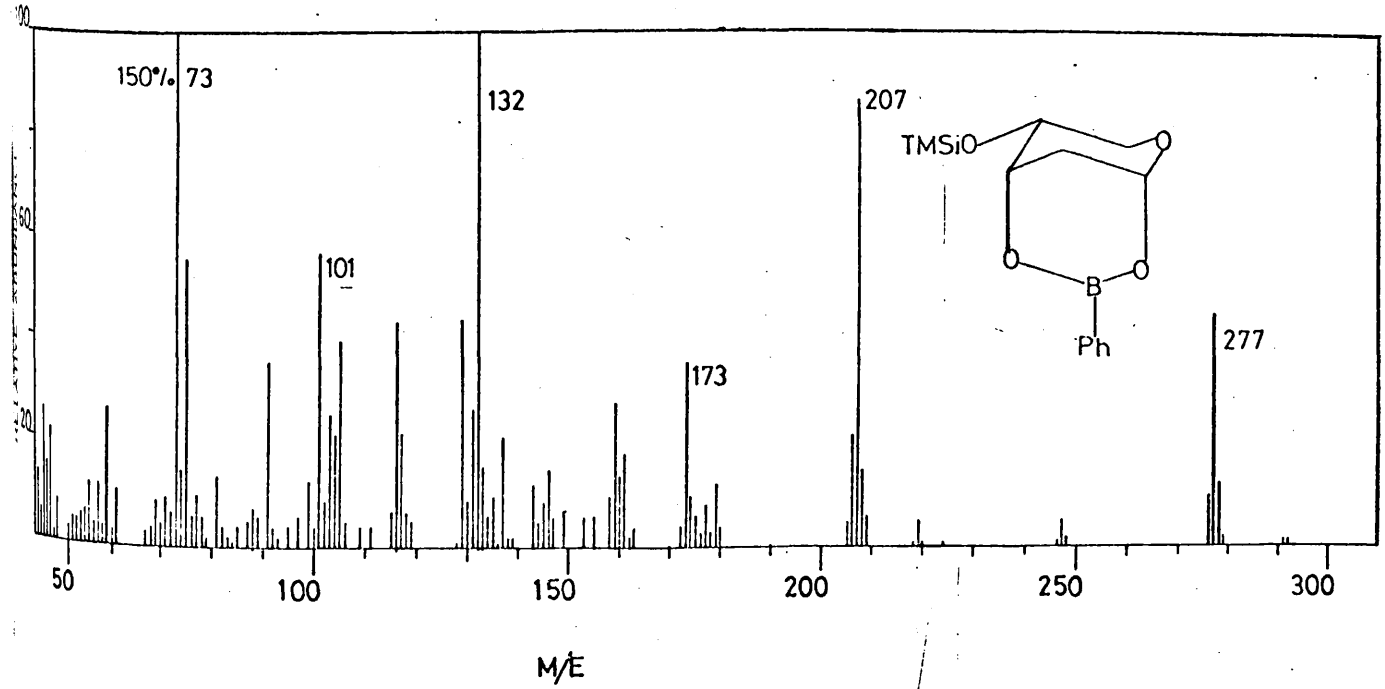
24. 2-deoxy-D-ribopyranose 3,4-methylboronate TMSi ether.



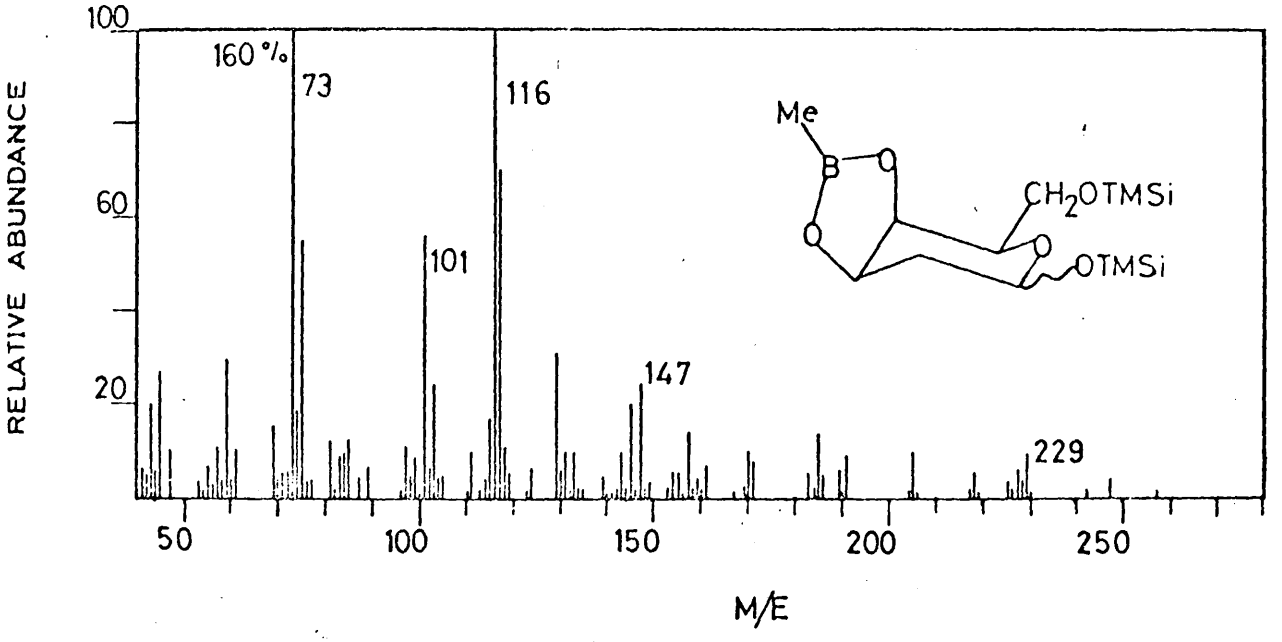
25. 2-deoxy-D-ribopyranose 3,4-phenylboronate TMSi ether.



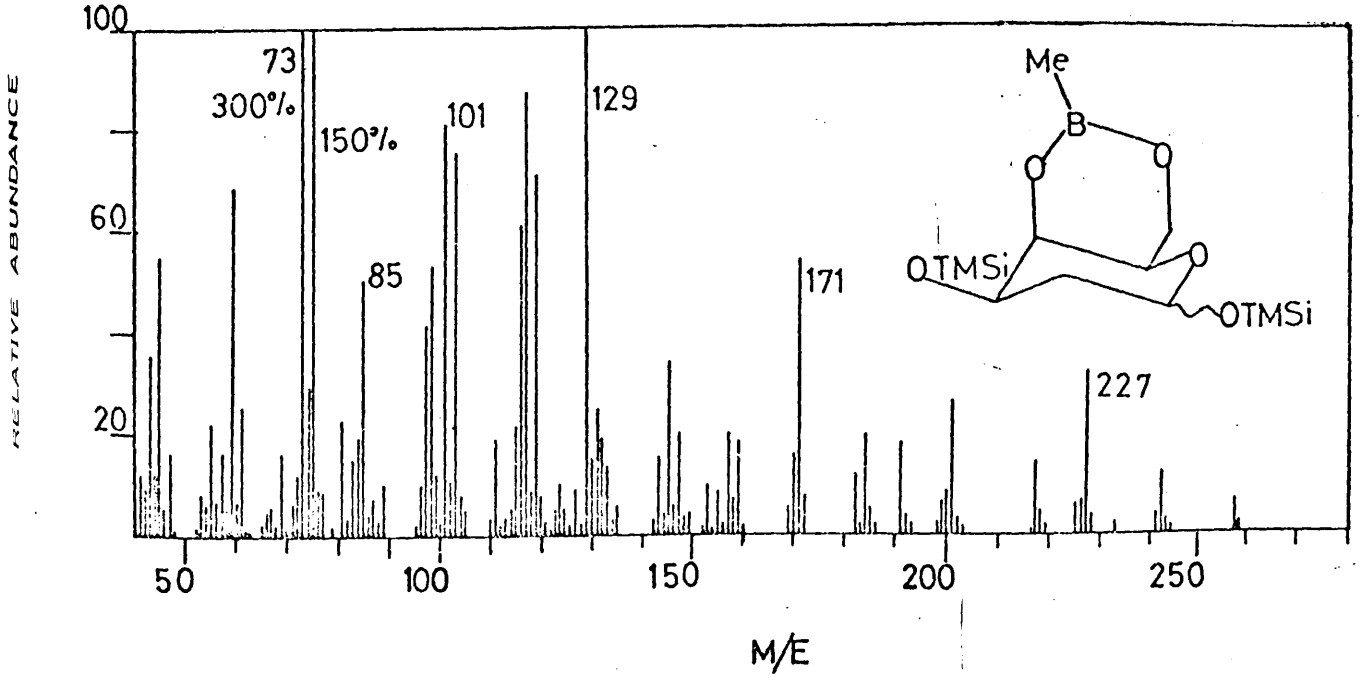
26. 2-deoxy-D-ribopyranose 1,3-phenylboronate TMSi ether.



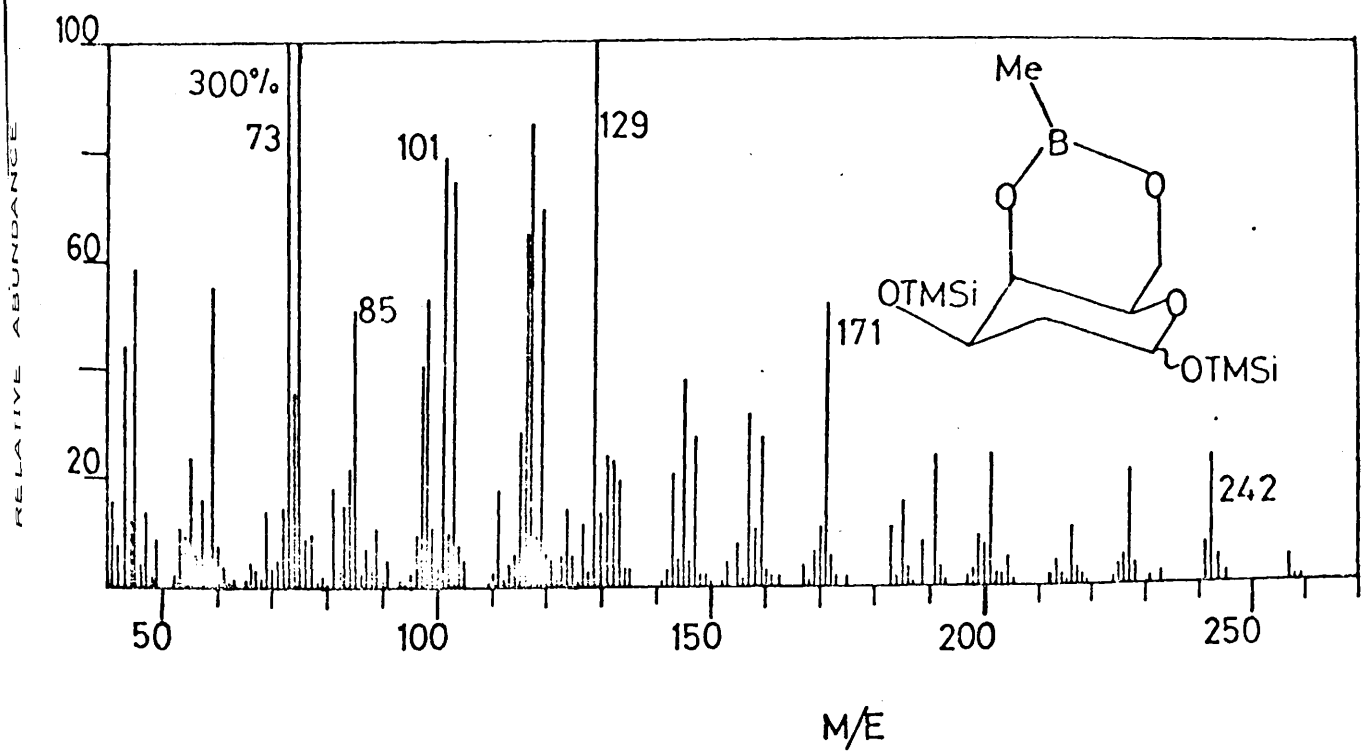
27. 2-deoxy-D-galactopyranose 3,4-methylboronate TMSi ether.



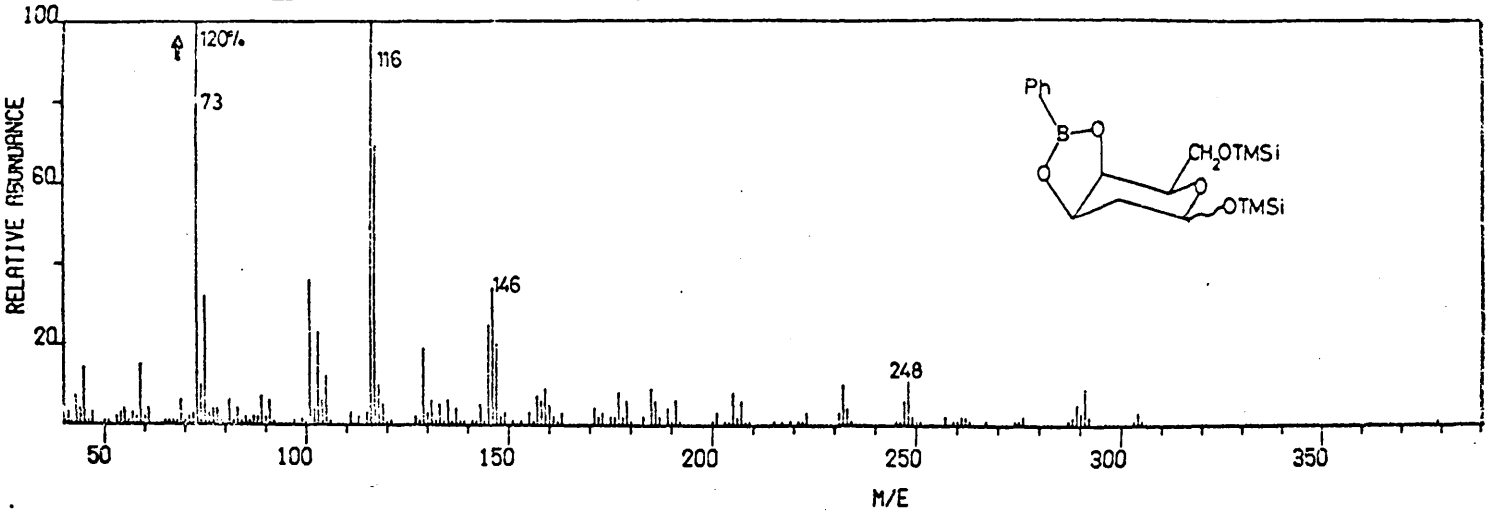
28. 2-deoxy-D-galactopyranose 4,6-methylboronate TMSi ether.



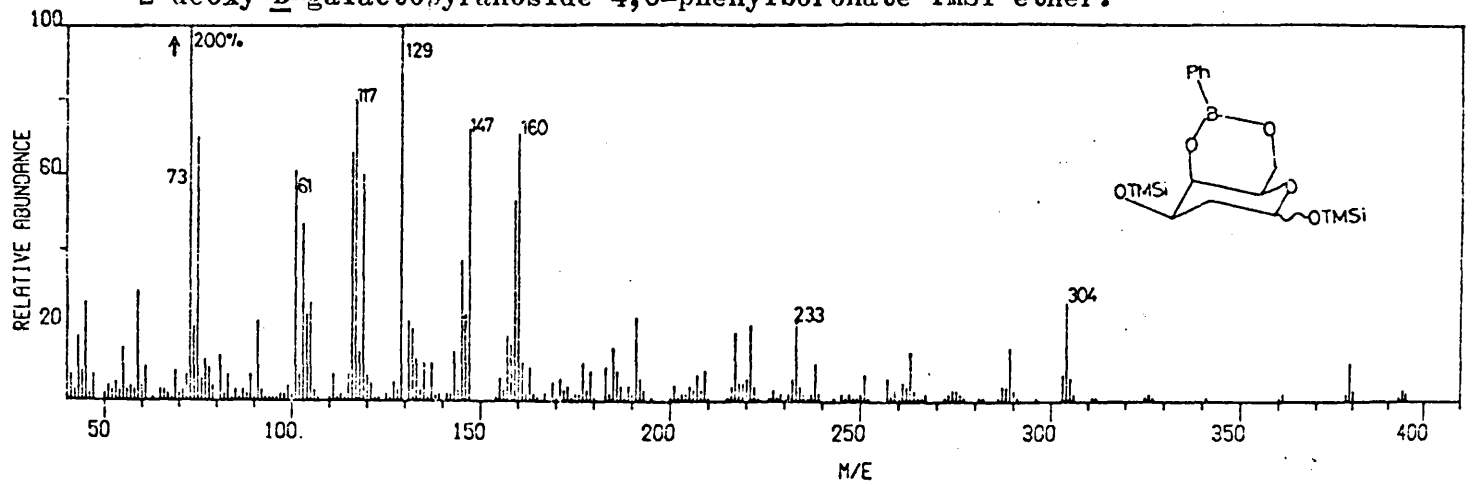
29. 2-deoxy-D-galactopyranose 4,6-methylboronate TMSi ether.



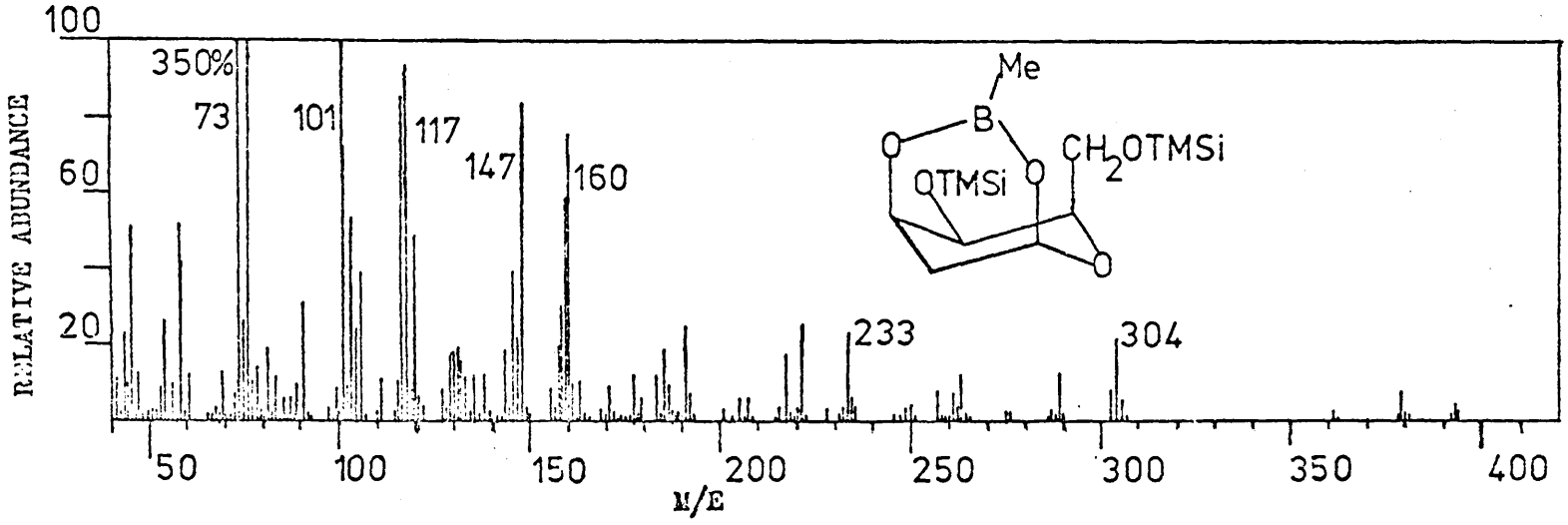
30. 2-deoxy-D-galactopyranoside 3,4-phenylboronate TMSi ether.



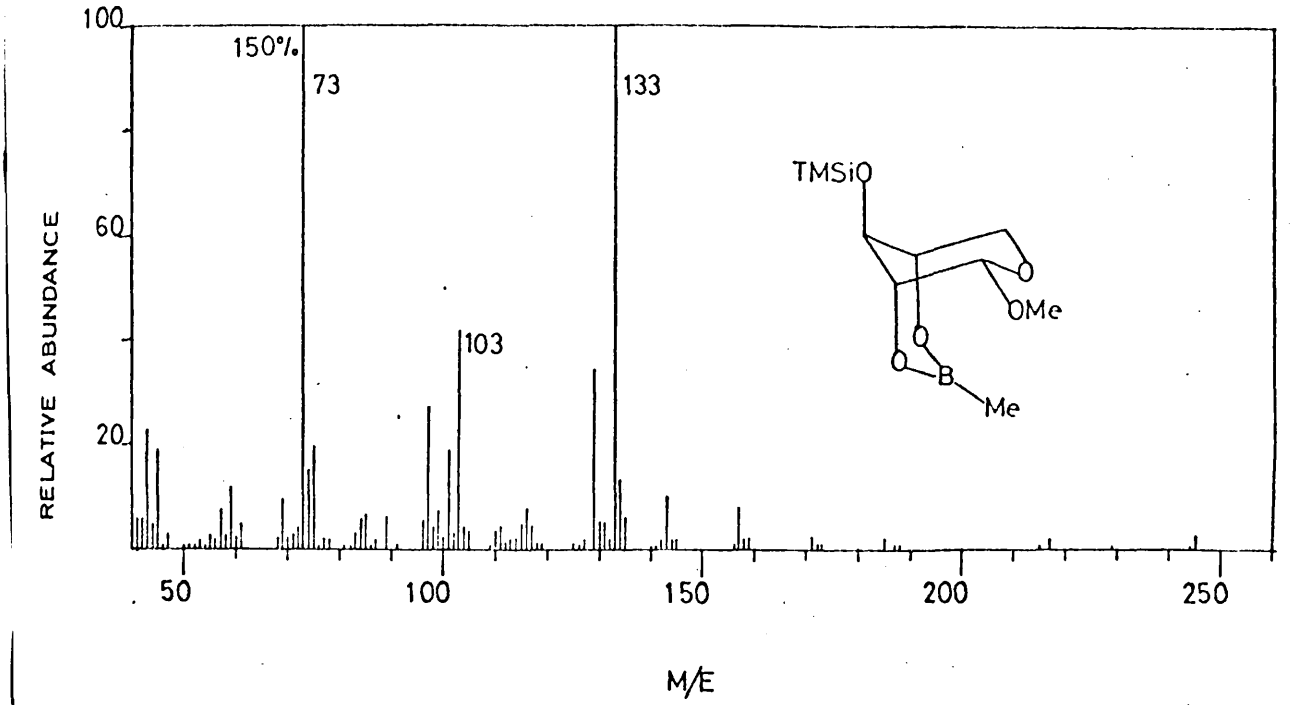
31. 2-deoxy-D-galactopyranoside 4,6-phenylboronate TMSi ether.



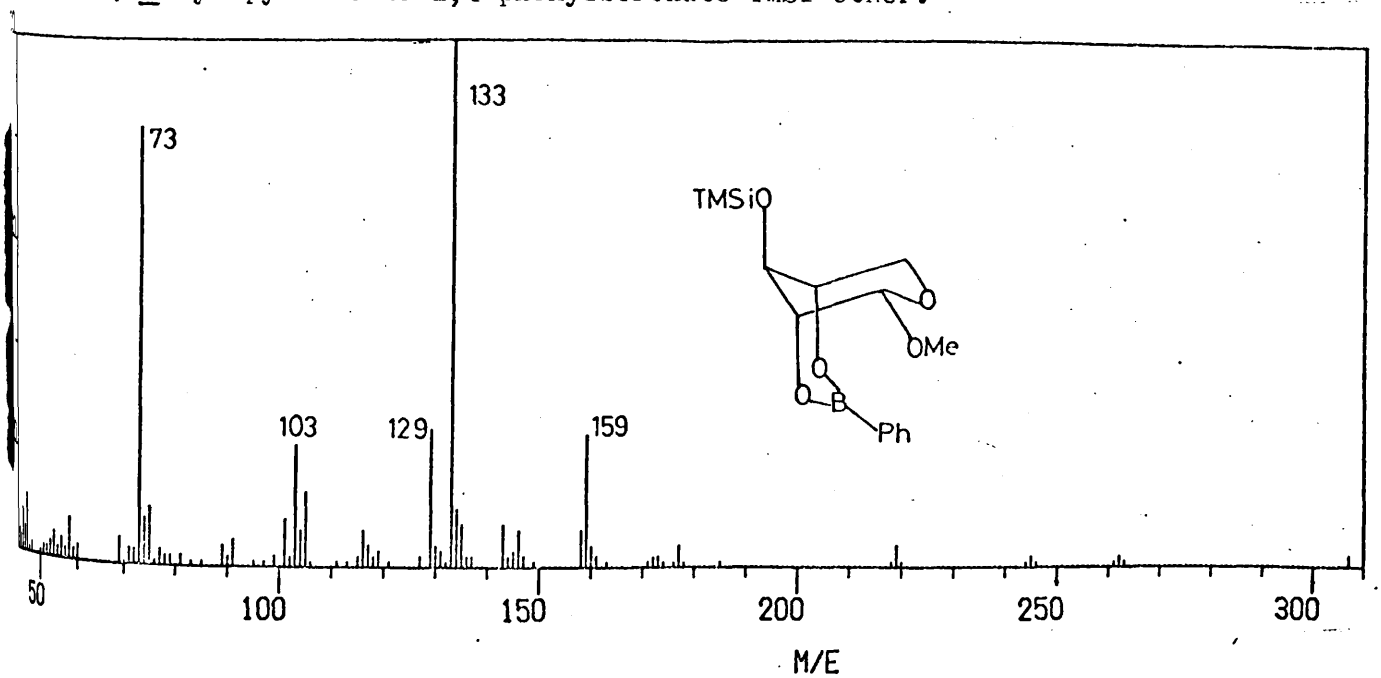
32. 2-deoxy-D-galactopyranoside 4,6-methylboronate TMSi ether.



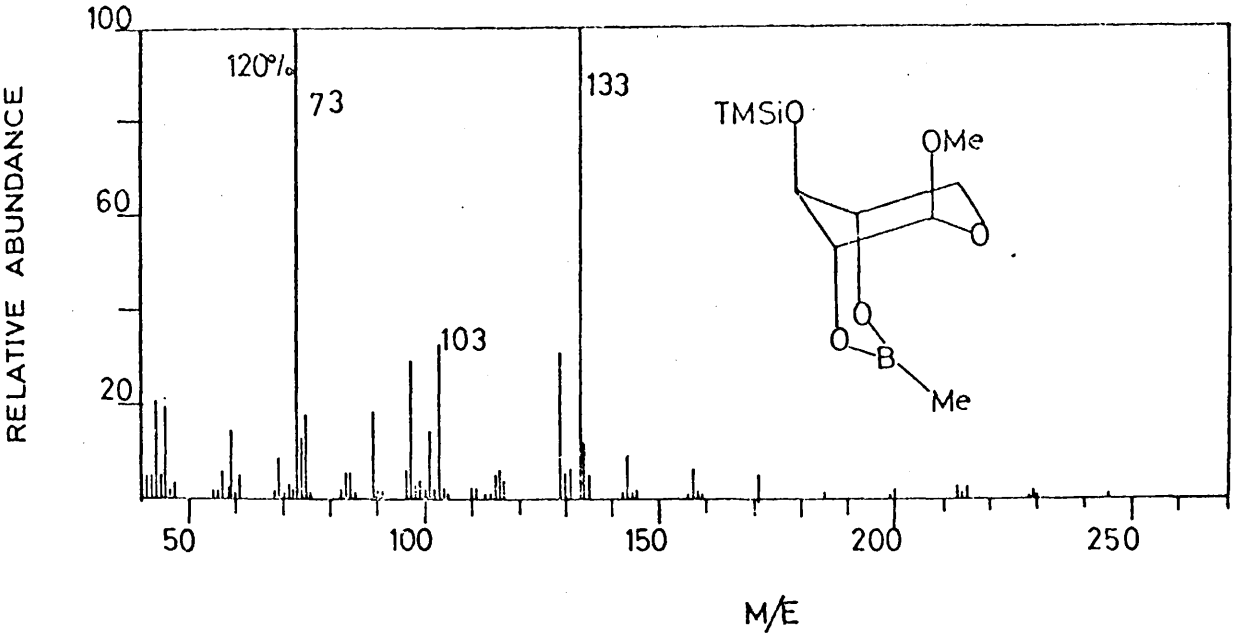
33. Me α-D-xylopyranoside 2,4-methylboronate TMSi ether.



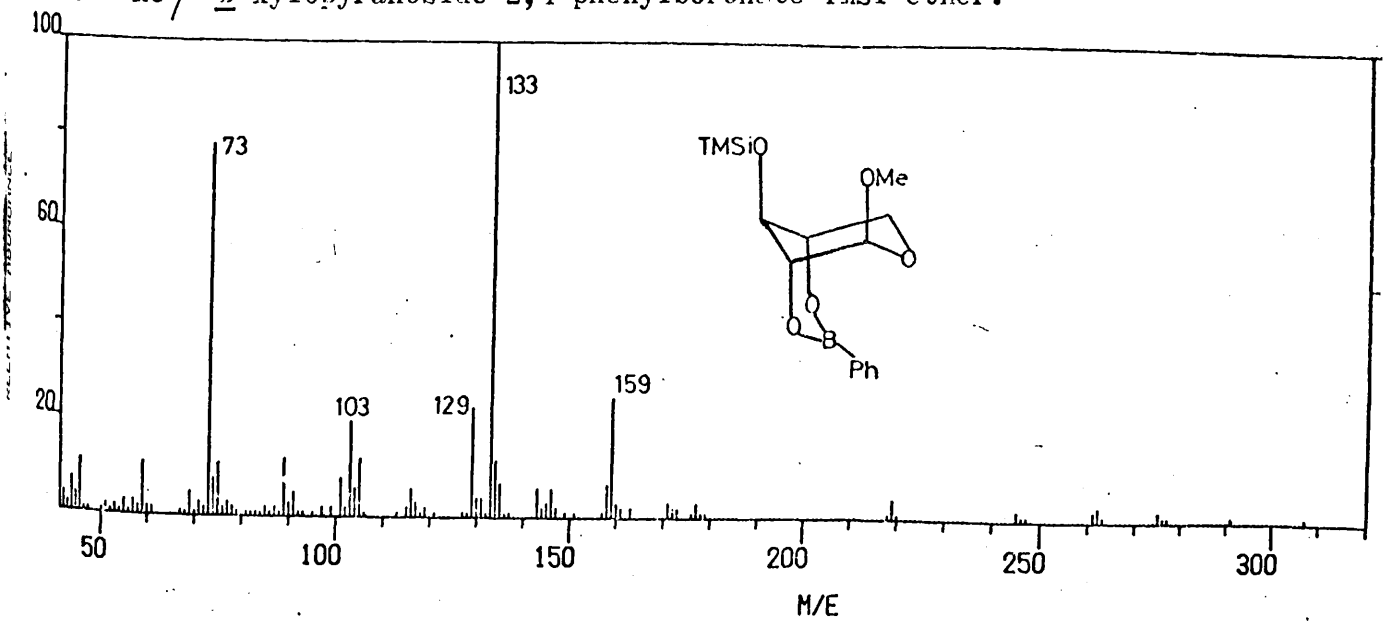
34. Me α-D-xylopyranoside 2,4-phenylboronate TMSi ether.



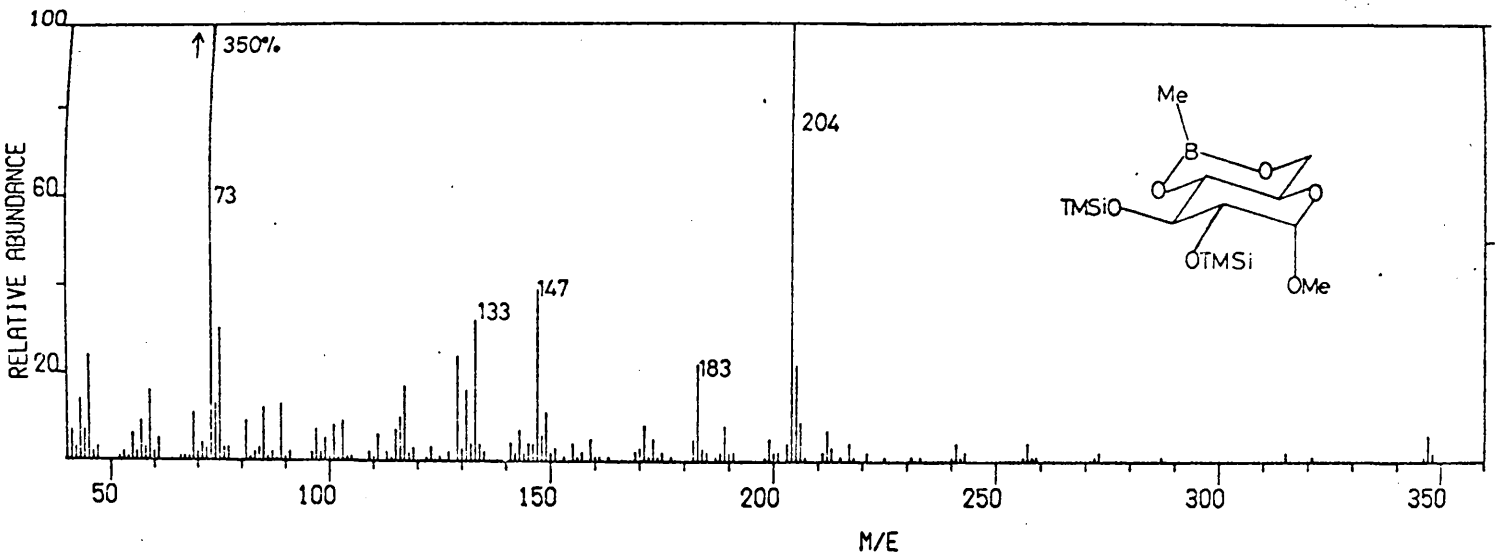
35. Me β-D-xylopyranoside 2,4-methylboronate TMSi ether.



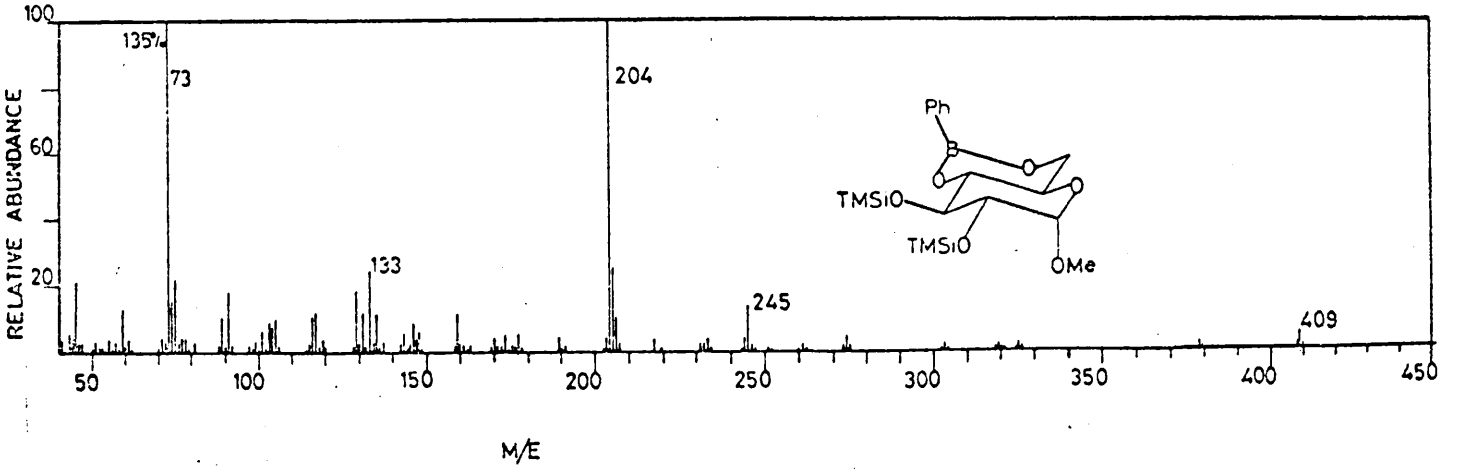
36. Me β-D-xylopyranoside 2,4-phenylboronate TMSi ether.



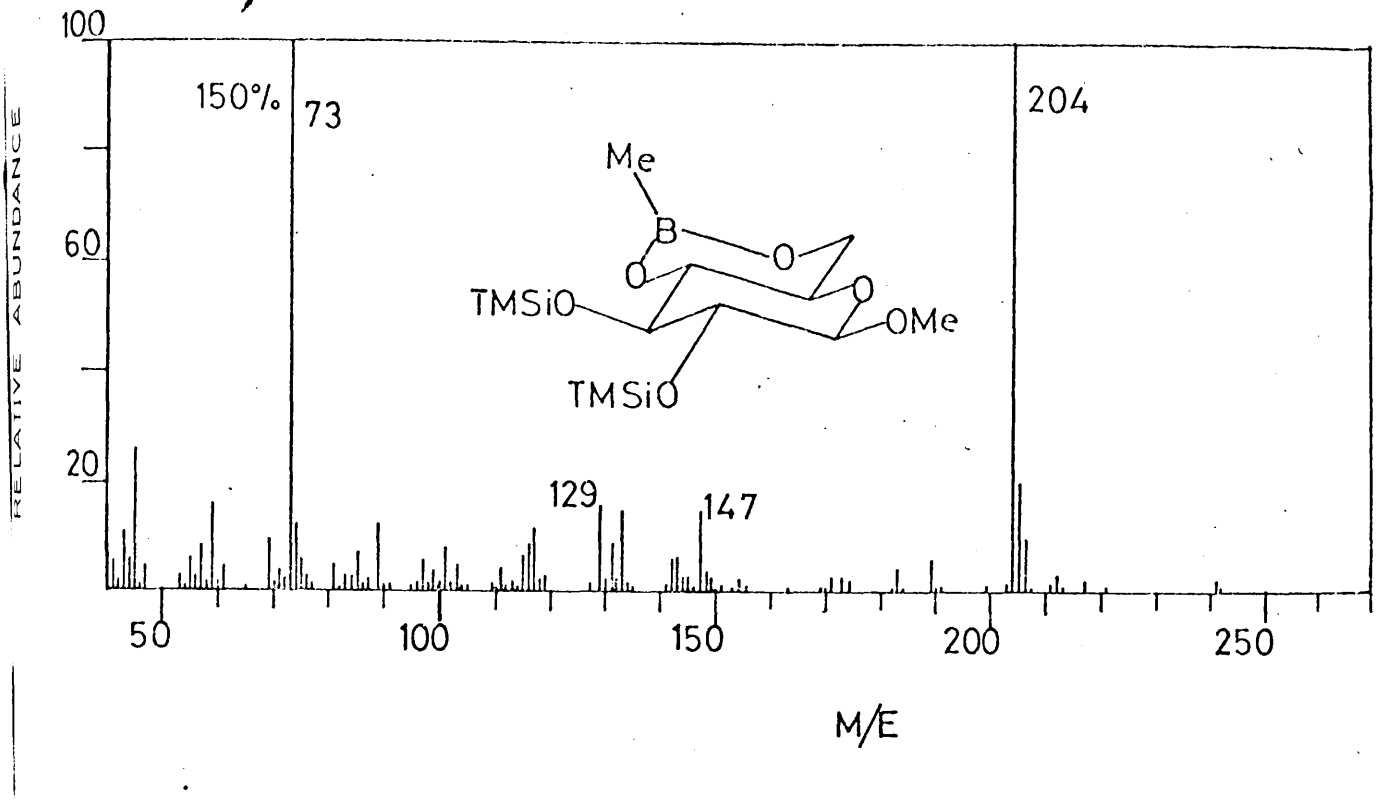
37. Me α-D-glucopyranoside 4,6-methylboronate TMSi ether.



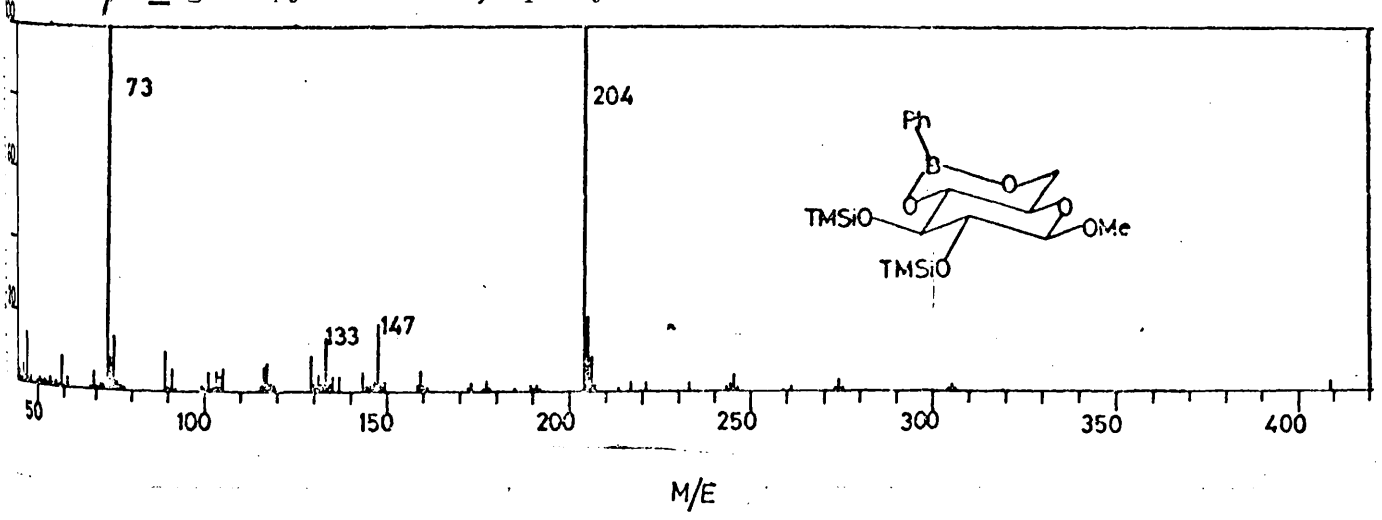
38. Me α-D-glucopyranoside 4,6-phenylboronate TMSi ether.

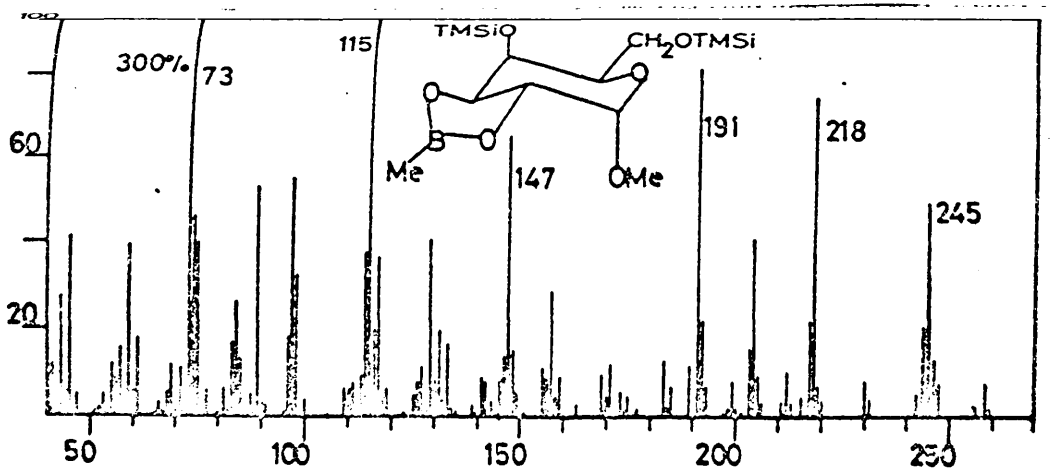


39. Me β-D-glucopyranoside 4,6-methylboronate TMSi ether.

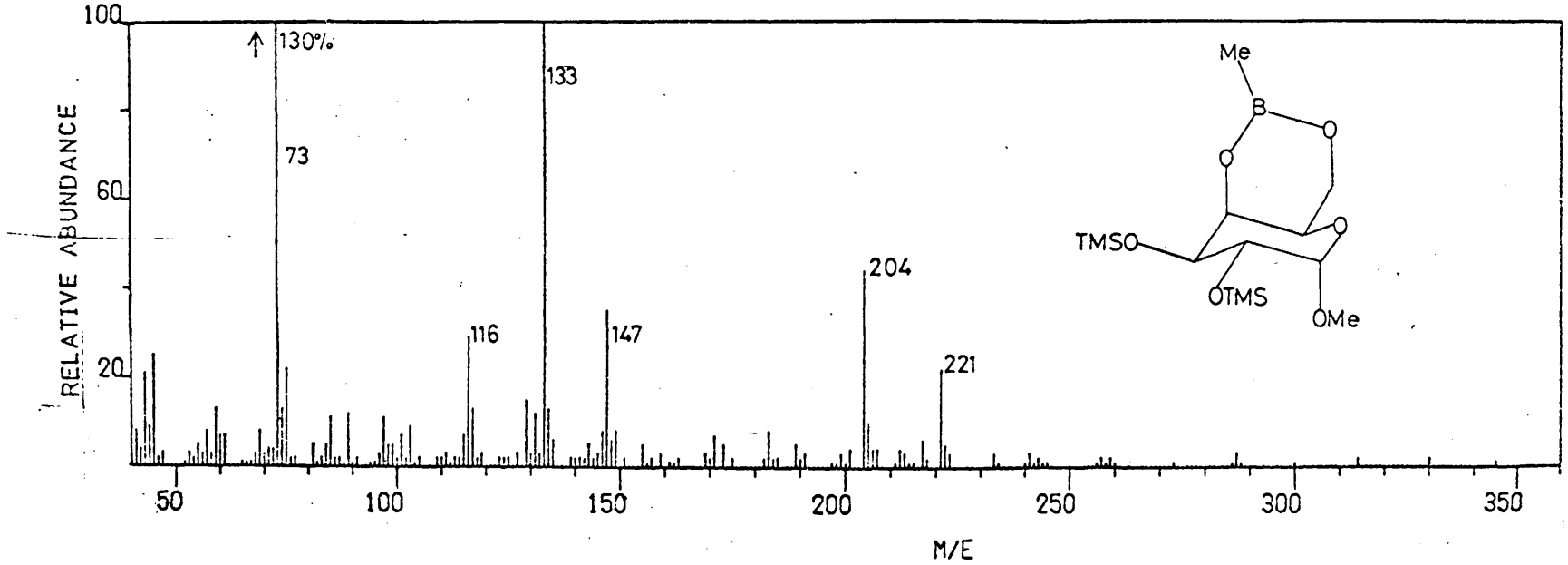


40. Me β-D-glucopyranoside 4,6-phenylboronate TMSi ether.

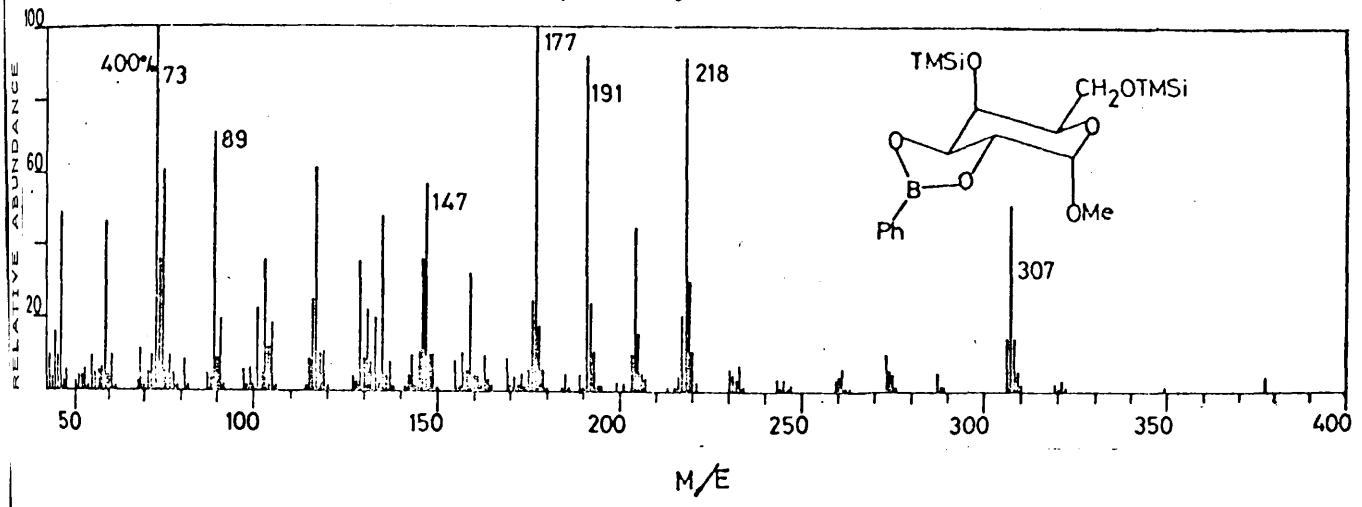




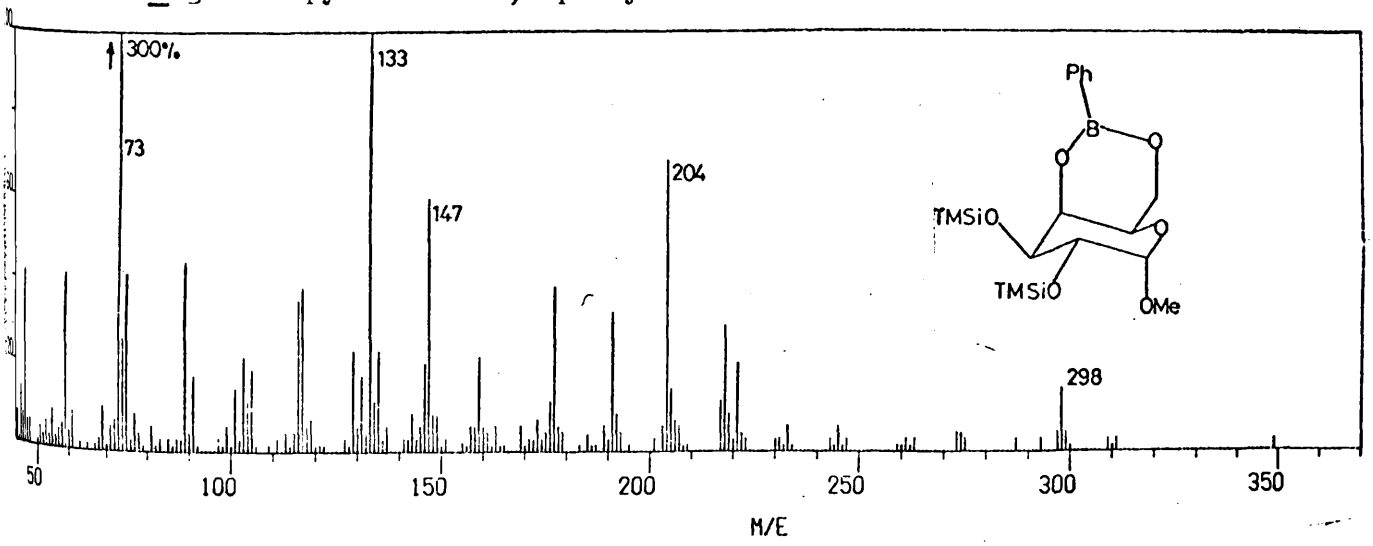
42. Me α-D-galactopyranoside 4,6-phenylboronate TMSi ether.



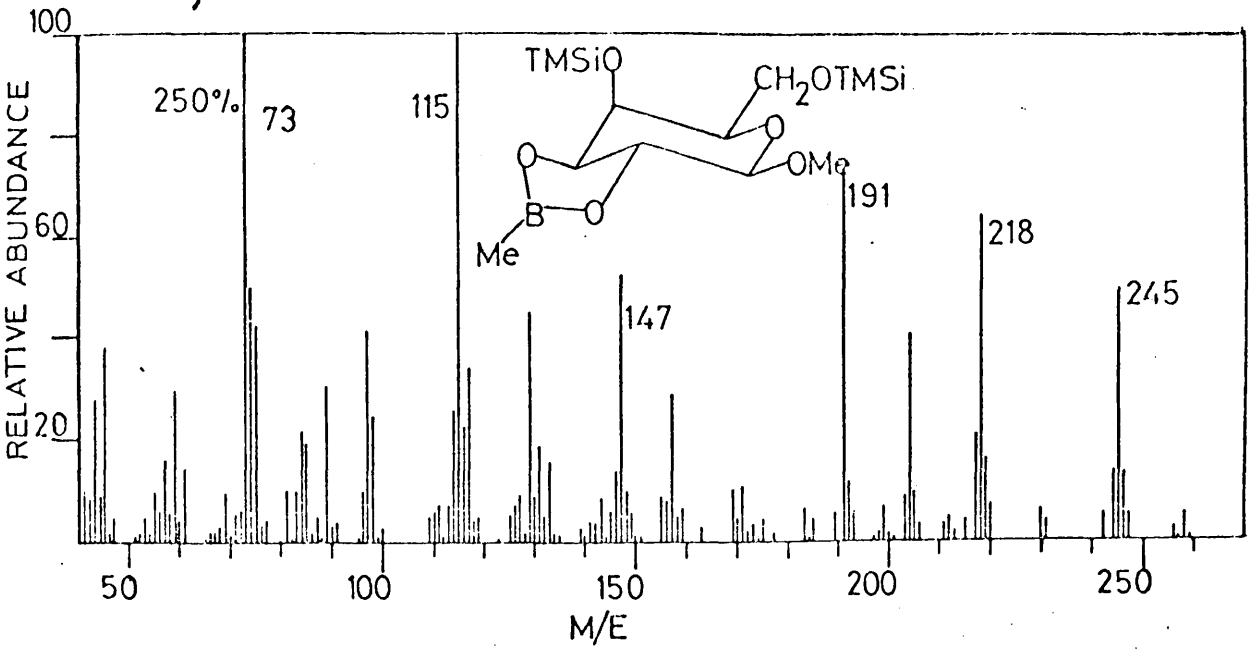
43. Me α-D-galactopyranoside 2,3-phenylboronate TMSi ether.



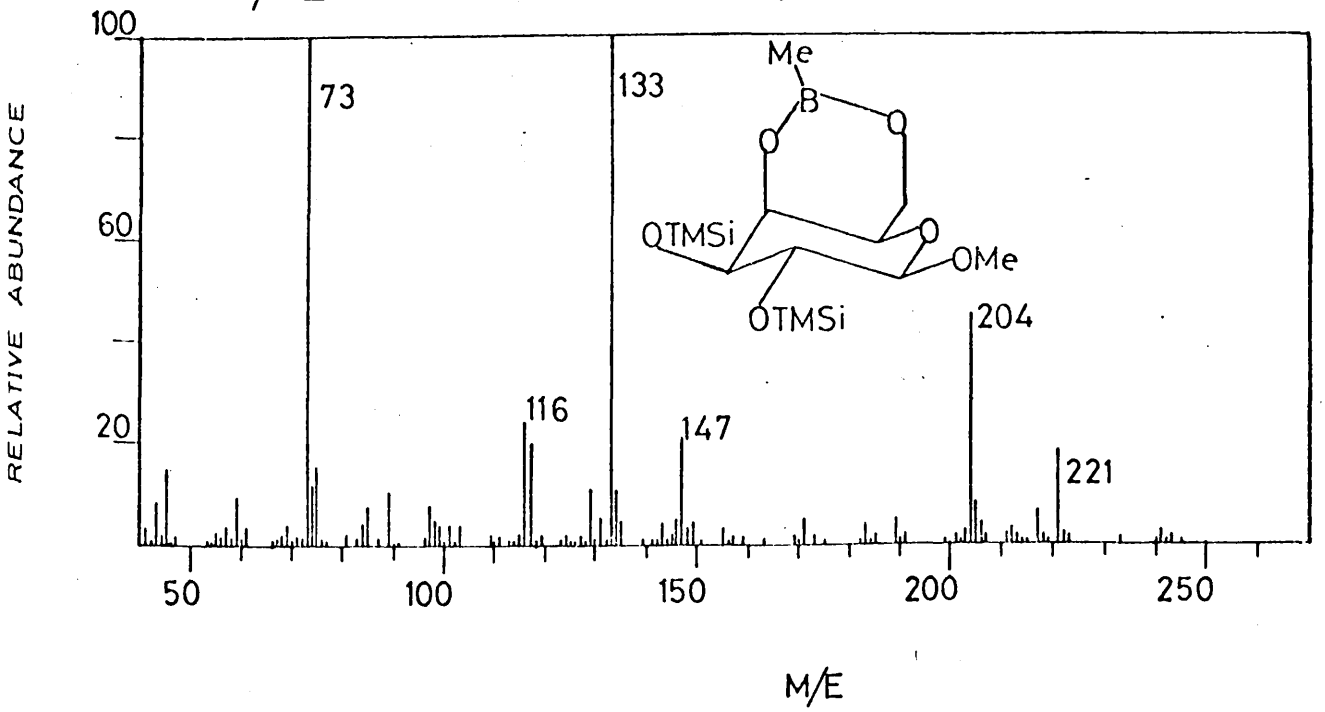
44. Me α-D-galactopyranoside 4,6-phenylboronate TMSi ether.



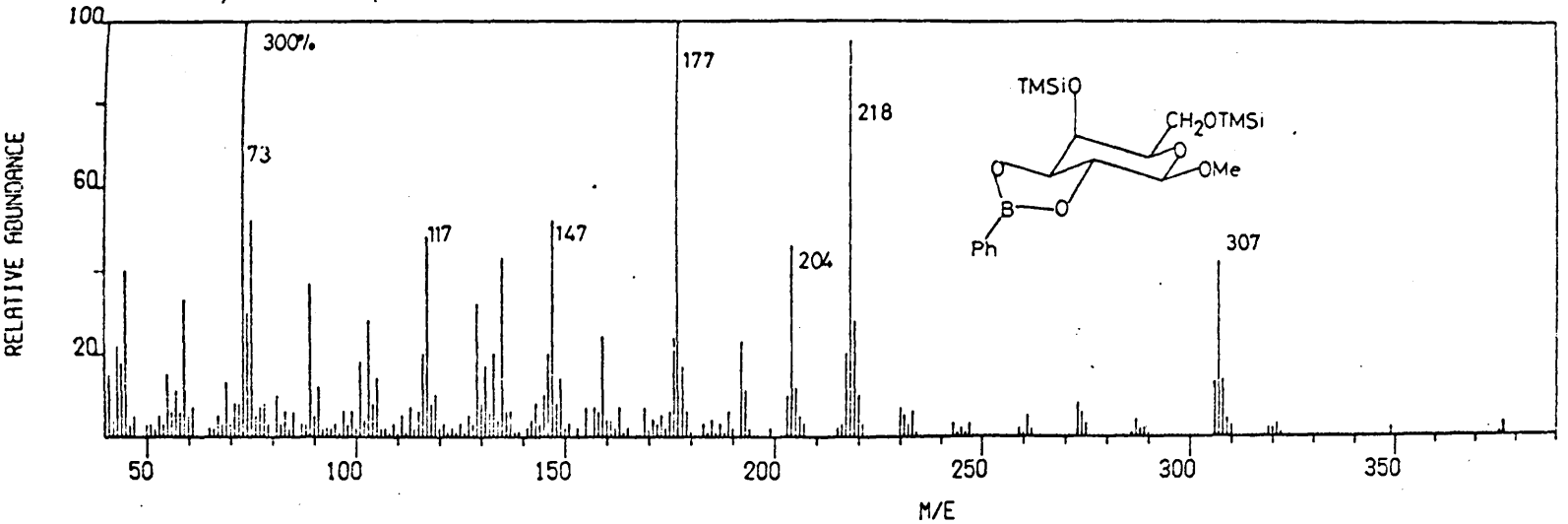
45. Me β-D-galactopyranoside 2,3-methylboronate TMSi ether.



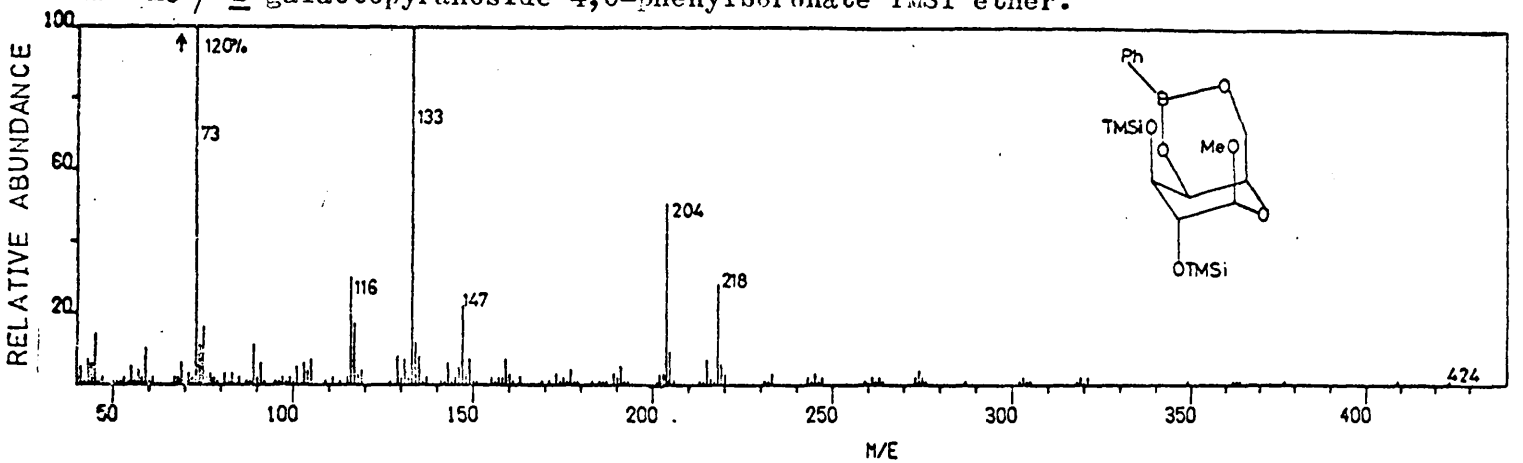
46. Me β-D-galactopyranoside 4,6-methylboronate TMSi ether.



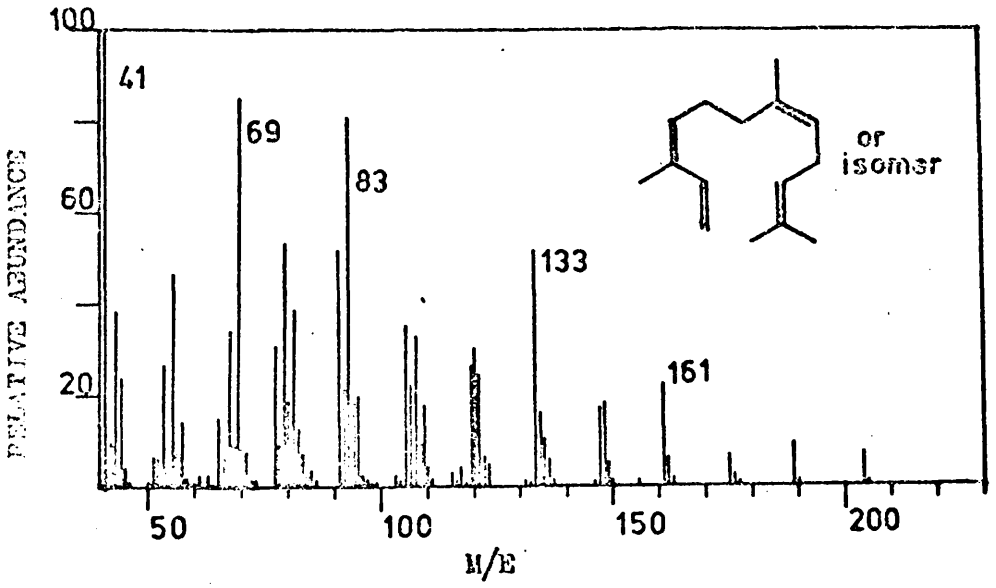
47. Me β-D-galactopyranoside 2,3-phenylboronate TMSi ether.



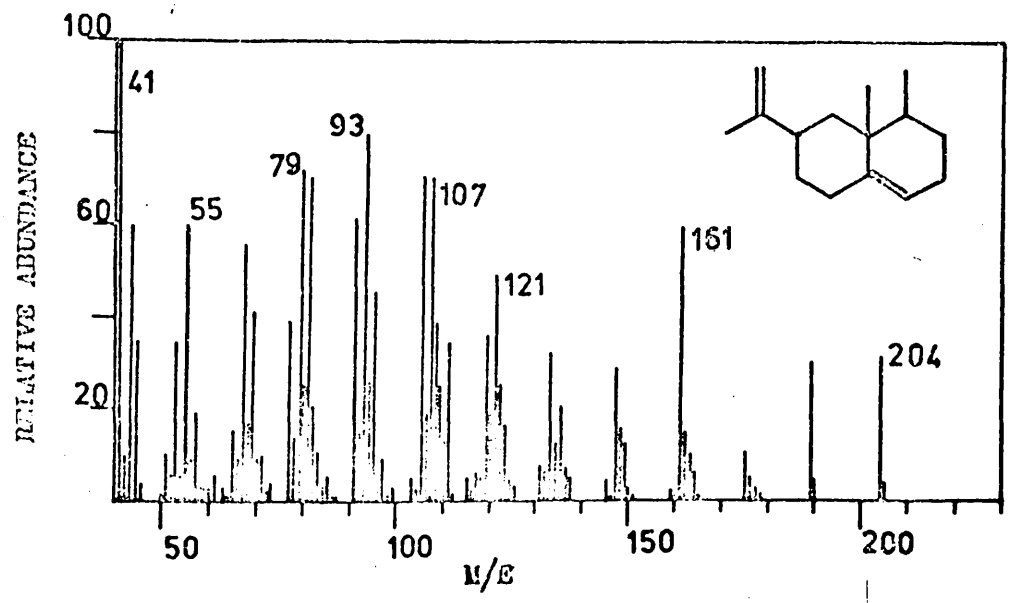
48. Me β-D-galactopyranoside 4,6-phenylboronate TMSi ether.



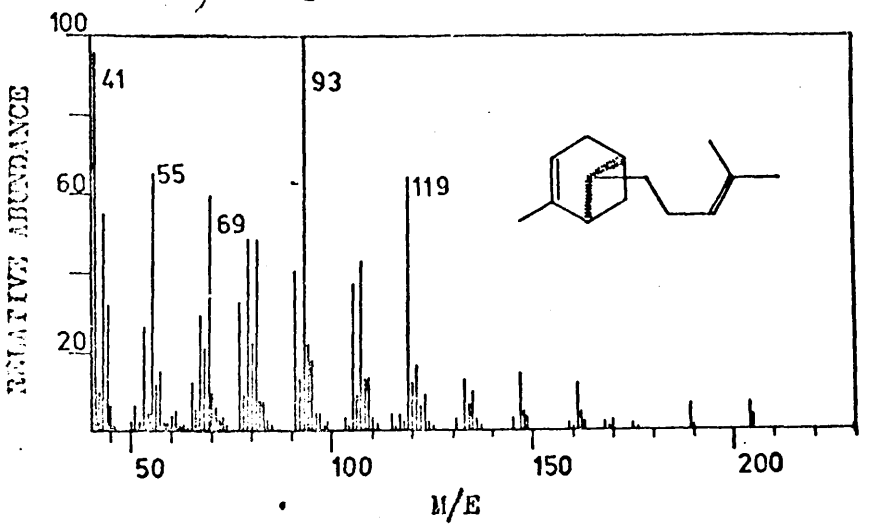
49. VL1 Farnesene.



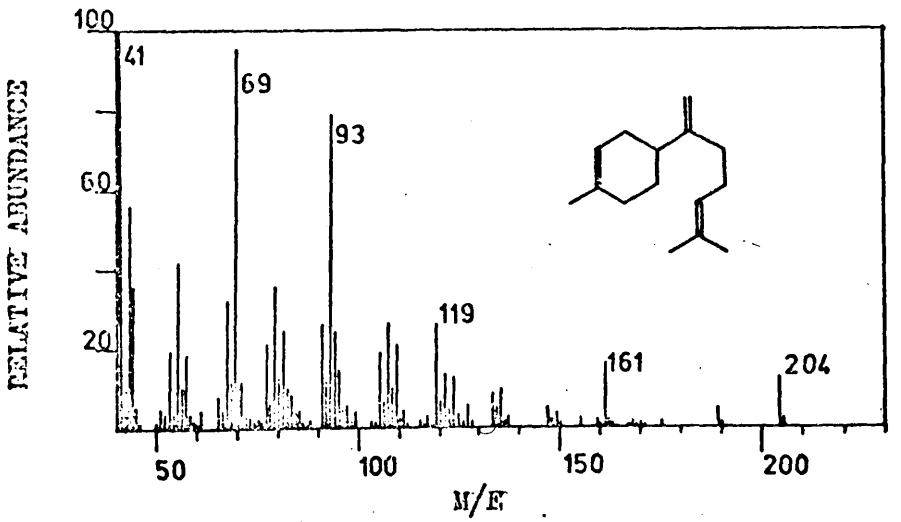
50. VL2 Eremophilene.



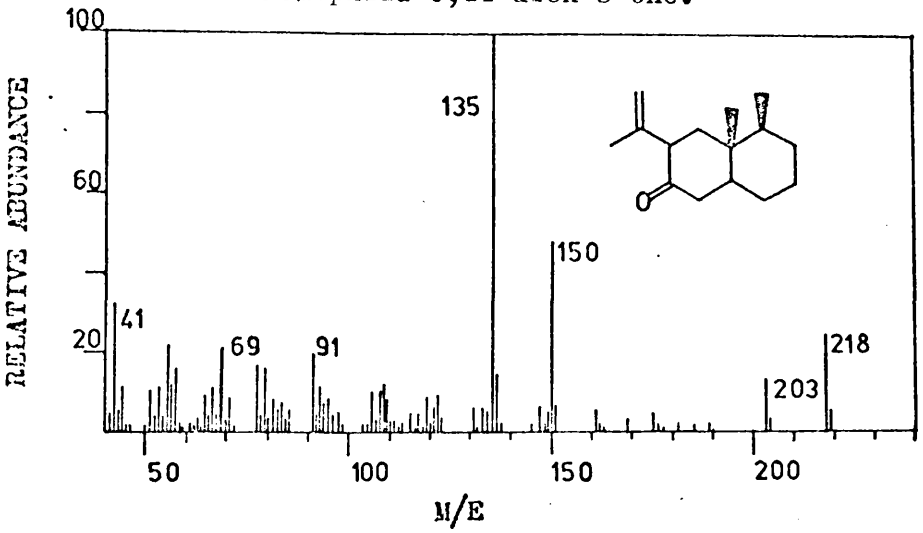
51. VL3 β-bergamotene.



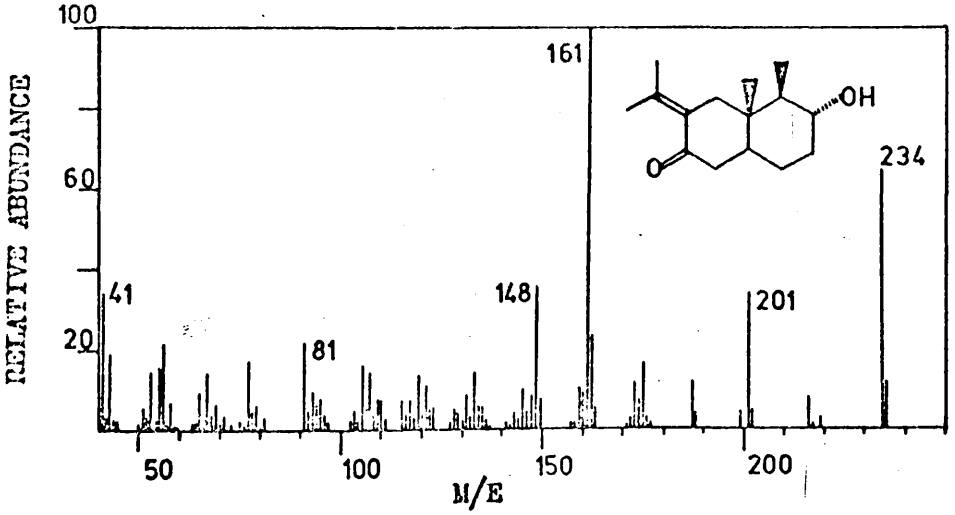
52. VL4 Bisabolene.



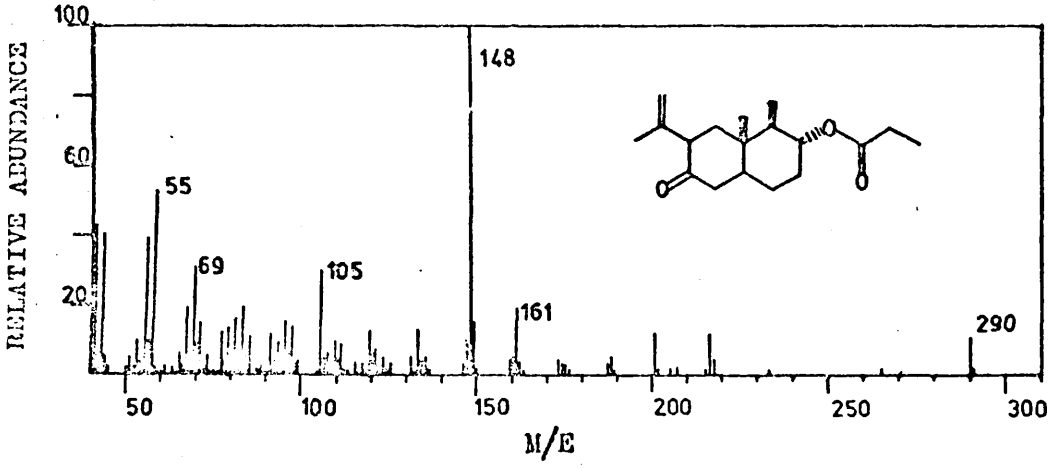
53. VR4 Eremophila-9,11-dien-8-one.



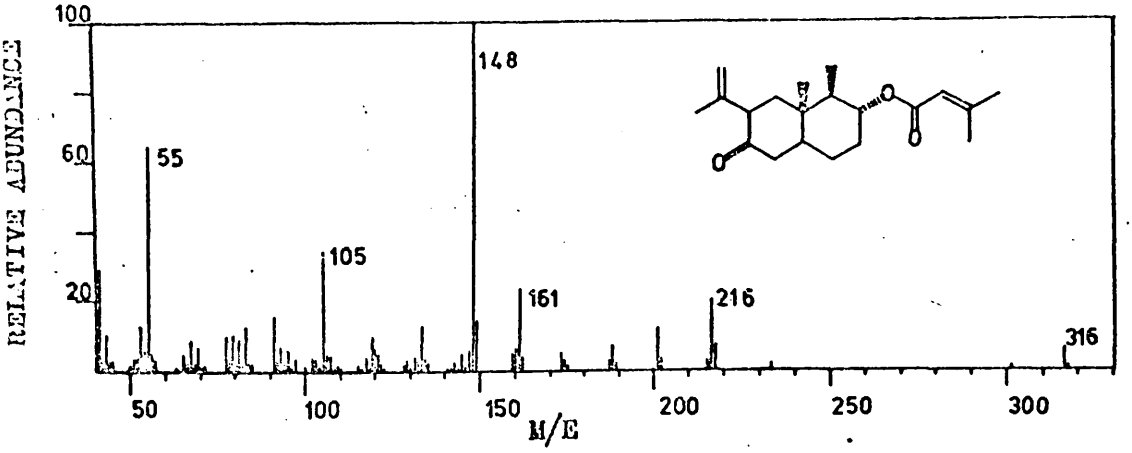
54. Isopetasol.



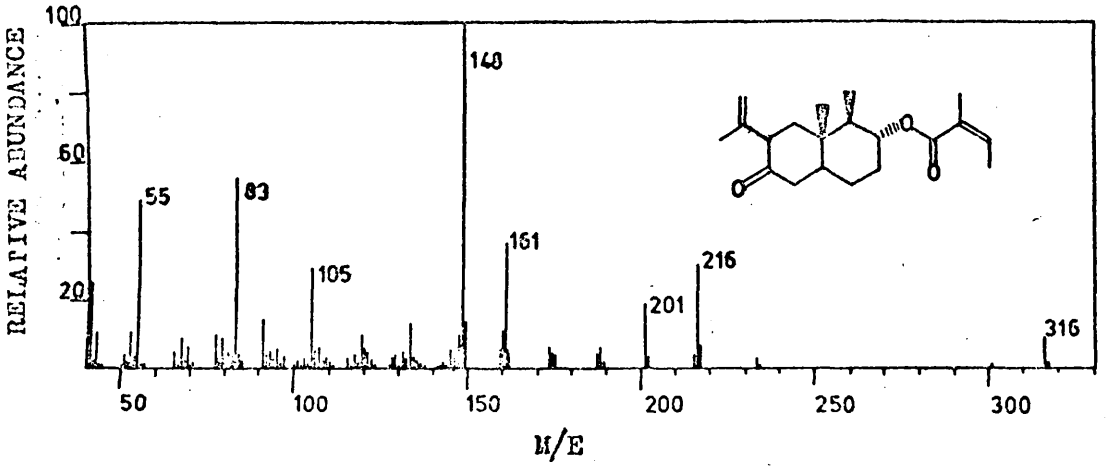
55. VR8 Petasyl propionate.



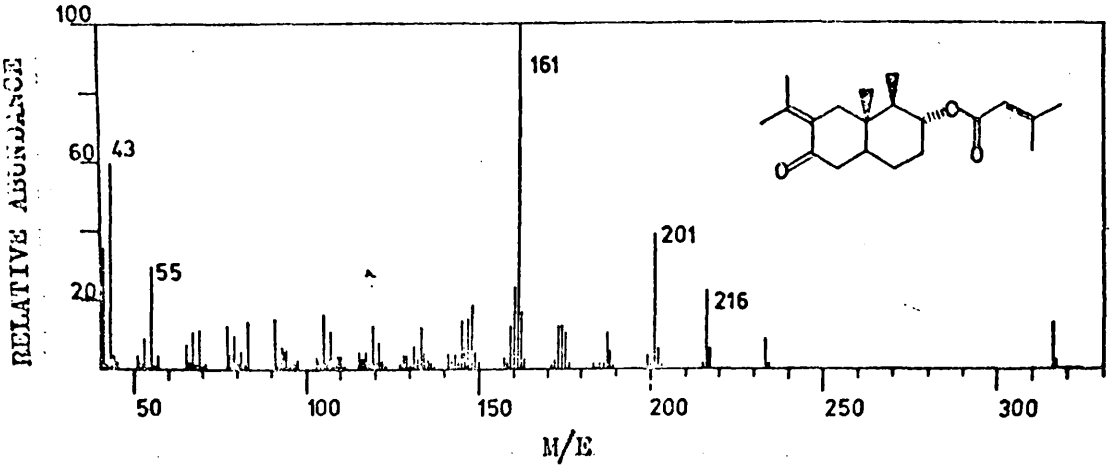
56. VR10 Petasyl dimethylacrylate.



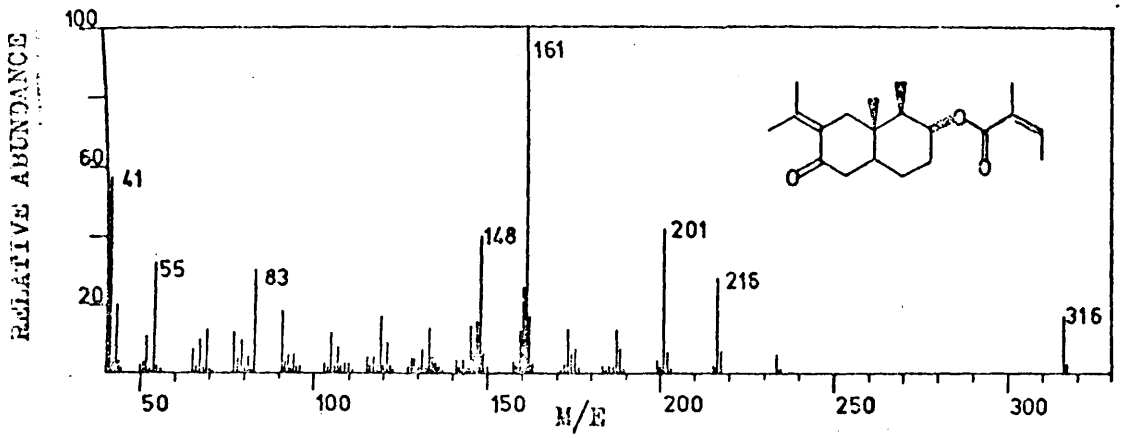
57. VR12 Petasin.



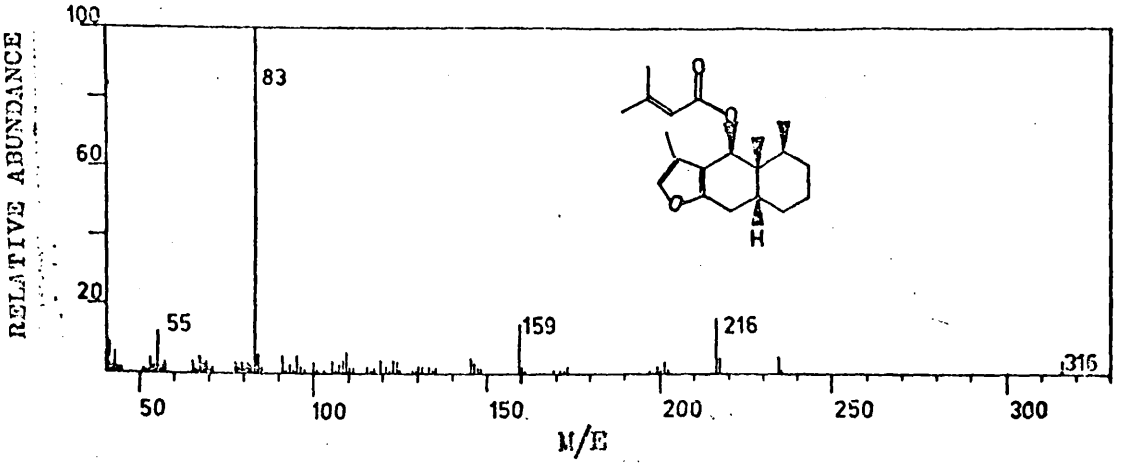
58. VR11 Isopetasyl dimethylacrylate.



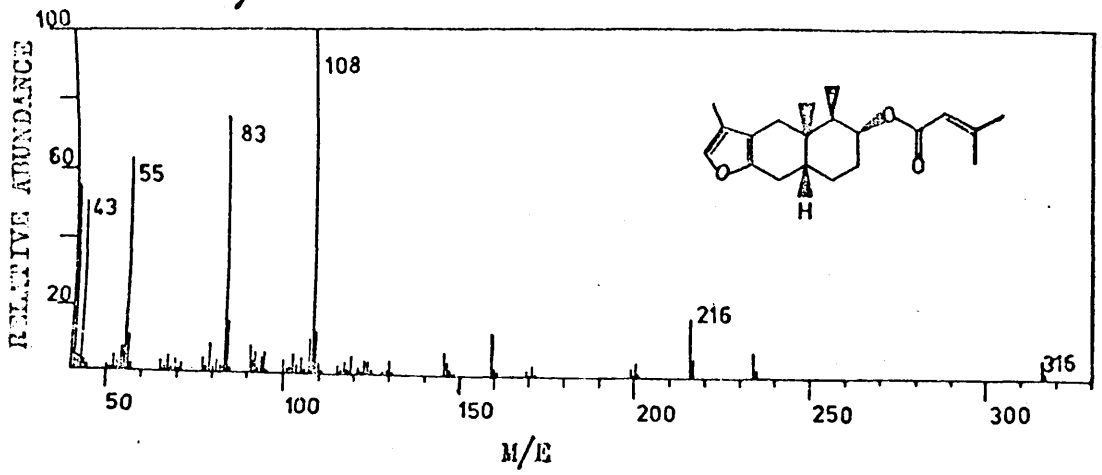
59. Isopetasin.



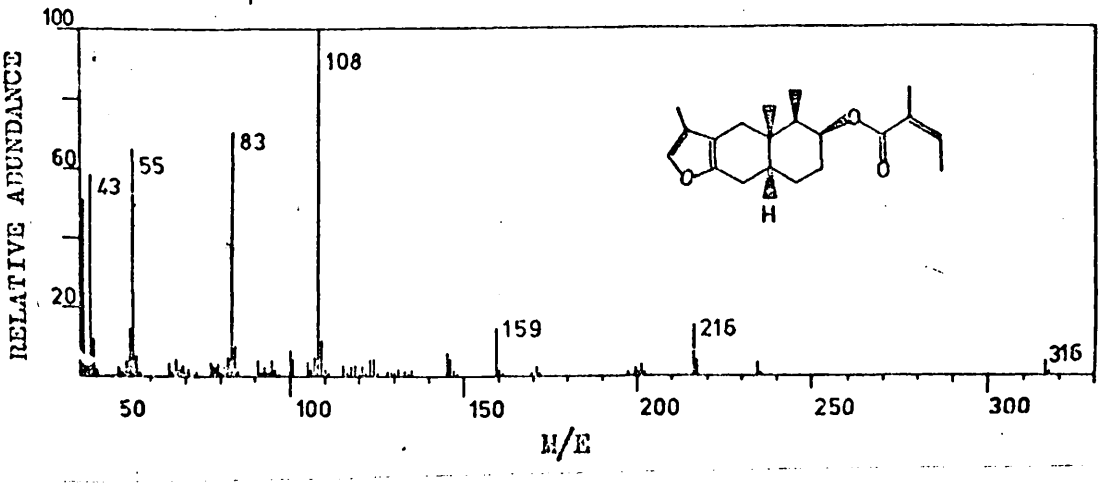
60. TN3 2β-dimethylacryloyloxy-furanoeremophilane.



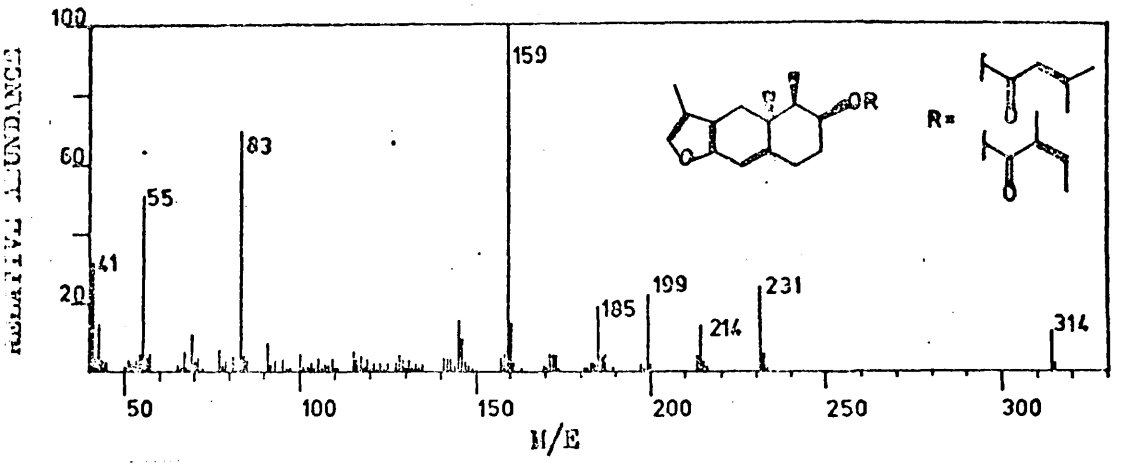
61. TR3a 5β-dimethylacryloyloxy-furanoeremophilane.



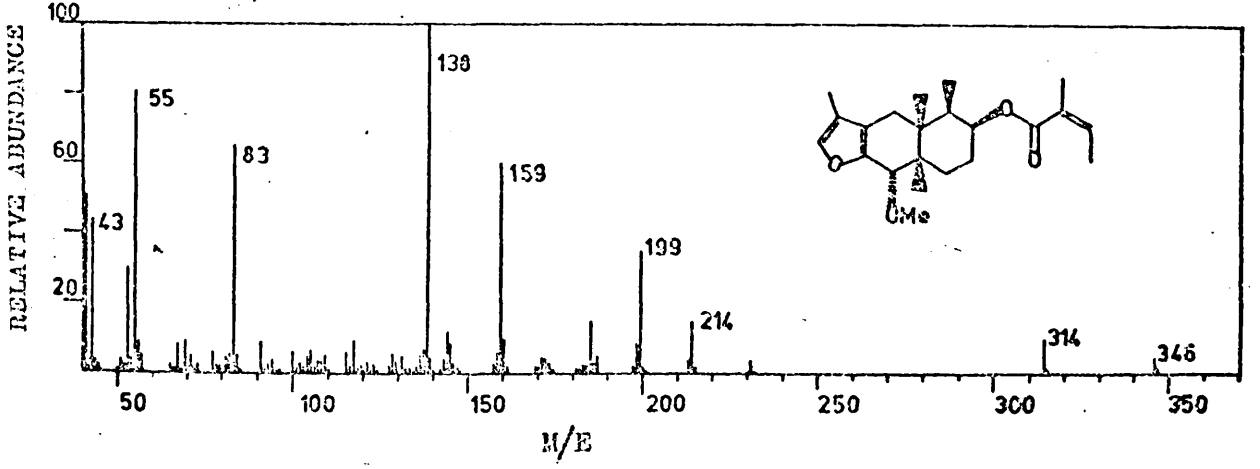
62. TR3b 5β-angeloyloxy-furanoeremophilane.



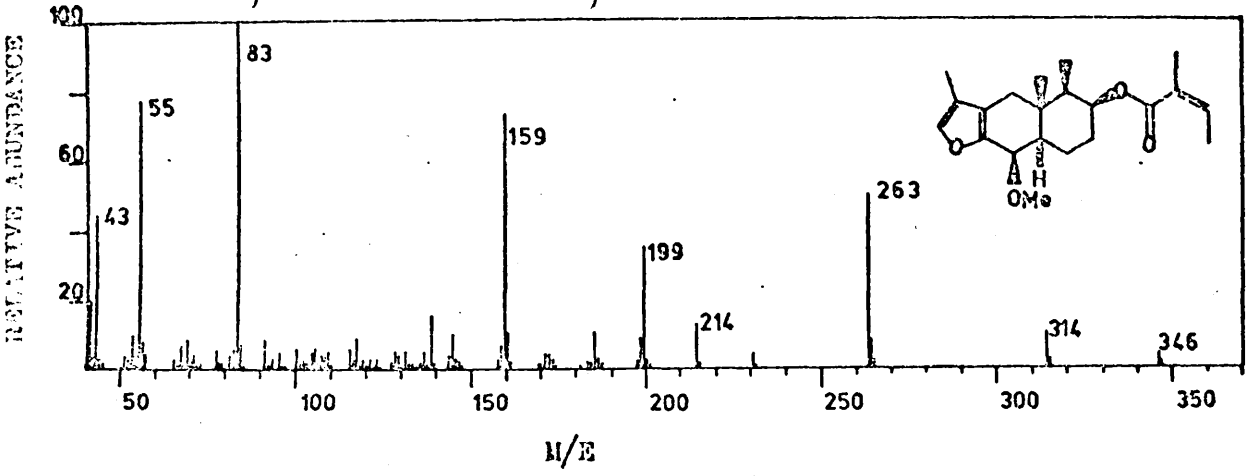
TR4 5β-dimethylacryloyloxy- and
63. 5β-angeloyloxy-furanoeremophilane.



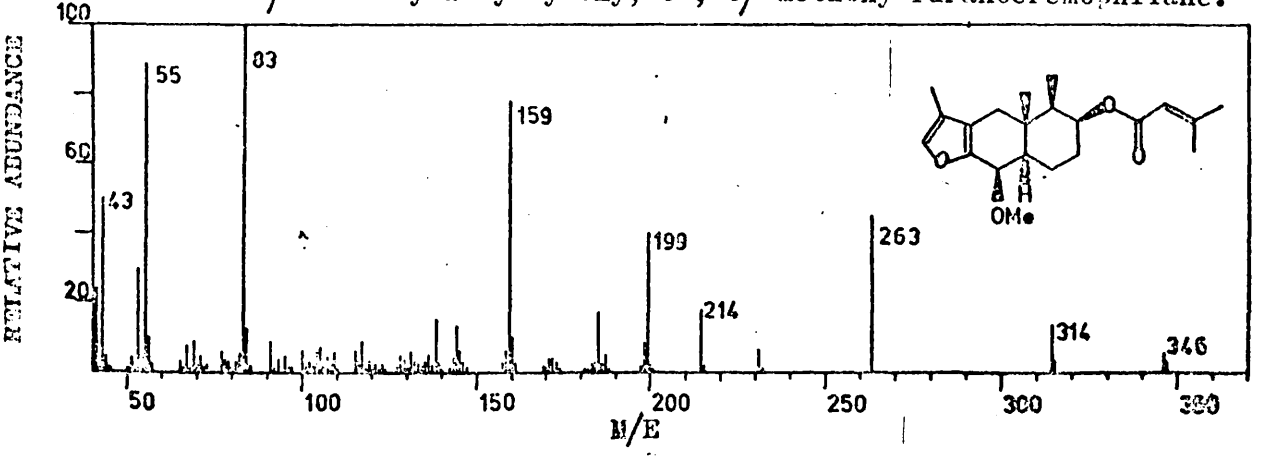
64. TR4a 5β-angeloyloxy, 9α-methoxy-furanoeremophilane.



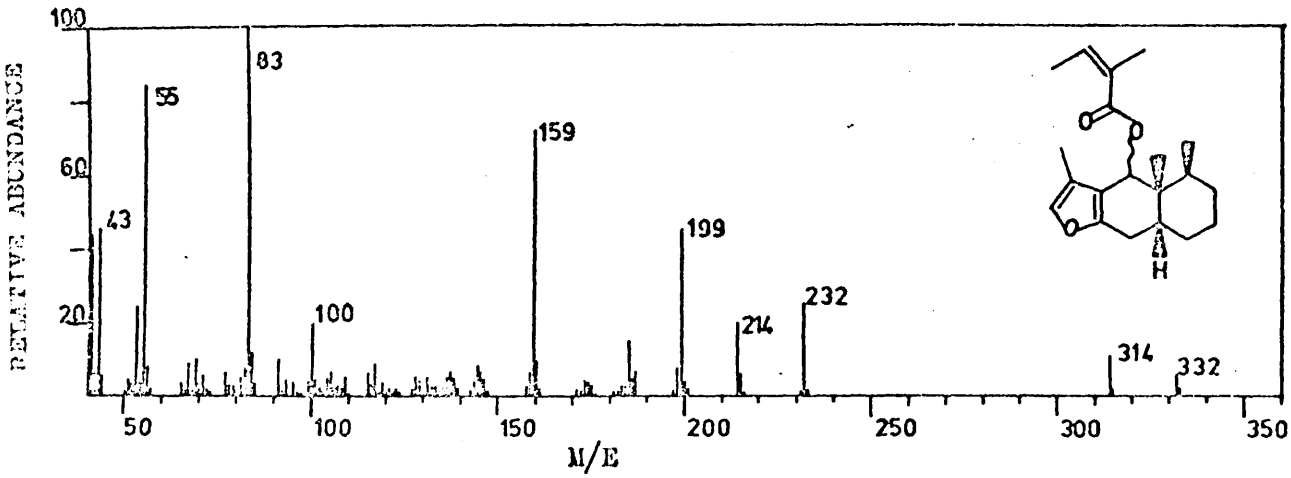
65. TR4b 5β-angeloyloxy, 8α, 9β-methoxy-furanoeremophilane.



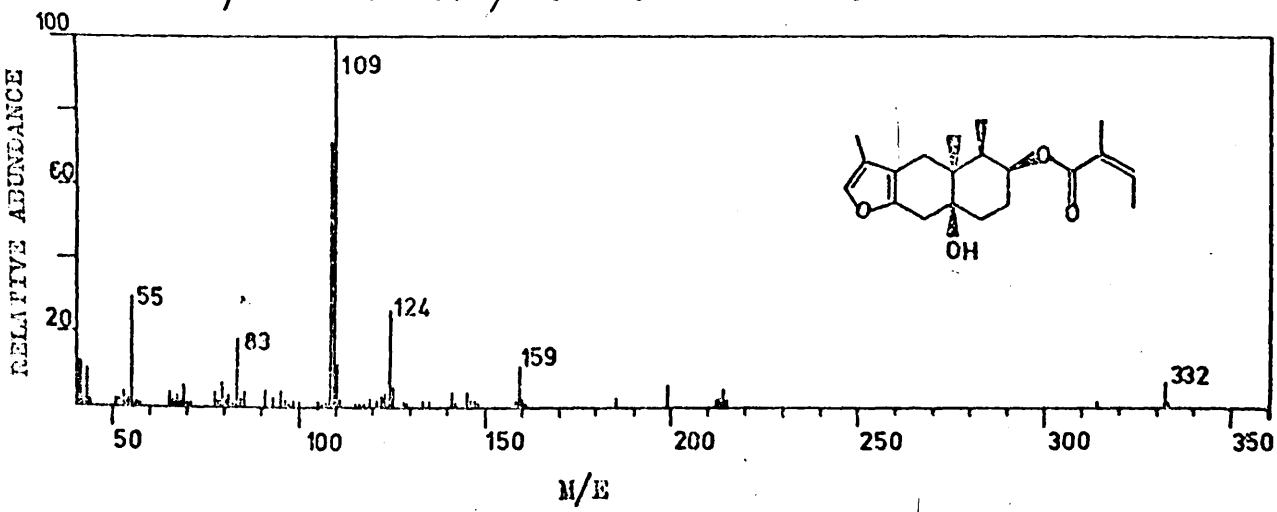
66. TR4c 5β-dimethylacryloyloxy, 8α, 9β-methoxy-furanoeremophilane.



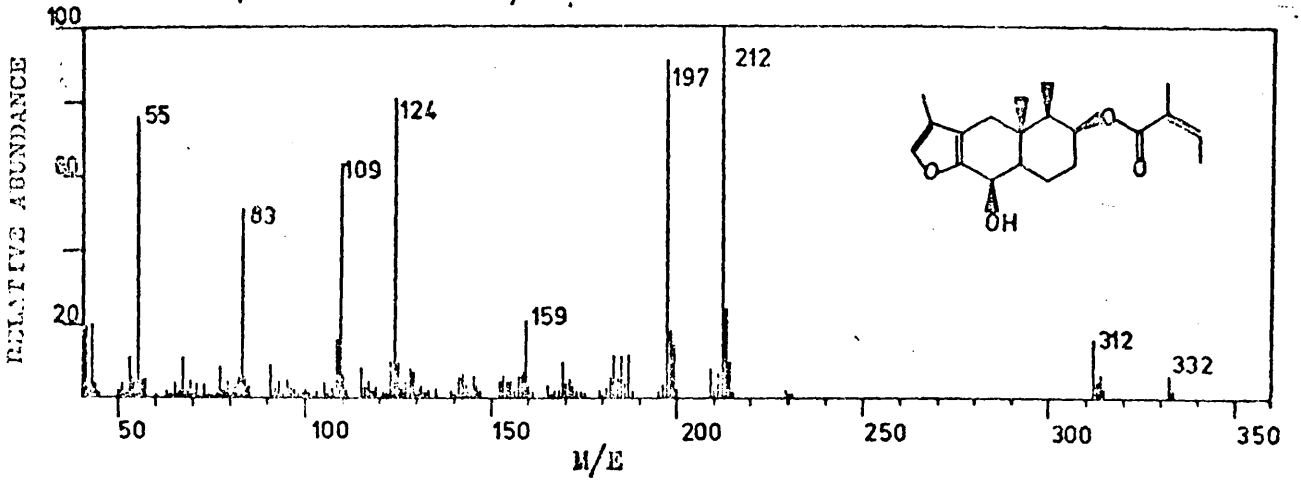
67. TR4d/e 2-angeloyloxy, 8β-hydroxy-furanoeremophilane.



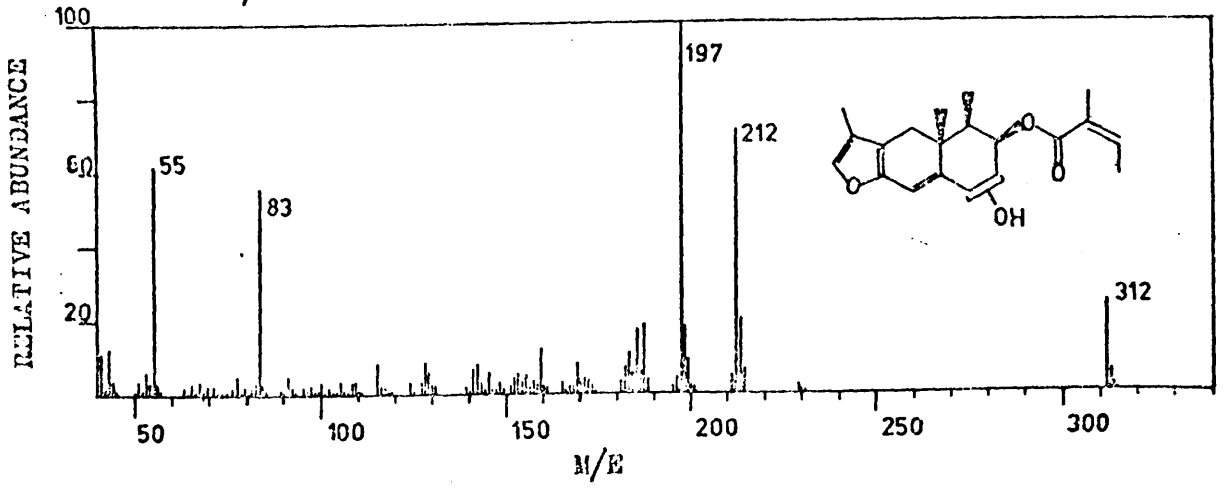
68. TR5 5β-angeloyloxy, 8β-hydroxy-furanoeremophilane.



69. TR6 5β-angeloyloxy, 9β-hydroxy-furanoeremophilane.



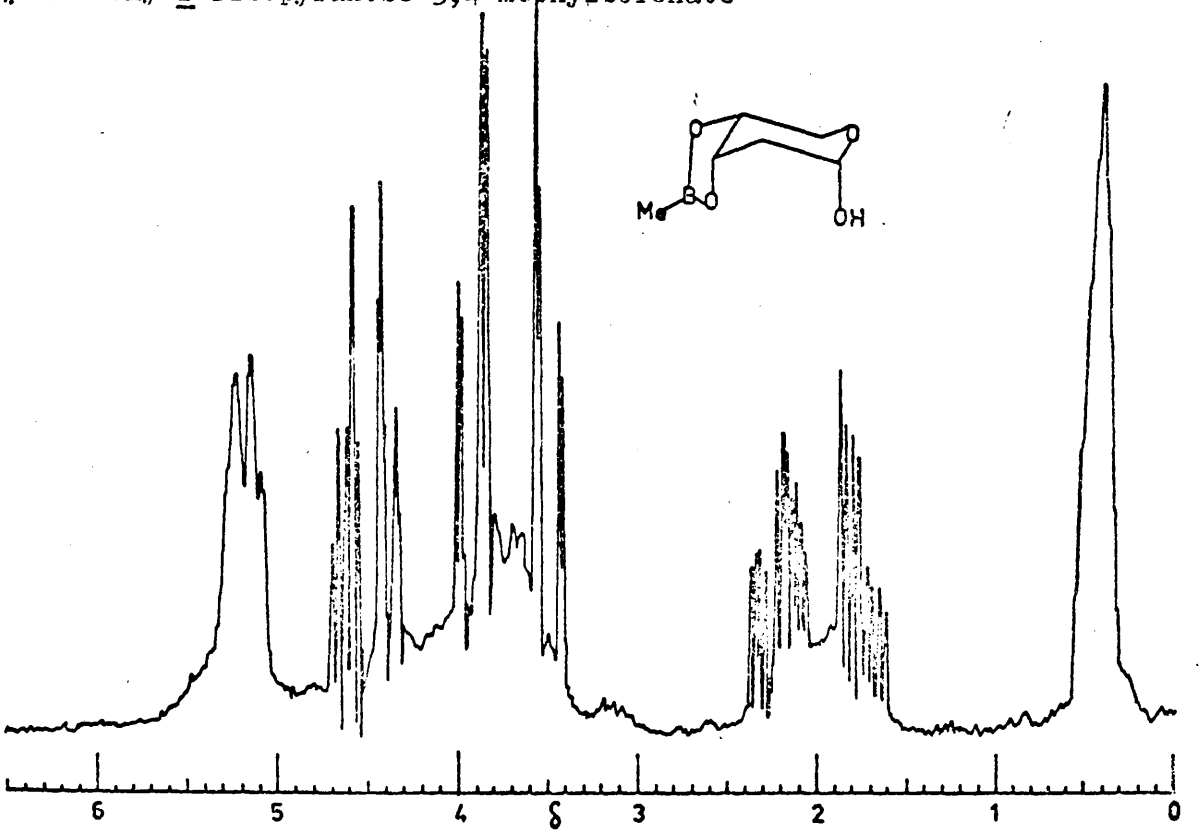
70. TR7 5β-angeloyloxy, (6 or 7)-hydroxy-furanoeremophilane.



APPENDIX II

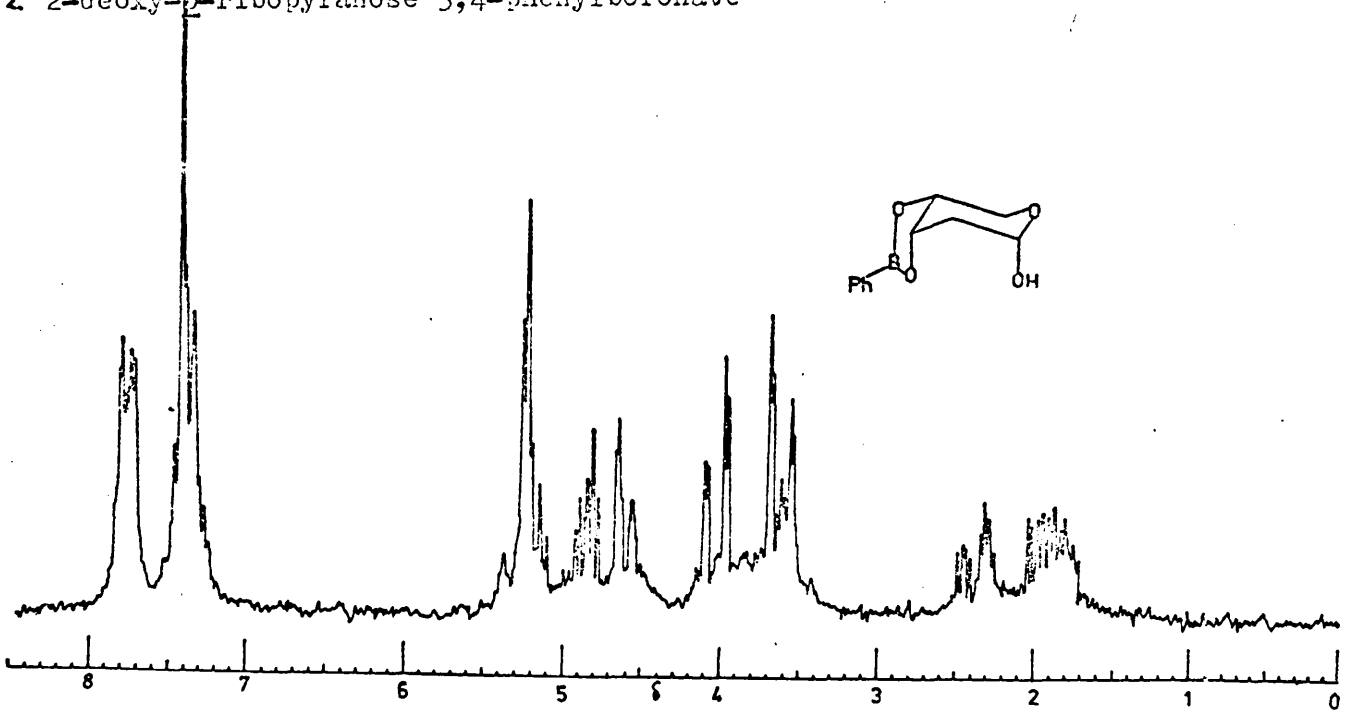
Nuclear Magnetic Resonance Spectre

1. 2-deoxy-D-ribopyranose 3,4-methylboronate

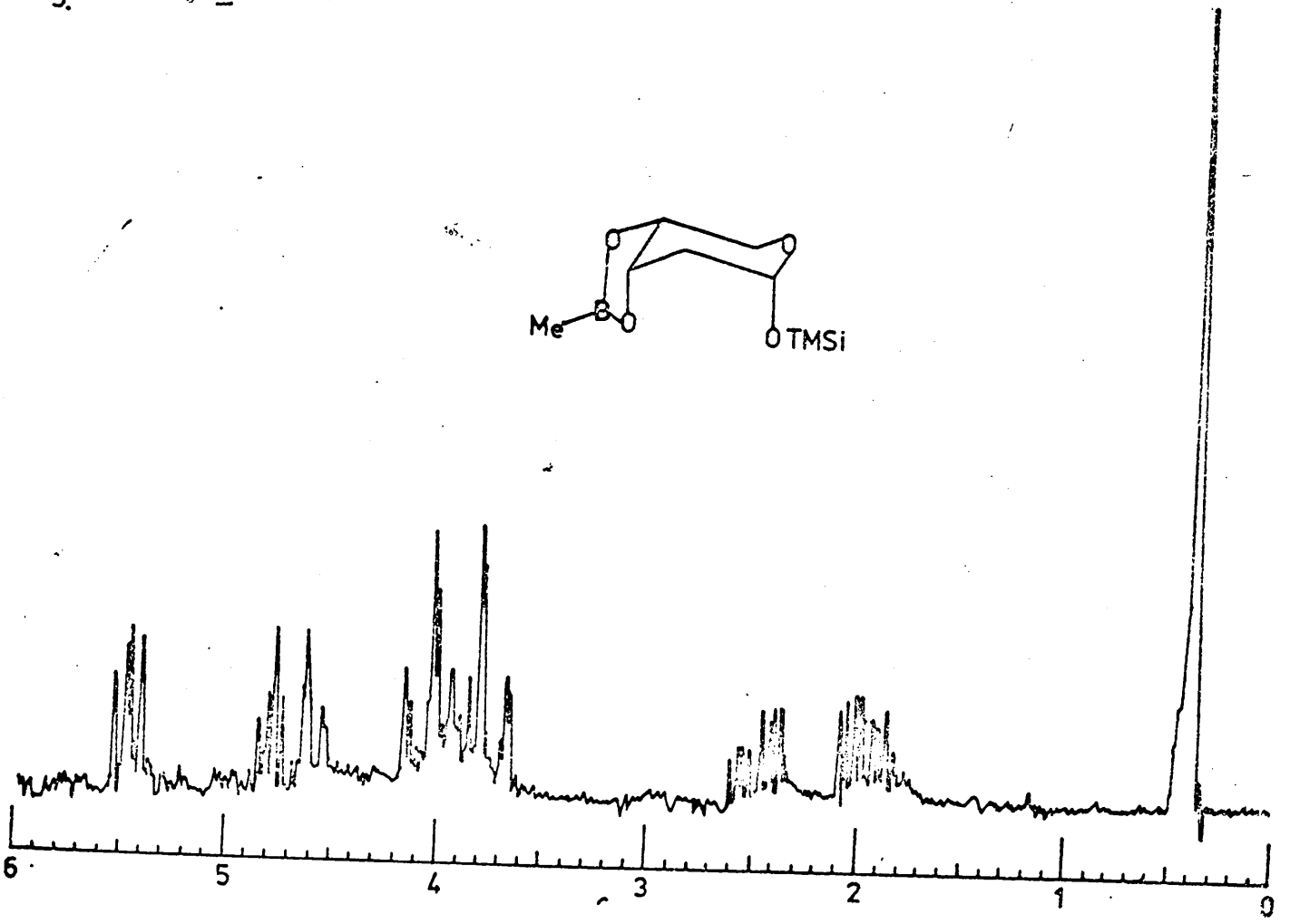


III 1

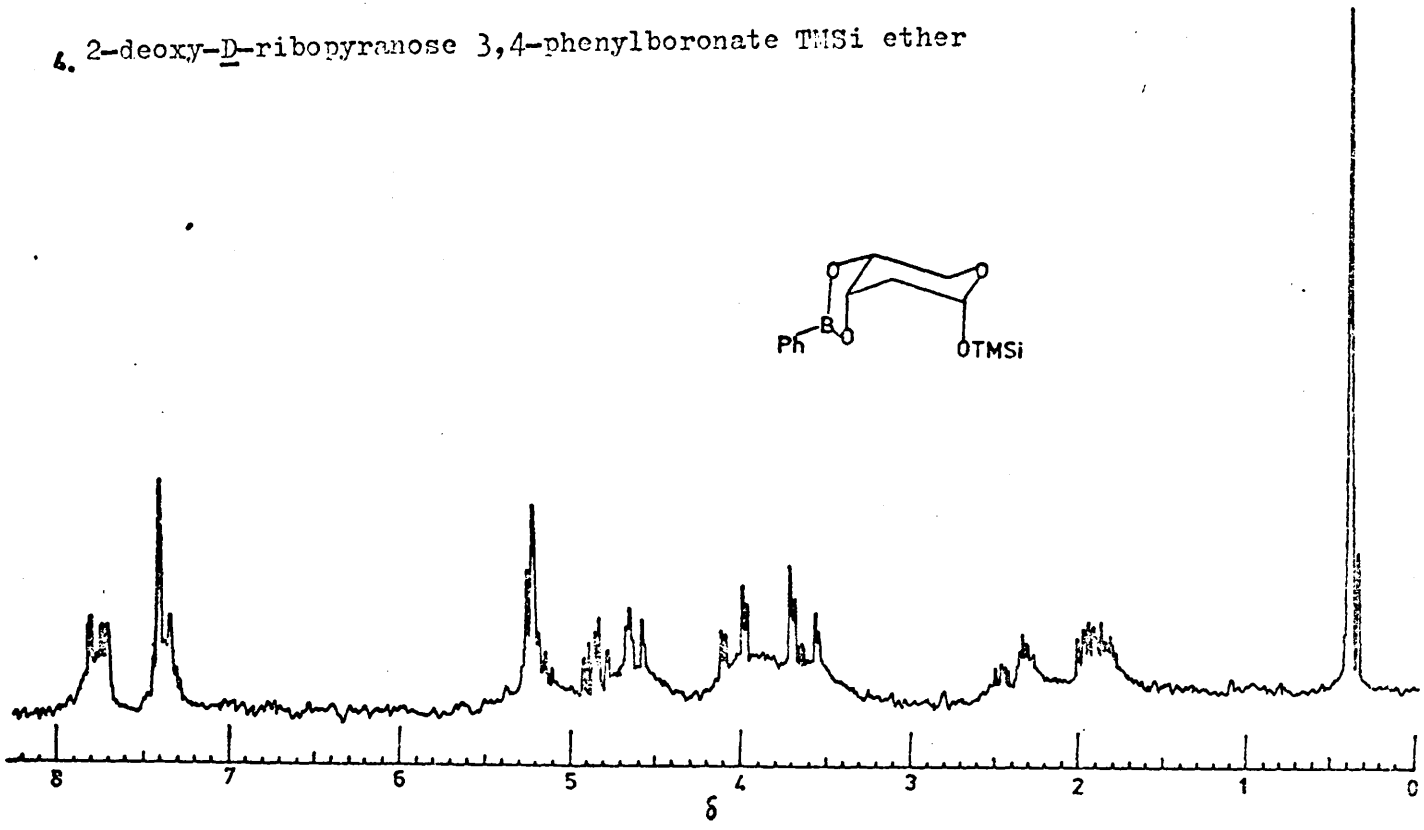
2. 2-deoxy-D-ribopyranose 3,4-phenylboronate



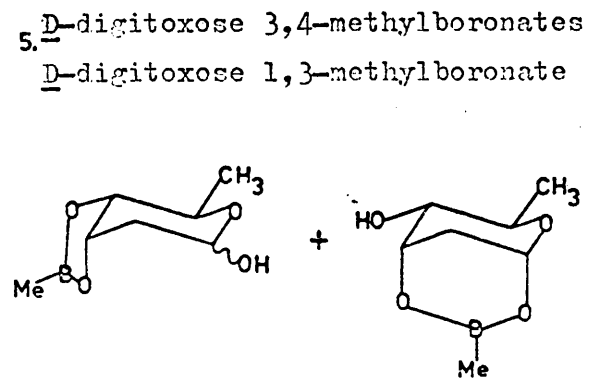
3. 2-deoxy-D-ribopyranose 3,4-melhylboronate TMSi ether



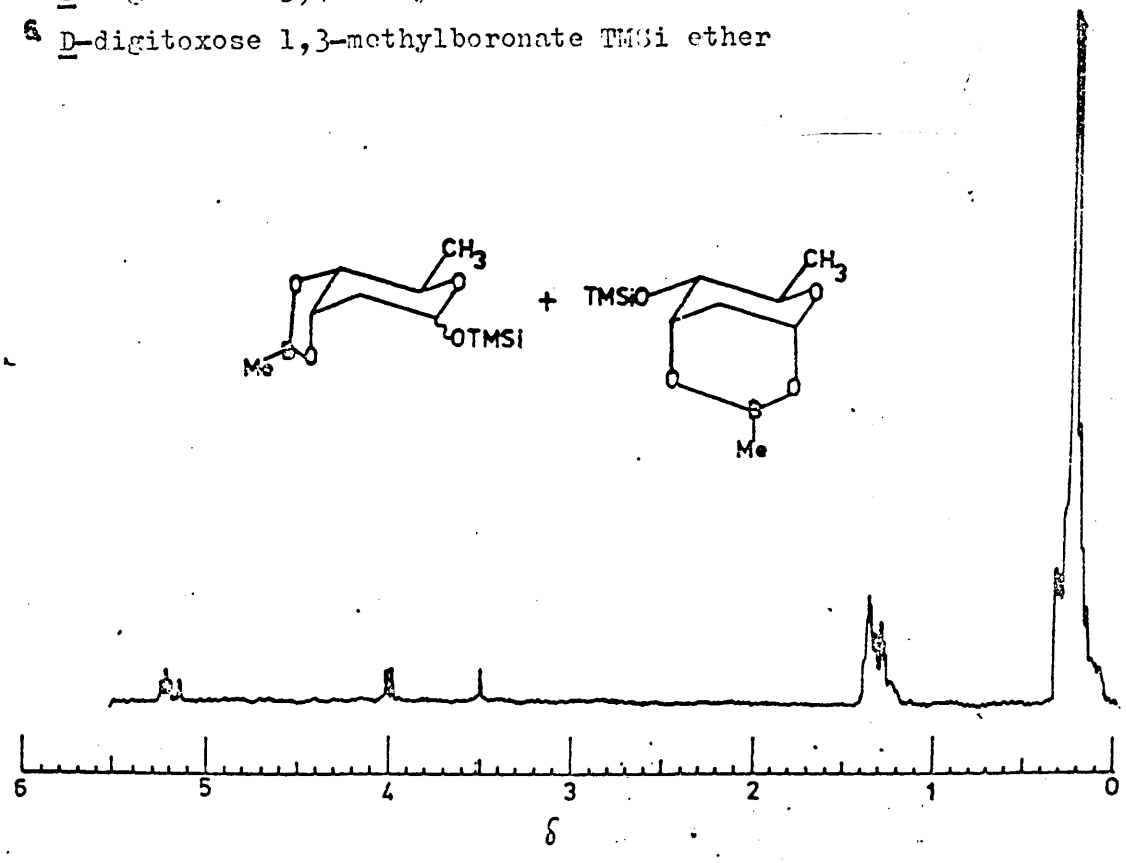
4. 2-deoxy-D-ribopyranose 3,4-phenylboronate TMSi ether



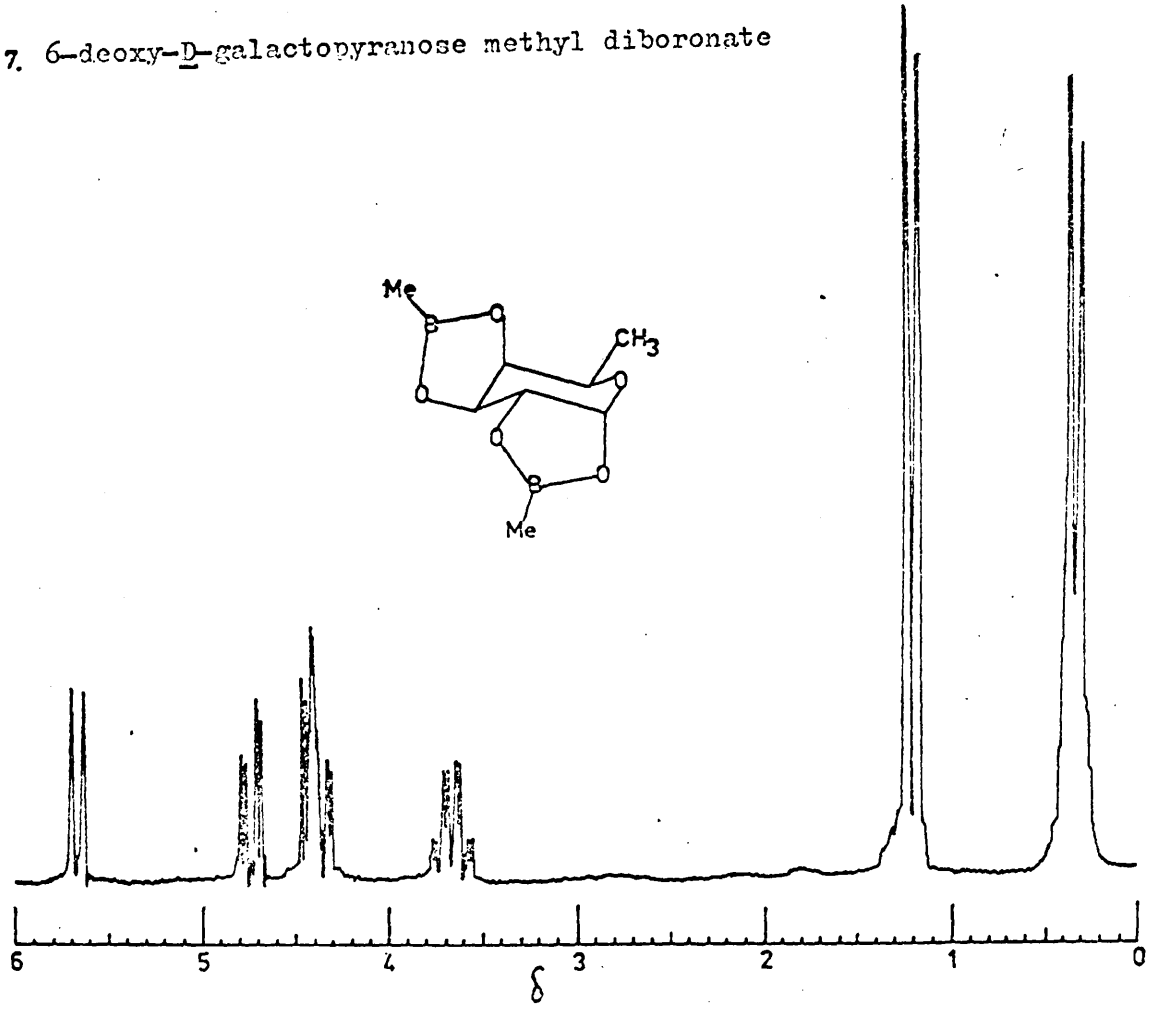
5. D-digitoxose 3,4-methylboronates +
D-digitoxose 1,3-methylboronate



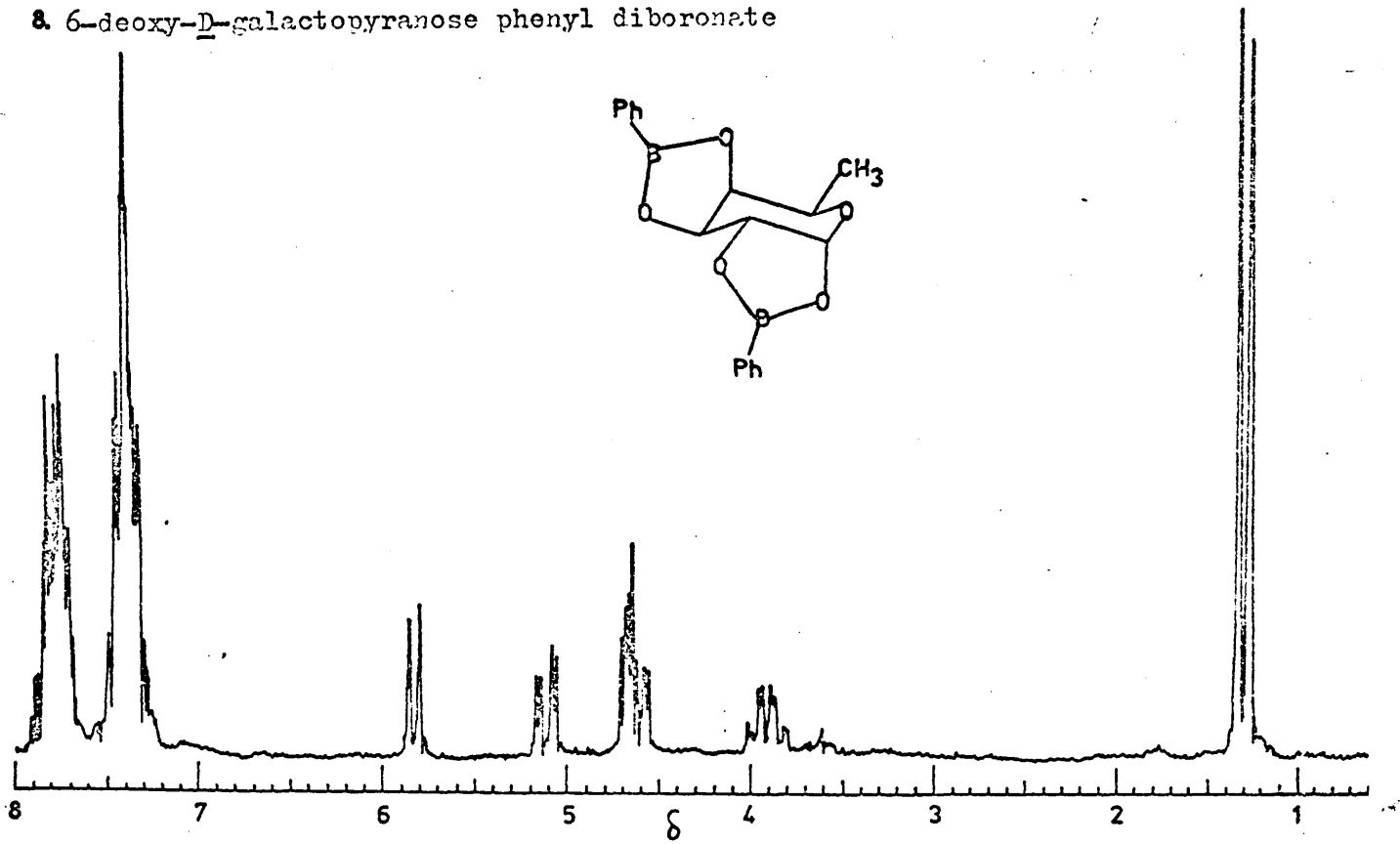
D-digitoxose 3,4-methylboronate TMSi ether +
D-digitoxose 1,3-methylboronate TMSi ether



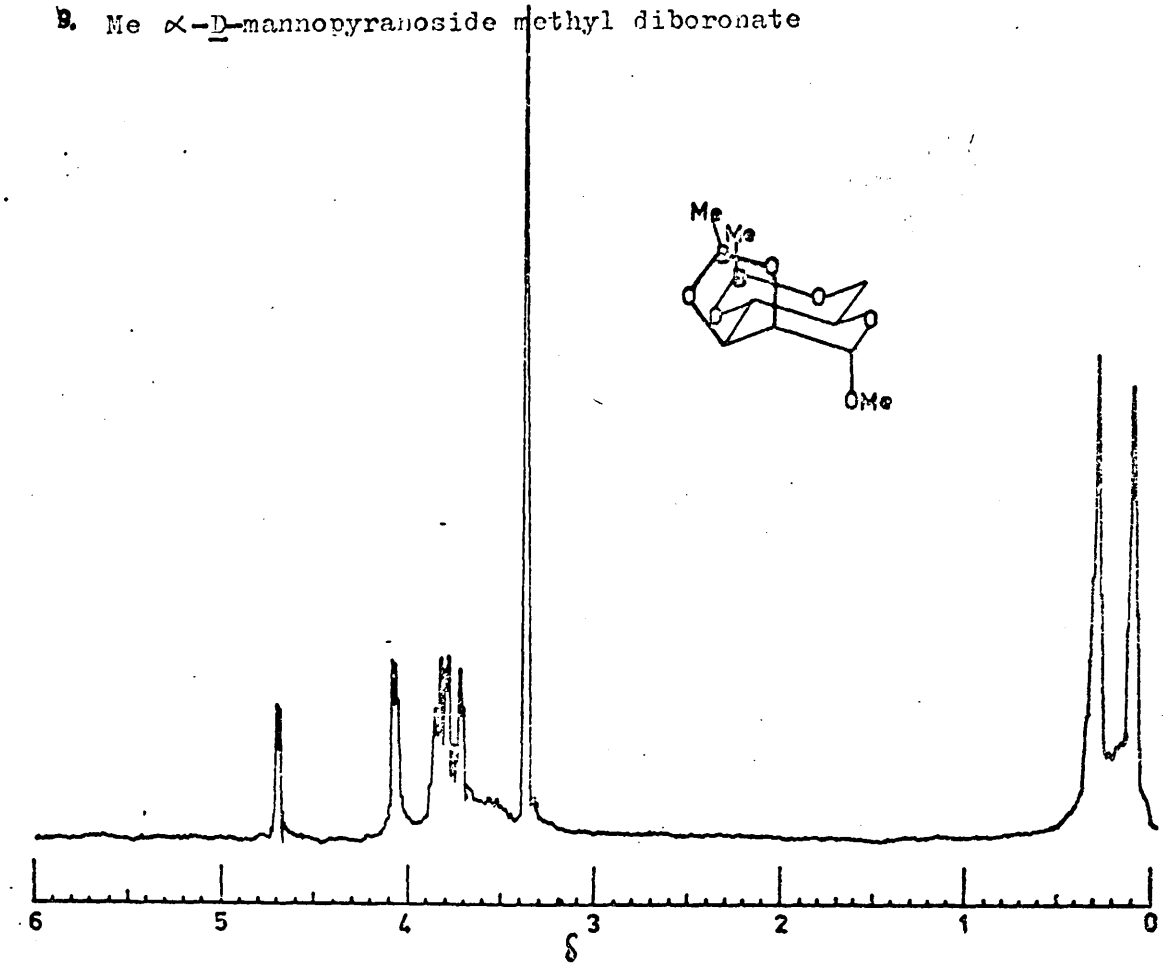
7. 6-deoxy-D-galactopyranose methyl diboronate



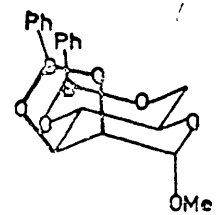
8. 6-deoxy-D-galactopyranose phenyl diboronate

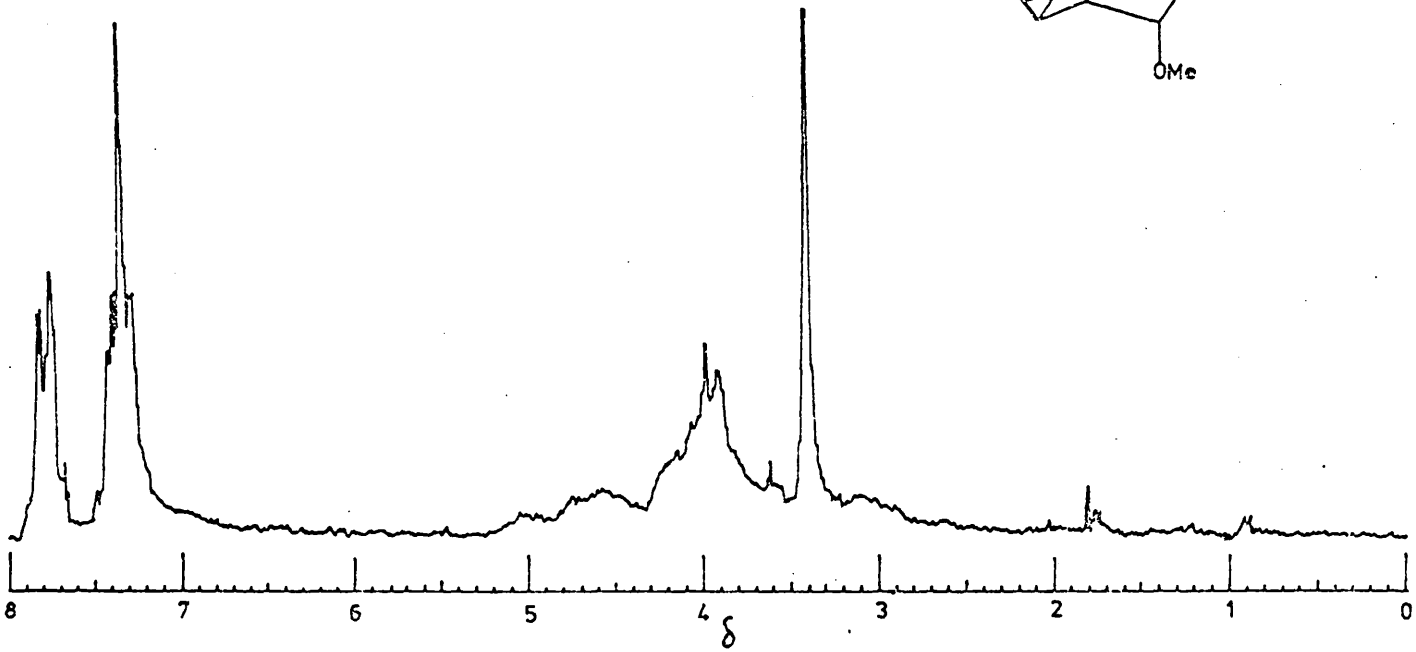


b. Me ∝-D-mannopyranoside methyl diboronate



10. Me α-D-mannopyranoside phenyl diboronate





APPENDIX III

GLC and GC-MS Reference Data

HYDROCARBONS GROUP A

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
158	cubebene	$C_{15}H_{24}$	204		
	α-longipinene			1396	
	α-ylangene				
	β-elemene				
	α-bourbonene				
	α-copaene			1379	1447
	cyclosativene				
	longicyclene			1371	1465
	cyclocopacamphene				1467
	β-bourbonene			1386	1477
	β-farnesene			1307	1509
	sativene				
	cedrene			1390	
	cyperene			1398	1493

GROUP B

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
158	α-gurujene	$C_{15}H_{24}$	204	1413	1471
	caryophyllene			1418	1587
0[1]	caryophyllene			1425	
158	longifolene			1404	1520
	isosativene				
	calarene			1435	1513
	β-ylangene			1418	
	β-copaene			1422	
	α-cedrene			1415	1518
	thujopsene			1431	1540
	aromadendrene				
	γ-curcumene				1532
	β-cedrene			1421	1539
	ε-muurolene			1445	1552
	humulene			1447	1583
	β-santalene			1435	
	α-himalchalene			1444	1533

[1] "0" means that the Data were obtained by experiment.

GROUP C

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
158	δ-selinene	$C_{15}H_{24}$	204		
	γ-muurolene				1545
	γ-amorphene				1545
	α-amorphene				1535
	zizaene				1562
	β-bisabolene			1497	1548
	β-curcumene				1547
	α-zingaberene				
	valencene			1457	1581
	α-curcumene			1475	1557
	β-himalchalene			1491	1578
	β-selinene				1598
	γ-bisabolene				
	α-muurolene			1495	1559

GROUP D

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
158	α-pyrovativene	$C_{15}H_{24}$	204		
	α-selinene				
	ε-bulgarene				
	δ-cadinene			1504	
	γ-cadinene			1506	1587
	β-vetivene				

ALCOHOLS

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
0	nerolidol	$C_{15}H_{26}O$	222	1545	1685
0	guaiol	"	"	1565	1740
0	cedrol	"	"	1565	1830
0	ledol	"	"	1570	1830
0	hinesol			1600	1850
0	daucol	$C_{15}H_{26}O_2$	238	1600	1885
0	eudesmol	$C_{15}H_{26}O$	222	1620	-
0	caparrapidiol	$C_{15}H_{28}O_2$	240	1660	-
0	∫-cadinol	$C_{15}H_{26}O$	222	1660	1910
0	drimenol	"	"	1715	2015

KETONES

Reference	Compound	Formula	M.W.	Retention Index	
				1%-SE-30	1%-QF-1
0	zerumbone	$C_{15}H_{22}O$	218	1690	2320
0	carissone	$C_{15}H_{24}O_2$	236	1860	2610
0	isopetasol	$C_{15}H_{22}O_2$	234	1935	-

SESQUITERPENE HYDROCARBONS

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
164	calamenene	$C_{15}H_{22}$	202	159	157, 41, 142, 160, 105, 143.
175	alloaromadendrene	$C_{15}H_{24}$	204	161	41, 91, 93, 105, 107, 69.
172	alloaromadendrene			161	41, 91.
172	aromadendrene			161	204, 41.
171	bazzanene		204	109	108, 67, 93, 41, 55, 81.
173	bergamotene			93	41, 119, 69, 55, 79, 107.
175	α-bergamotene			93	119, 41, 69, 55, 107, 79.
176(3340)	α-trans-β-bergamotene			119	93, 41, 69, 91, 107, 55.
172	β-bergamotene			41	69, 93, 148.
173	bisabolene			41	69, 93, 204, 94, 55, 73.
172	α-bisabolene			93	41, 121, 80.
177	α-bisabolene			93	41, 81, 109, 119, 121, 204.

* B.P. = Base Peak

SESQUITERPENE HYDROCARBONS Contd...

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
176	α-bisabolene	$C_{15}H_{24}$	204	93	119, 41, 105, 109, 121, 107.
172	β-bisabolene			69	41, 93.
175	β-bisabolene			69	93, 41, 94, 67, 204, 79.
172	α-bulnesene	$C_{15}H_{24}$	204	107	93, 108.
172	γ-cadinene			161	105, 41, 136.
173	γ-cadinene			161	41, 204, 57, 91, 79, 105.
175	γ-cadinene			161	105, 93, 95, 41, 119, 79.
172	δ-cadinene			161	134, 204, 134.
173	δ-cadinene			161	134, 204, 41, 105, 91, 119.
178	δ-cadinene			161	134, 105, 119, 204, 41, 91, 81.
175	δ-cadinene			161	134, 204, 119, 105, 91, 81.
173	calarene			161	204, 41, 105, 91, 55, 189.
172	sesqui-2-carene			93	41, 121.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
173	caryophyllene	$C_{15}H_{24}$	204	41	69, 93, 81, 57, 133, 51.
172	caryophyllene			41	93, 69, 148, 120.
176	caryophyllene			41	79, 91, 93, 53, 77, 67.
175	caryophyllene			79	91, 39, 53, 77, 67, 93.
176(3476)	caryophyllene			133	93, 79, 91, 105, 81, 77.
174	α-caryophyllene			93	80, 121, 41, 92, 147, 107.
172	cedrene			119	93, 204.
173	α-cedrene			119	41, 93, 204, 69, 103, 55.
175	α-cedrene			119	93, 105, 41, 69, 91, 161.
174	α-cedrene			93	41, 105, 204, 69, 161, 91.
173	β-cedrene			204	181, 69, 41, 93, 55, 120.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
172	α-chamigrene	$C_{15}H_{24}$	204	136	121, 93, 136.
172	β-chamigrene			189	41, 93.
176(138)	clovene			161	41, 189, 91, 105, 79, 55.
172	clovene			161	189, 41, 189.
173	α-copaene			161	119, 105, 41, 204, 93, 89.
175	α-copaene			119	105, 161, 93, 91, 41 92.
178	copaene			119/ 105	161, 93, 41, 91, 92
172	α-cubebene			161	105, 119.
173	α-cubebene			161	119, 105, 204, 41, 121, 91.
175	α-cubebene			105	161, 119, 91, 41, 93, 81.
175	β-cubebene			161	105, 91, 120, 41, 119, 81.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
173	β-cubebene	$C_{15}H_{24}$	204	161	119, 105.
172	α-cuprenene			119	121, 204, 136.
172	α-cuprenene			119	93, 105.
175	4,10-dimethyl-7-isopropyl-bicycle (4,4,0)-1,4,decadiene			119	105, 161, 204, 121, 91, 41.
172	α-elemene			161	41, 119.
173	α-elemene			121	41, 93, 55, 57, 110, 67.
172	β-elemene			93	41, 81, 68.
173	β-elemene			41	68, 81, 93, 67, 55, 57.
172	γ-elemene			121	93, 105, 136.
175	γ-elemene			121	93, 107, 41, 105, 67, 91.
172	δ-elemene			121	93, 136.
173	δ-elemene			136	121, 93, 41, 91, 204, 161.
175	δ-elemene			121	93, 136, 41, 161, 91, 43.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
173	eremophilene	$C_{15}H_{24}$	204	41	204, 123, 121, 93, 79, 119.
177	eremophilene			93	107, 41, 161, 79, 91, 105.
172	farnesene			69	41, 93, 120.
175	α-farnesene			93	41, 69, 55, 79, 107, 119.
173	trans-β-farnesene			41	69, 93, 55, 81, 53, 67.
172	α-ferulene			105	161, 91.
173	guaiene			161	105, 204, 41, 119, 91, 109.
172	α-guaiene			105	147, 107, 148.
175	α-guaiene			105	107, 147, 93, 41, 79, 91.
175	δ-guaiene			107	93, 108, 79, 41, 81, 105.
175	α-gurujene			204	161, 105, 189, 41, 119, 91.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
172	α-gurujene	$C_{15}H_{24}$	204	204	161, 105.
175	β-gurujene			161	41, 105, 91, 119, 93, 107.
176(137)	β-gurujene			41	161, 105, 79, 77, 93, 55/107.
174	β-gurujene			161	91, 105, 79, 77, 103, 107.
172	α-himalchalene			93	41, 94, 134.
175	α-himalchalene			93	94, 119, 41, 79, 91, 105.
175	β-himalchalene			119	204, 121, 134, 105, 93, 133.
172	β-himalchalene			119	204, 134.
176(3474)	humulene			80	41, 121, 93, 107,
172	humulene			93	41, 80.
175	humulene			93	80, 121, 41, 92, 107, 147.
176(136)	humulene			41	93, 53, 67, 91, 79, 77.
173	α-humulene			93	41, 80, 121, 53, 204, 55.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
177	β-humulene	$C_{15}H_{24}$	204	161	41, 105, 81, 91, 119, 79.
174	isolongifolene			161	41, 175, 105, 148, 133, 119.
172	khusimene			134	91, 93, 135, 133.
172	longicyclene			94	105, 91, 163.
175	longifolene			161	94, 91, 93, 107, 95, 105.
172	longifolene			41	161, 91, 94.
174	longifolene			161	94, 91, 93, 107, 105, 95.
172	longipinene			91	119, 105, 204.
175	α-muurolene			105	161, 204, 93, 94, 81, 119.
172	α-muurolene			105	161, 41, 94.
178	α-muurolene			105	94, 93, 161, 41, 204, 89.
174	α-muurolene			161	94, 93, 204, 41, 81, 91.
175	γ-muurolene			161	105, 119, 93, 204, 91, 79.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
174	γ-muurolene			105	93, 119, 41, 91, 79, 204.
174	ε-muurolene			81	41, 91, 93, 105, 79, 67.
172	α-patchoulene			135	93, 107, 108.
172	β-patchoulene			189	161, 204.
175	β-patchoulene			161	189, 119, 105, 93, 204, 41.
172	γ-patchoulene			204	161, 41.
173	santalene			94	122, 41, 55, 93, 79, 69.
175	α-santalene			94	93, 41, 95, 69, 121, 107.
172	α-santalene			41	94, 93.
40(3439)	α-santalene			93	94, 41, 107, 121, 91, 69.
172	β-santalene			94	93, 41, 122.
175	β-santalene			94	122, 41, 93, 79, 55, 91.
175	epi-β-santalene			94	122, 41, 93, 79, 69, 67.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
172	α-selinene	$C_{15}H_{24}$	204	189	204, 161, 189.
173	α-selinene			41	55, 204, 189, 52, 81, 93.
173	β-selinene			41	204, 81, 55, 107, 93, 67.
40(135)	β-selinene			93	41, 80, 121, 107, 55, 109.
174	β-selinene			80	41, 121, 107, 55, 109, 79.
172	β-selinene			204	41, 105.
173	δ-selinene			204	189, 161, 41, 91, 105, 55.
175	thujopsene			119	123, 105, 121, 93, 41, 107.
172	thujopsene			119	123, 121.
176(1746)	thujopsene			121	119, 133, 93, 189, 105, 107.
174	thujopsene			119	123, 41, 105, 121, 93, 55.
172	valencene			204	161, 41.
172	vetivenene			204	161, 91.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
172	zingiberene	$C_{15}H_{24}$	204	93	119, 41.
173	∝-zingiberene			93	119, 41, 69, 84, 56, 204.
178	ylangene			105	119, 93, 120, 161, 41, 91.
172	α-ylangene			105	119, 93, 120.
172	β-ylangene			161	41, 204, 120.
173	farnesane	$C_{15}H_{32}$	212	57	43, 71, 55, 52, 89, 69.
173	bisabolane	$C_{15}H_{30}$	210	41	69, 93, 204, 94, 55, 73.
173	elemane			69	41, 55, 43, 56, 83, 111.
173	humulane			41	55, 57, 69, 59, 45, 67.
173	cadinane	$C_{15}H_{28}$	208	165	109, 41, 95, 55, 208, 83.
173	caryophyllane			41	68, 152, 55, 69, 82, 83.
173	eremophilane			208	41, 55, 95, 81, 109, 69.

SESQUITERPENE HYDROCARBONS Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
173	santalane	$C_{15}H_{28}$	208	123	41, 95, 55, 43, 208, 81.
173	selinane			41	55, 208, 109, 96, 83, 69.
173	bergamotane			41	55, 69, 95, 81, 43, 83.
173	cedrane	$C_{15}H_{26}$	206	82	41, 206, 56, 69, 96, 122.
173	copane			163	41, 81, 107, 55, 93, 67.
173	dihydrovalancene			206	41, 95, 163, 91, 81, 121.

LACTONES

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
165	hyposantonin	$C_{15}H_{18}O_2$	230	158	215, 230, 119, 159, 115, 171.
160	costunolide	$C_{15}H_{20}O_2$	232	53	41, 123, 39, 81, 121, 105.
0(73/768)	furanoliguranone			108	232, 109, 41, 79, 77, 91.
167	bakkenolide-A	$C_{15}H_{22}O_2$	234	124	109, 111, 123, 91, 79, 85.
170	linderalactone	$C_{15}H_{16}O_3$	244	244	161, 199, 133, 148, 91, 105.
176(350)	achillin	$C_{15}H_{18}O_3$	246	246	91, 173, 172, 217, 77, 135.
11(935)	6-epi-α-santonin			246	173, 83, 85, 41, 108, 91.
165	α-santonin			90	91, 173, 246, 135, 175, 121.
176(934)	α-santonin			246	173, 41, 91, 134, 172, 77.
165	β-santonin			90	91, 173, 135, 246, 142, 122.
176(936)	β-santonin			246	173, 41, 135, 91, 172, 231.

LACTONES

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
165	C -santonin	$C_{15}H_{18}O_3$	246	90	246, 173, 91, 145, 108, 135.
165	D -santonin			90	246, 173, 145, 135, 159, 134.
176(939)	6-deoxy-geigerin	$C_{15}H_{20}O_3$	248	133	248, 41, 55, 79, 91, 206.
176(941)	1,2,-dihydro-α-santonin			248	192, 41, 55, 124, 233, 136.
176(378)	arglanine	$C_{15}H_{18}O_4$	262	247	229, 201, 183/248, 262, 211.
176(937)	artemisin			91	41, 71, 262, 69, 135, 77.
165	artemisin			90	135, 123, 262, 244, 149, 124.
176(938)	geigerin	$C_{15}H_{20}O_4$	264	151	95, 69, 55, 41, 67, 173.
176(940)	isophoto-α-santonic lactone			43	41, 264, 55, 193, 91, 124.
176(377)	vulgarin			249	203, 175/231, 250/185.
163	gaillardin	$C_{15}H_{22}O_5$	306	188	53, 91, 55, 105, 189, 228.

KETONES

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
176(358)	aristolone	$C_{15}H_{22}O$	218	218	203, 77, 91, 147, 161, 105/119.
177	eremophila-9,11-dien-8-one			218	135, 150, 203, 161, 147, 97.
177	eremophila-7(11),9,-dien-8-one			218	161, 147, 162, 203, 175, 149.
175	germacrone			107	135, 121, 67, 41, 93, 91.
177	isopetasol	$C_{15}H_{22}O_2$	234	234	161, 162, 139, 149, 187, 147.
177	petasol			166	234, 122, 161, 94, 123, 201.
177	fukinone	$C_{15}H_{24}O$	220	109	220, 110, 96, 68, 108, 111.
0(73/1096)	carissone	$C_{15}H_{24}O_2$	236	59	218, 43, 41, 163, 203, 147.
176(1180)	cyperolone			43	41, 91, 133, 179, 236, 161.
176(347)	allopeucenin	$C_{15}H_{16}O_4$	260	205	260, 217, 245, 123, 176.

KETONES Contd....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
176(762)	allopeucenin	$C_{15}H_{16}O_4$	260	205	260, 217, 43, 206, 41, 245.
176(346)	isopeucenin			205	260, 217, 245, 176, 192, 123.
176(763)	isopeucenin			205	260, 217, 204, 206, 41, 43.
176(342)	peucenin			205	217, 260, 245, 192, 123, 231.
176(761)	peucenin			205	217, 260, 245, 206, 49.
176(348)	karenin	$C_{15}H_{14}O_5$	274	259	274, 233, 231, 245, 203.

ALCOHOLS

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
175	cis,trans,farnesol	$C_{15}H_{24}O$	220	69	41, 84, 81, 55, 105, 67.
175	trans, trans farnesol			69	84, 41, 81, 55, 39, 67.
175	α-santalol			94	93, 121, 43, 41, 122, 107.
176(140)	santalol			93	94, 121, 41, 79, 167, 91.
176(1749)	santalol			93	121, 94, 55, 91, 43, 107.
178	ylangenol			91	135, 93, 105, 177, 41, 81.
176(1220)	β-bisabolol	$C_{15}H_{26}O$	222	81	93, 41, 69, 119, 121, 82.
175	bulnesol			135	59, 107, 93, 161, 105, 81.
176(147)	δ-cadinol			161	43, 119, 41, 105, 204, 95.
0(73/462)	δ-cadinol			161	43, 119, 105, 95, 41/91.
176(145)	α-caryophyllene alcohol			41	109, 135, 189, 43, 95, 55.

ALCOHOLS Contd/....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
175	cedrenol	$C_{15}H_{26}O$	222	95	41, 43, 150, 151, 69, 55.
0(73/935)	cedrol			95	150, 151, 43, 41, 69/81.
175	cedrol			95	150, 151, 43, 41, 69, 81.
176(141)	cedrol			95	150, 151, 41, 64, 43, 81.
0(73/760)	drimenol			109	124, 41, 69, 55, 81, 95.
176(148)	drimenol			109	124, 41, 69, 55, 81, 95.
175	β-eudesmol			59	149, 164, 108, 109, 41, 81.
176(145)	eudesmol			59	149, 109, 164, 41/43, 81.
176(150)	farnesol			69	41, 43, 93, 55, 81, 68.
176(1747)	farnesol			69	93, 41, 55, 119, 79, 107.
175	guaiol			161	59, 107, 105, 93, 81, 163.

ALCOHOLS Contd/....

Reference	Compound	Formula	M.W.	B.P.	Strongest Ions
0(73/955)	guaiol	$C_{15}H_{26}O$	222	161	59, 107, 105, 43, 93, 81.
176(143)	ledol			43	41, 69, 122, 109, 81, 107.
0(73/957)	ledol			43	41, 69, 109, 81/122, 55.
0(73/764)	nerolidol			69	41, 93, 43, 71, 55, 81.
176(149)	nerolidol			69	41, 93, 45, 71, 43, 55.
176(142)	patchouli alcohol			41	222, 98, 81, 83, 138, 55.
175	torreyol			161	43, 119, 41, 105, 95, 81.
175	hexahydronerolidol	$C_{15}H_{32}O$	228	73	43, 55, 69, 57, 41, 81.
177	isopetasol	$C_{15}H_{22}O_2$	234	234	161, 162, 139, 149, 187, 147.
177	petasol			166	234, 122, 161, 94, 123, 201.
176	daucol	$C_{15}H_{26}O_2$	238	151	43, 194, 41, 136, 93, 133.
0(73/961)	daucol			151	93, 43, 194, 41, 136, 55.