

SOME STUDIES OF
ORGANIC MASS SPECTROMETRY

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Abstract

The existing information on the behaviour of methyl ethers and acetates of pyranosides under electron impact is reviewed and certain fragmentation schemes have been reappraised.

The mass spectra of several derivatives of fusicoccin, a naturally occurring glycoside, are reported. A part structure, which depends on mass spectral evidence, is deduced for the molecule.

The principles laid down in the review are used to rationalise the fragmentations of the acetates of some partially methylated glucosides. Particular reference is made to the effect of the position of attachment of methoxy groups to the sugar ring.

The methods of preparation, and the mass spectral decompositions of tetra-acetyl glycopyranosides are discussed. Eight fragmentation sequences are found in the mass spectra of these compounds, and the relative abundances of certain series of ions are related to the structure of the aglycone. The number of aglycone ions in the mass spectra of substituted phenolic glycosides is correlated with the Hammett substituent constants.

Deuterium labelling and high resolution mass spectrometry have been used to elucidate the fragmentation mechanisms for 4,6-O-benzylidene derivatives of glucosides. Most ions

are shown to be formed by rearrangement processes.

The behaviour of allylic acetates under electron impact conditions is described, with emphasis on the mechanism of loss of acetic acid. For cinnamyl acetates this is accompanied by cyclisation to an indene ion. Pyrolysis of cinnamyl acetate gives an analogous reaction and indene is formed as the major product.

To my parents.

"Oh!let us never,never doubt
What nobody is sure about!"

From "More Beasts for Worse Children-
The Microbe"
Hilair Belloc,1897.

Acknowledgments

My post graduate research has been carried out under the supervision of Dr.E.S.Waight,to whom I would like to express my gratitude for his guidance and freely-given advice.

I thank Professor D.H.R.Barton,F.R.S.for the valuable opportunity of working in the organic chemistry research laboratories of Imperial College.

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Introduction

The basic principles of mass spectrometry have been known since about 1920, but it is only within the last 10-15 years that the technique has been found of use to the organic chemist. A large amount of structural information can be obtained using very small quantities of compound, and because of this, mass spectrometry is rapidly becoming the most useful physical-organic technique. However, in contrast to nuclear magnetic resonance spectroscopy (n.m.r.), for which chemical shifts can be calculated from tables of data, it is often difficult to predict the mass spectral fragmentations of a system that has not been previously studied. Therefore at the present time a large amount of work is being carried out to elucidate the fragmentation modes of a number of different classes of organic compounds. This thesis is an example of this type of work. At the outset, when the mass spectrum of fusicoccin was being evaluated, it was found that little was known about the fragmentations of glycosides. This prompted the investigation of a number of model glycosides, and later benzylidene acetals and allylic acetates were also studied.

An A.E.I. MS9 double focusing mass spectrometer was used to record the mass spectra and some of the following comments, such as those relating to metastable ions and mass measurement are dependent upon this instrumentation.

When the vapourised compound is irradiated by a beam of electrons, positive ions are produced according to the equation:



Energy in excess of that required for ionisation is imparted to the molecular ion $M^{+\bullet}$ by the collision, and this causes the ion to fragment. The energy of the electron beam is normally 70eV but this can be lowered to a value which is just above the ionisation potential of the molecule. Only a small amount of excess energy is then given to the ion and fragmentation is reduced.

The distribution of the charge over the molecular ion is the subject of controversy but it is conventionally shown as being localised at one particular site. This is often an heteroatom and fragmentation mechanisms can be successfully rationalised on this basis. In the mechanisms described in this thesis, a single headed arrow $\rightarrow$ indicates a single electron shift, whereas a double headed arrow $\longleftrightarrow$ infers a two electron shift.

Unless otherwise stated, the intensities of ions are referred to as a percentage of the intensity of the base peak in the mass spectrum. The alternative method, which is sometimes used, is designated $\% \sum_n^M$, and is:

$$\frac{\text{Height of peak} \times 100\%}{\text{Total heights of peaks between } M^+ \text{ and } m/e \text{ } n}$$

A low resolution mass spectrum gives the masses of ions as integral units, but the MS9 has a facility for high resolution peak matching. The mass of an unknown ion can be obtained to an accuracy of at least ten parts per million by comparison with an ion of known mass in the spectrum of a standard such as heptacosafuoro-tri-n-butylamine.

Fragment ions that are observed in mass spectra are due to decompositions that occur in the source before acceleration. If an ion M_1 decomposes to an ion M_2 in the field-free region between the electrostatic and magnetic analysers, a small broad peak, known as a metastable peak, is found in the mass spectrum. The m/e value at which this occurs (shown as m^* throughout the thesis) is given by the equation:

$$m^* = \frac{(M_2)^2}{M_1}$$

PART 1

Glycosides.

1,1 A Review of the Mass Spectrometric Fragmentations of some Pyranose Sugars

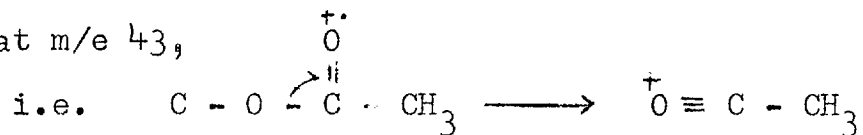
The use of mass spectrometry for the investigation of carbohydrates was limited by the low volatility of these compounds until relatively recently. With the introduction of the direct insertion (probe) technique and by the use of derivatives with increased volatility it has become possible to record the mass spectra of many sugar derivatives. Reviews by Djerassi et al⁽¹⁾ and Kochetkov and Chizhov⁽²⁾ have appeared on this subject. Therefore this brief critical review will only be concerned with the mass spectrometric behaviour of the methyl ethers and acetates of pyranoses that is pertinent to the work to be described later.

Acetylated Monosaccharides

In 1963 Heyns and Müller⁽³⁾ reported the mass spectrum of glucose penta-acetate without any suggested mechanisms for its fragmentations, but the Biemann group published a more extensive treatment of hexose and pentose acetates which included the study of some trideuterioacetates.⁽⁴⁾ They observed that the stereochemistry of the sugar had little effect on the mass spectrum, so that the α and β epimers of glucopyranose and mannopyranose penta-acetates could only be distinguished by small differences in peak heights. The fragmentation sequences that were observed for pent-acetyl- β -D-glucopyranose using an heated stainless steel inlet system are described below.

In common with most polyacetyl compounds the base peak

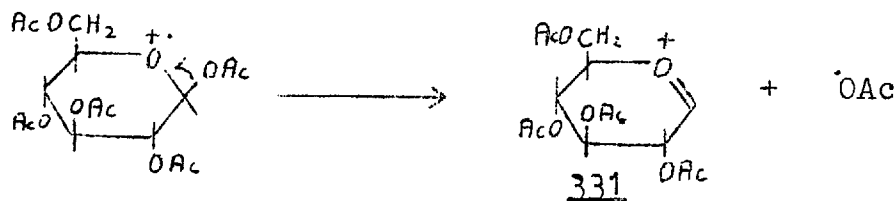
is at m/e 43,



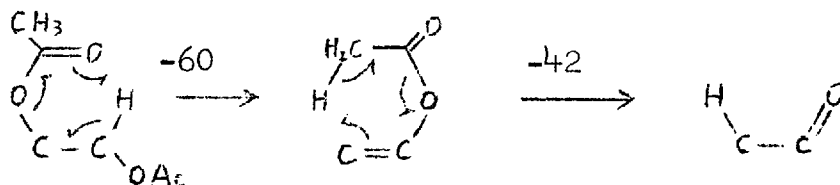
and there are oxonium ions of mass 103, $(\text{CH}_3\text{CO})_2\overset{+}{\text{O}}\text{H}$ and 145, $(\text{CH}_3\text{CO})_3\overset{+}{\text{O}}$. The mode of formation of the latter two ions is not known.

The electron impact induced fragmentations of cyclic sugars can often be rationalised on the basis of ionization on the ring oxygen atom followed by bond fission in one of several different directions.

Series A. This is initiated by the loss of the substituent attached to C(1) to give a pyronium ion of mass 331.



The pyronium ion then decays by losses of acetic acid (60 m.u.) and ketene (42 m.u.) to give peaks at m/e values of 271, 229, 211, 187, 169 and 109. The suggested mechanisms for these losses are:

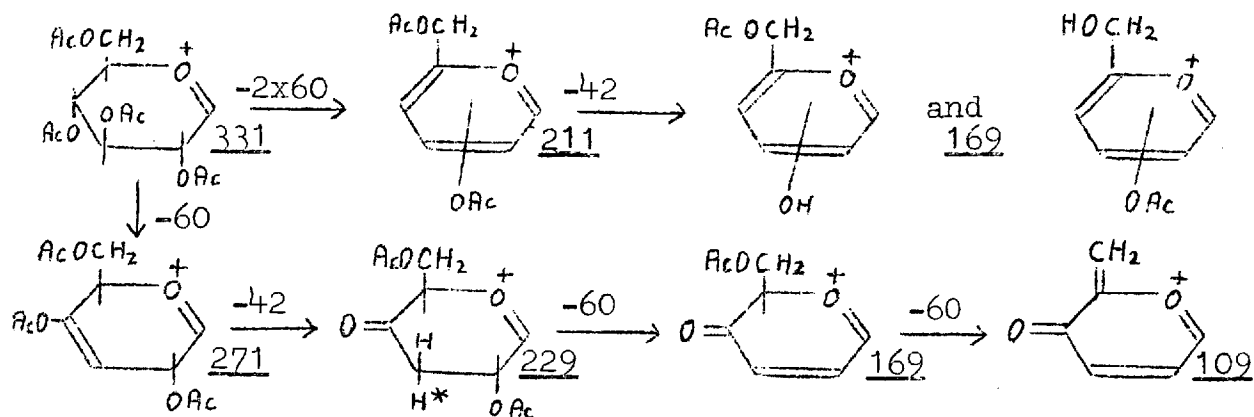


and a simple 1,2-elimination of ketene was rejected in the belief that only vinyl acetates readily lose ketene.

The mass spectrum of the penta- $[\text{}^2\text{H}_3]$ -acetyl analogue showed a shift of m/e 169 to 172 and 173 and m/e 109 was shifted to m/e 110 to an extent of 50%. This was said to be

due to the abstraction of either a proton or a deuteron in the elimination of acetic acid from the ion of mass 236 (equivalent to m/e 229 in undeuterated compound).

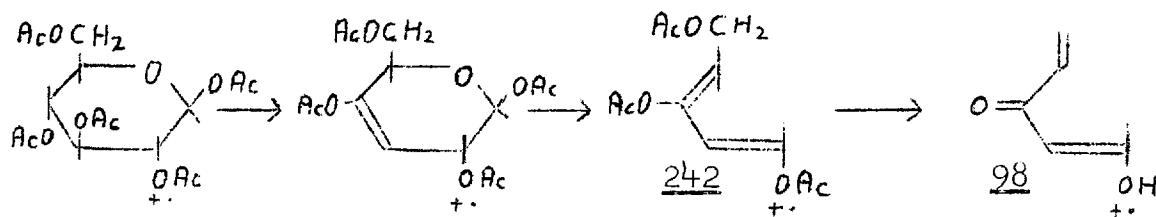
Thus fragmentation sequence A was thought to be:



It was noted that the series A fragmentation becomes more pronounced when a methoxy group is attached to C(1) and still more with phenoxy at this position. This reflects the stability of the radical that is lost.

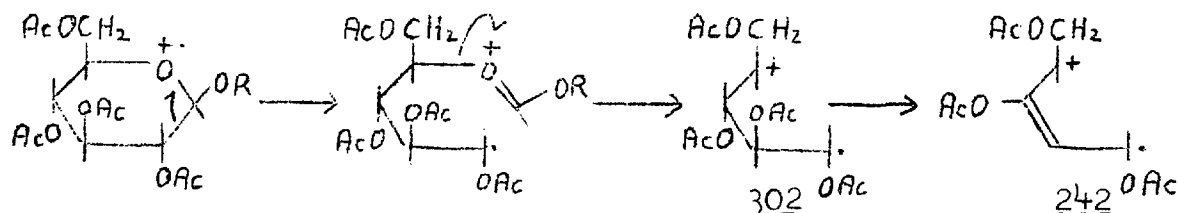


Series B. Biemann originally suggested that this was a fragmentation of the "retro Diels-Alder" type.

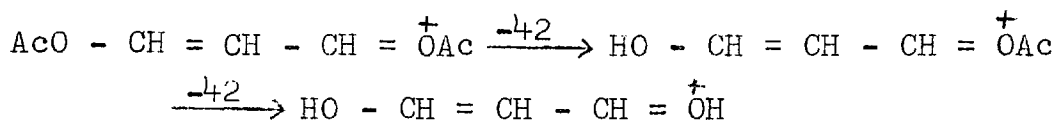


This gives an ion m/e 242 which then decomposes by the elimination of acetic acid and ketene to give ions m/e 200, 140 and 98. Since m/e 242 is shifted to lower mass by 58 m.u. in the mass spectrum of tetra-acetyl-6-desoxy- α -D-glucopyranose it was obvious that C(5) and C(6) are present in

this ion. Biemann and De Jongh modified this mechanism in a paper on methyl tetra-acetyl glycosides.⁽⁵⁾ Deuterium labelling showed that an M-60 ion had not lost any of the acetoxy groups but had lost C(1) and its substituents. Therefore the following scheme was proposed for the formation of m/e 242:



Series C. Peaks were observed at m/e 157, 115 and 73 which are due to fragments containing three of the ring carbon atoms. They have been given the structures:

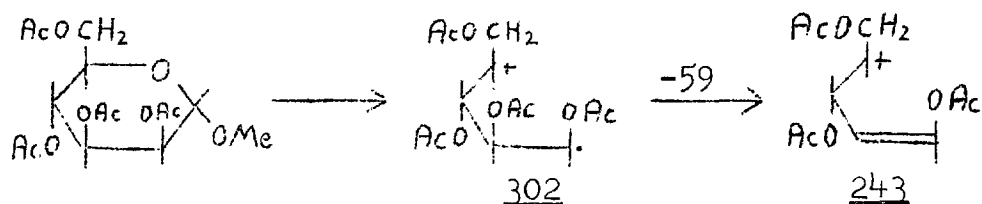


It was argued that because tetra-acetyl pentopyranoses and penta-acetyl fructopyranose also give a peak at m/e 157, this fragment must contain C(2), C(3) and C(4).

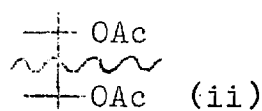
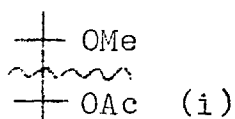
The second paper from the Biemann group extended these findings to the acetates of partially methylated sugars.⁽⁵⁾ Methyl tetra-acetyl glycopyranosides gave M+1 and M+43 ions. This phenomenon was not normally observed in the work which is described later, and seems to be somewhat dependent on the conditions under which the mass spectrum is obtained.

Series D. Methyl tetra-acetyl- α -D-mannopyranoside fragments to give the peaks in series A, B and C described above, but in addition there is a series of peaks beginning with m/e 243.

The ion of mass 302 (M-60) was shown by deuterioacetylation not to be formed by 1,2 elimination of acetic acid and De Jongh and Biemann therefore postulated the following mechanism for the formation of m/e 302 and 243:

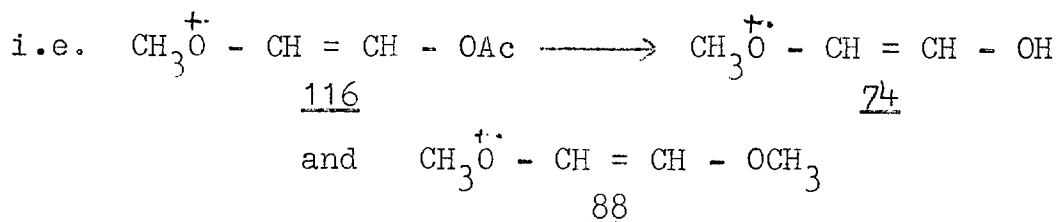


Compared with the 1-acetoxy compound, additional stabilisation of the positive charge at C(1) is provided by the methoxy group. The initial step of fission between C(1) and C(2) is the same as that which is currently accepted for the series B fragmentation (see page 20) but the distribution of charge and free electron is in the opposite sense. Thus depending on the site of localisation of the positive charge, subsequent fragmentation can lead to ions at either m/e 302 and 243 or m/e 288 and 242. Another example of charge stabilisation by a methoxy group being greater than stabilisation by an acetoxy group is provided by the mass spectra of partially methylated alditol acetates.⁽⁶⁾ The primary fragmentation of the carbon chain takes place preferentially next to a methoxy group with retention of the charge on the fragment carrying the methoxy group. Thus the ions resulting from fission (i) are more abundant than those arising from fission (ii).

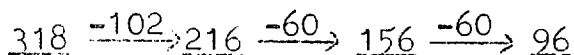


The paper by Biemann et al⁽⁵⁾ also reported the mass spectra

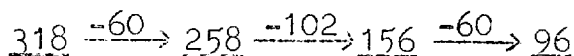
of some acetylated 2 and 3-O-methyl pyranoses and some more highly methylated carbohydrates. They were observed to fragment according to the schemes outlined above, with appropriate shifts due to the differing substituents. With an increasing degree of methylation ions containing only two of the carbon atoms from the sugar ring become more abundant.



De Jongh has investigated the mass spectra of acyclic sugars⁽⁷⁾ and some of his results can be illustrated by the fragmentations of aldehydo-D-arabinose tetra-acetate (molecular weight 318). The sequence suggested for loss of acetoxy groups from the molecular ion was:

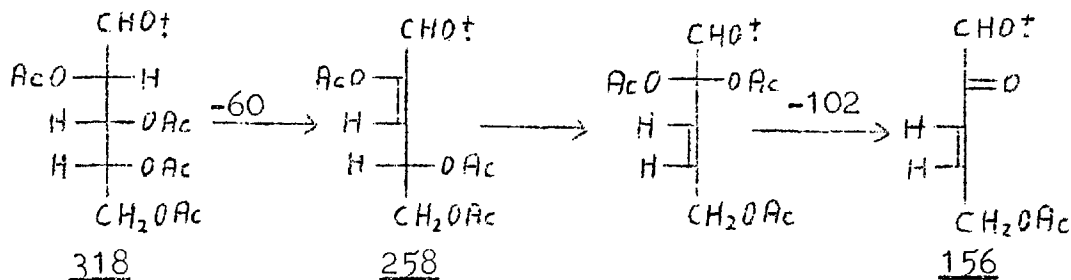


in which the loss of 102 m.u. was said to be the loss of acetic anhydride. In the light of subsequent investigations on polyacetates the sequence is more likely to be:

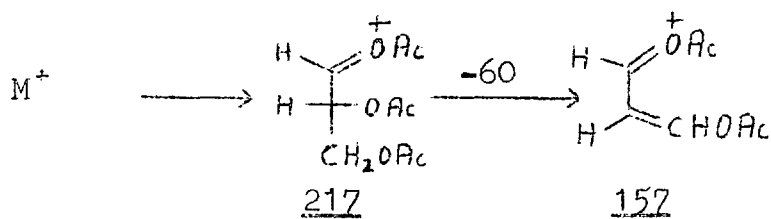


The ion of mass 258 is not observed and decays by the loss of 102 m.u. in a concerted elimination of acetic acid and ketene. This view is supported by the fact that the published spectrum of the tetra- $\left[{}^2\text{H}_3\right]$ -acetate shows a shift of m/e 216 to 223 (i.e. separate losses of $\text{CD}_3\text{CO}_2\text{H}$ and CD_2CO) and of m/e 156 to 159 (i.e. loss of $\text{CD}_3\text{CO}_2\text{H}$ followed by $\text{CD}_3\text{CO}_2\text{D}$ and CD_2CO). The peak at an m/e value of 216 is therefore on a

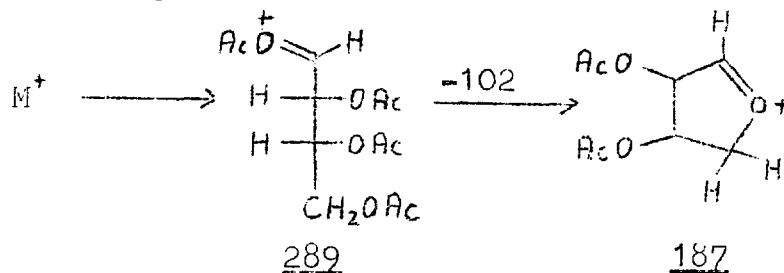
different fragmentation pathway. A possible mechanism for the former series of ions is.



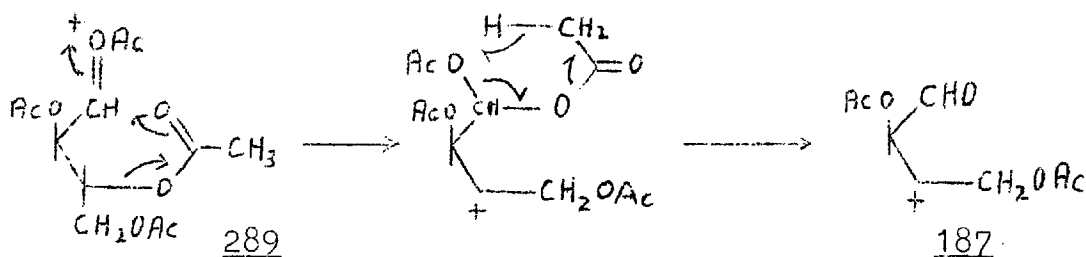
Peaks of series C at m/e 157, 115 and 73 are observed but they are formed by a different mechanism from the one proposed for the cyclic compounds.



Fission of the chain between C(1) and C(2) gives an ion which then loses 102 mass units. This loss is confirmed by a metastable ion and by the loss of 108 m.u. in the spectrum of the trideuterioacetylated compound. The ions were given the structures:

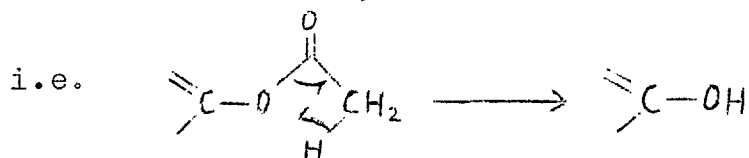


No mechanism for this process was given, nor proof of the structure of the resulting ion. A plausible mechanism, which is similar to some of those suggested by Heyns and Müller for the fragmentation of penta-acetyl- α -D-glucopyranose,⁽⁸⁾ is shown below.



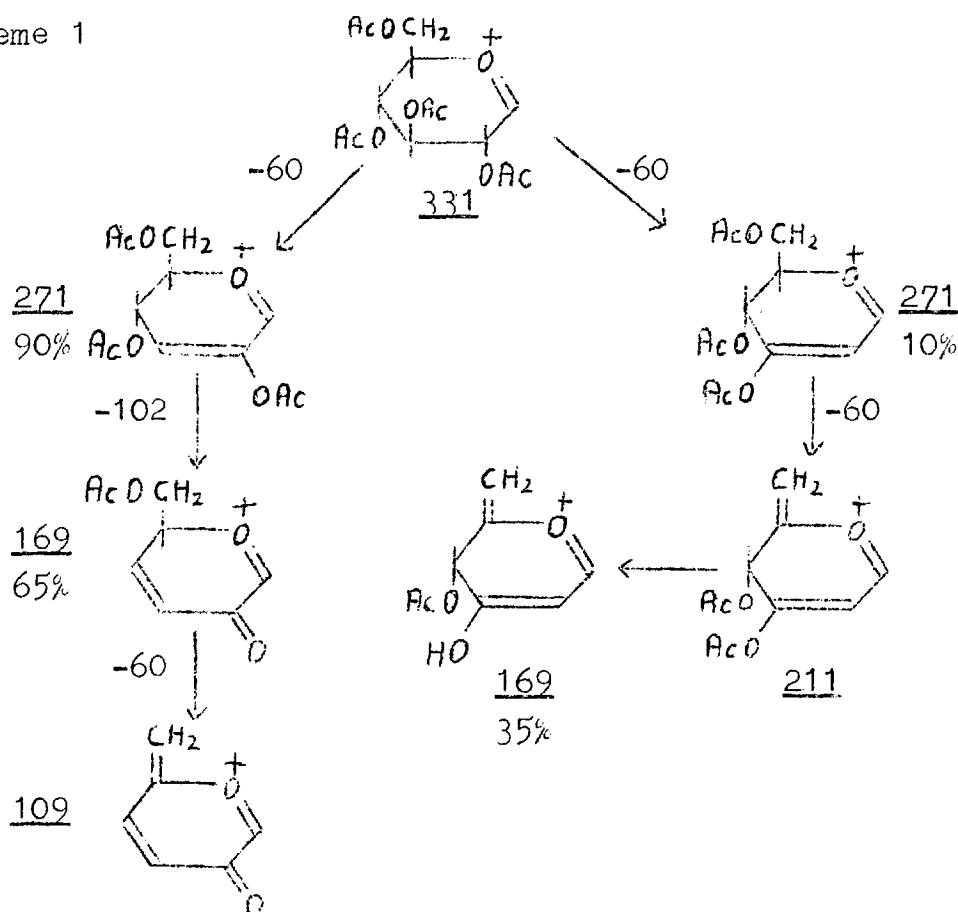
Such was the state of the published literature on acetyl sugars when the author began his work on the mass spectral fragmentations of tetra-acetyl glycopyranosides. During the course of these studies the single step loss of acetic acid and ketene was discovered and published in a communication by Smale and Waight.⁽⁹⁾ This was followed by a second paper by Heyns and Müller on peracetylated pentoses and hexoses.⁽⁸⁾

In the hexose series each of the acetoxy groups was selectively labelled as a trideuterioacetate and the ring protons were replaced by deuterium at C(2),C(3),C(4) and C(6). As a result of this Heyns and Müller were able to clarify many of the fragmentation schemes for penta-acetyl-D-glucose. Series A. The pyronium ion of mass 331 was shown to further fragment according to scheme 1. The loss of ketene in this type of sequence was stated on the basis of unpublished energy calculations to be by a four membered transition state.



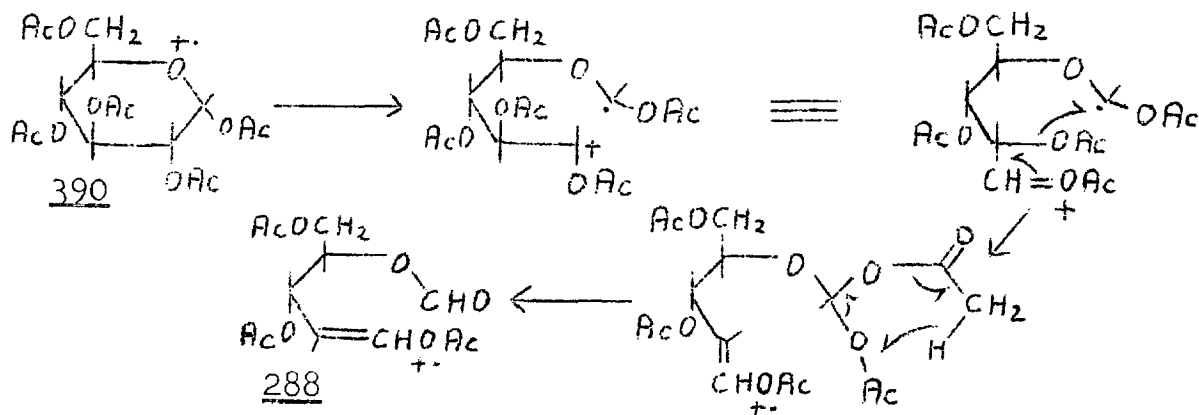
Empirically it can be seen that loss of ketene from the ion of mass 211 by a six membered transition state would result in the loss of conjugation with the formation of a keto group at C(3). Since Djerassi et al have demonstrated that keto-enol

Scheme 1

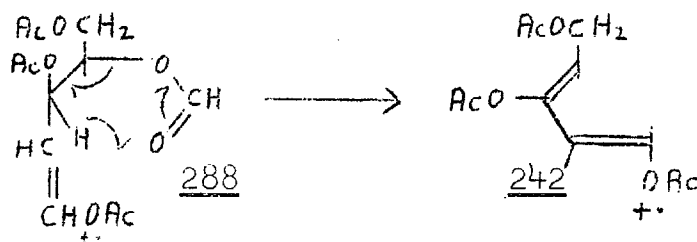


tautomerism does not occur readily in the mass spectrometer,⁽¹⁰⁾ the conjugation could not be expected to be regained and the process would be energetically unfavourable.

Series B. An ion was observed at m/e 288 which had lost the acetoxy groups from C(1) and C(3) and Heyns and Müller postulated the mechanism for this as:

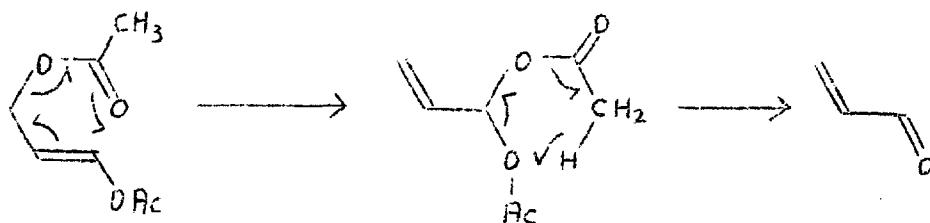


A metastable peak showed m/e 288 to be the precursor of the peak observed at m/e 242 and a proton was lost from C(4) in its formation. Therefore this ion is formed by a McLafferty rearrangement.

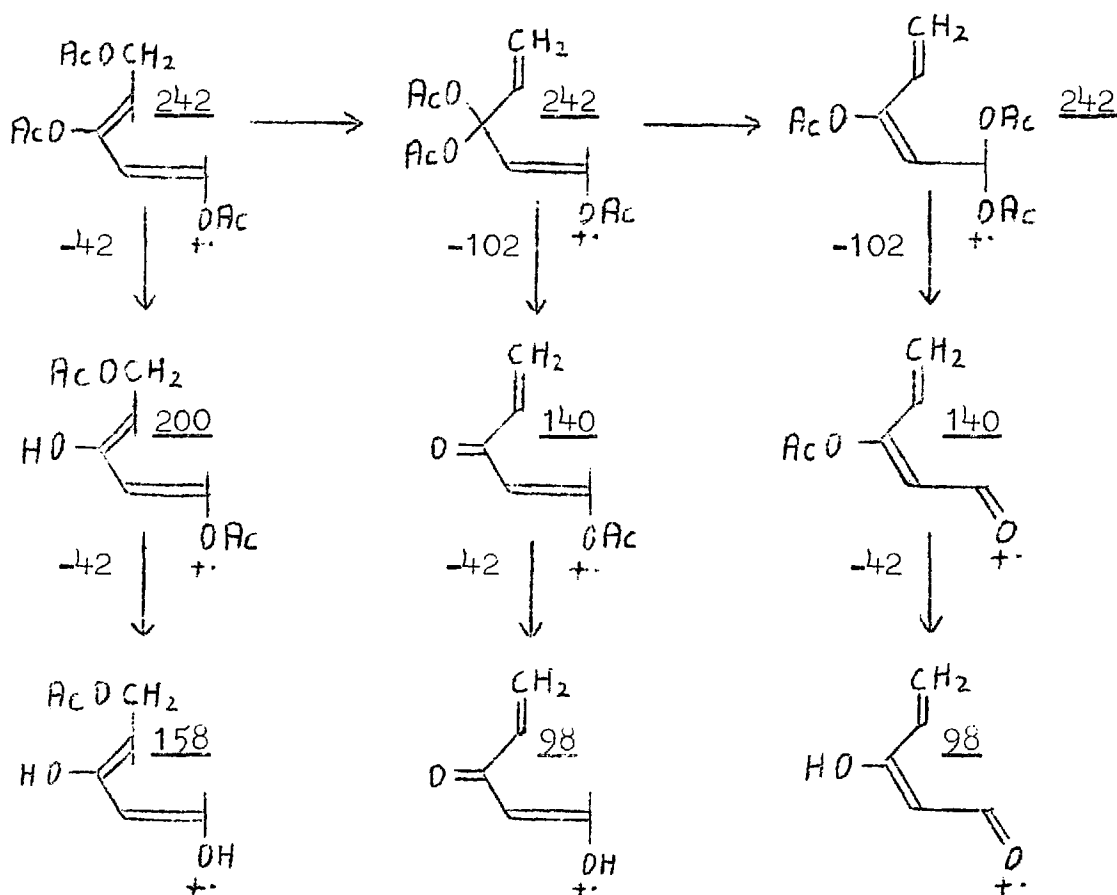


A mechanism of the Biemann type (page 14) would give identical shifts of m/e 242 to higher mass in the labelled compounds, but the existence of the metastable ion due to its formation from m/e 288 excludes this.

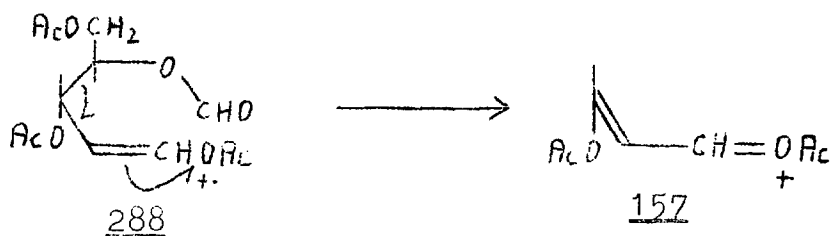
Deuterium labelling showed that the three acetate groups were equivalent in the subsequent decompositions of m/e 242 which include a one step loss of 102 m.u. An allylic rearrangement was postulated to account for the migration of acetate groups around the ion. This rearrangement also gives a geminal diacetate grouping which can lose acetic acid and ketene in a six membered transition state.



Therefore the following scheme was given for the fragmentations of the ion of mass 242 to give the peaks of series B at m/e 200, 158, 140 and 98.



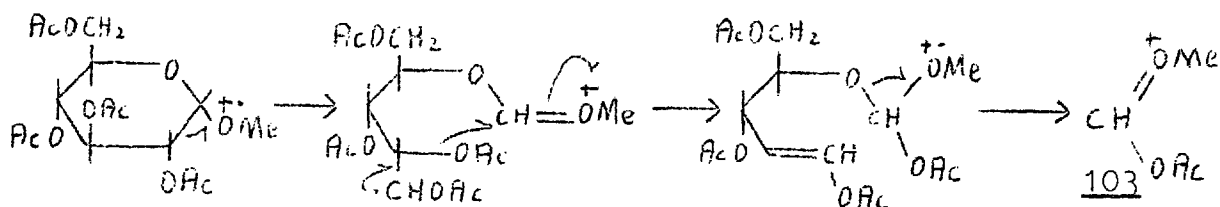
Series C. The ion observed at an m/e value of 288 (see series B) was stated to be the precursor of the series C ions of mass 157, 115 and 73.



The labelling experiments have also thrown a little more light on the origin of the oxonium ions m/e 145 and 103. In the case of the penta-acetyl hexoses the acetyl groups come mainly from C(2), C(4) and C(6) and tetra-acetyl pentoses lose acetyl groups from C(1), C(2) and C(3) to form these ions.

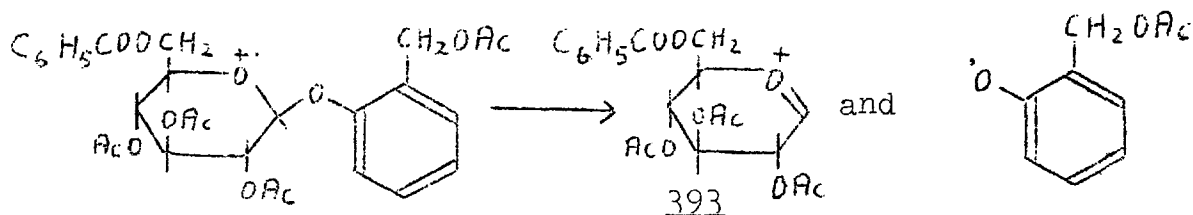
Heyns and Müller have also done some selective trideuterio-

acetylation of methyl tetra-acetyl-glucopyranoside.⁽⁸⁾ Under electron impact the C(1)-C(2) bond is broken, followed by migration of an acetoxy group from C(3) to C(1). This ion then not only decomposes to give the ions of series B and C but also forms an ion which partly contributes to the peak found at m/e 103.



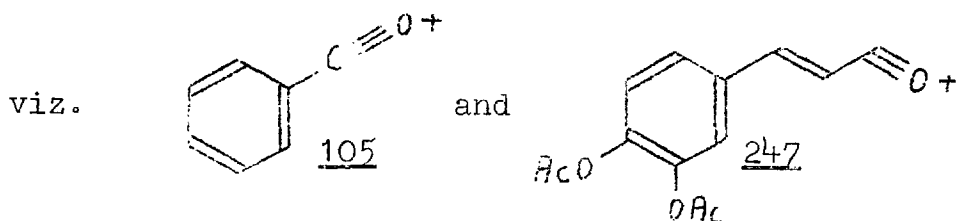
Naturally Occurring Glycosides

Several groups of workers have recently been applying mass spectrometry to the peracetates of natural glycosides. A paper by Pearl and Darling is concerned with the formation of the parent ion of series A.⁽¹¹⁾ Natural glycosides which have a substituent on the sugar ring have the m/e 331 ion shifted to a different mass, and so it is possible to deduce what the substituent is. Thus populin tetra-acetate gives a series A ion at m/e 393.



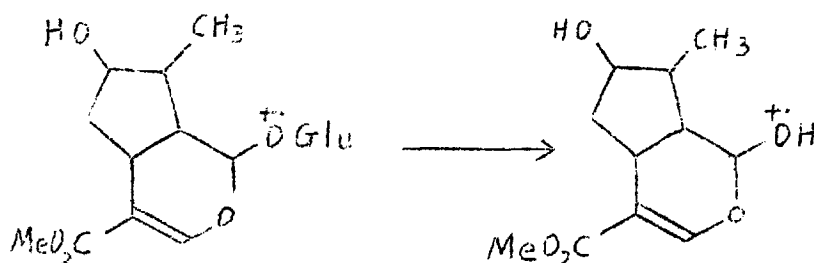
The work on the peracetates of acyl aroyl glucosides has been extended by Haslem.⁽¹²⁾ The aroyl substituent on the pyranose ring forms an aroyl ion which is prominent in the mass spectrum. Thus a benzoate such as populin tetra-

acetate gives an m/e 105 ion, and a dihydroxycinnamoyl ester such as 2-O-caffeoylarbutin hexa-acetate gives an ion at m/e 247 which loses ketene to form ions at m/e 205 and 163.



It is possible to deduce the point of attachment of the aroyl ester from the abundances of the various ions of series A. Heyns and Müller's decomposition sequence (page 20) gives two ions at m/e 169 in tetra-acetates, of which the most abundant one retains the substituent at C(6) and the minor one retains the substituent at C(2). Therefore populin tetra-acetate which has a C(6) substituent has a large peak at m/e 231 and one at m/e 169 which is only a quarter of the size. On the other hand, the 2-O-benzoyl isomer which is called tremuloidin has a large m/e 169 peak and a negligible one at m/e 231.

The mass spectra of some iridoids has been published by Bentley et al.⁽¹³⁾ The unacetylated glucosides give mass spectra which are mainly due to the aglycone, and the primary fragmentation is shown below for loganin.

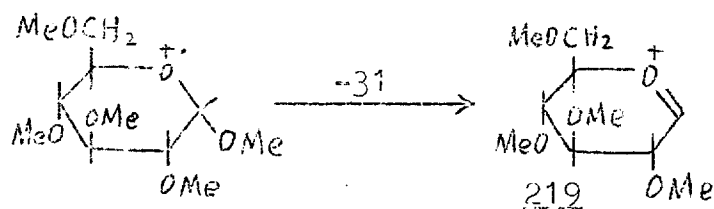


Most of the charge goes with the sugar portion of the acetylated iridoids and most of the fragmentation is due to series A, with a small contribution from series C.⁽¹⁴⁾

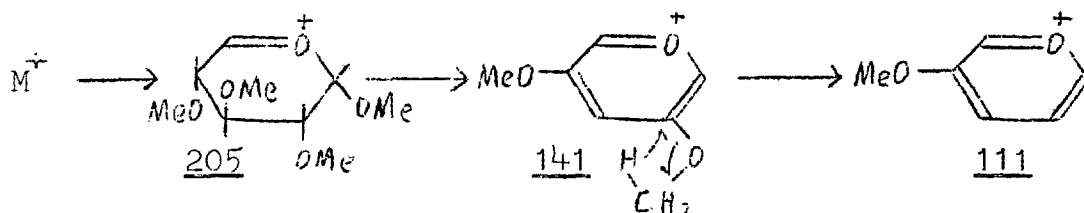
Permethylated Monosaccharides

Two groups of workers have been active in this field. The first comprehensive paper by Kochetkov⁽¹⁵⁾ made several false proposals for the structures of ions in the mass spectrum of methyl tetra-O-methyl- α -D-glucopyranoside. For instance, he postulated the ion of mass 75 as being $\text{CH}_3\text{O} - \text{CH} = \text{CH} - \overset{+}{\text{O}}\text{H}_2$ in which the two adjacent carbon atoms come from C(1), C(2), C(3) or C(4) on the sugar ring. However these conclusions have been rectified in ensuing papers.^(16,17) Concurrently with the work by the Russian group, Heyns *et al* produced papers on the mass spectra of permethylated sugars.⁽¹⁸⁾ The important fragmentation mechanisms elucidated by these workers are summarised below for methyl tetra-O-methyl- α -D-glucopyranoside (molecular weight 250).

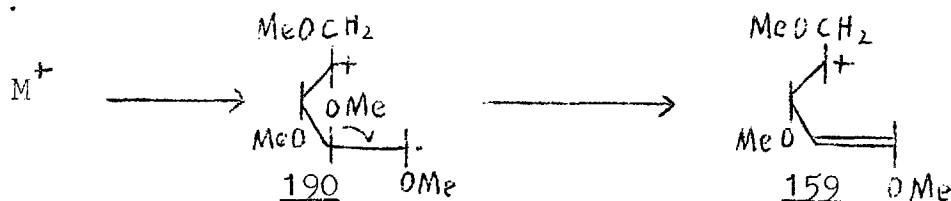
Ions of series A, due to the loss of the glycosidic methoxy group followed by sequential losses of methanol giving ions at m/e 219, 187 and 155 are present, but are not very abundant.



A similar series of ions is formed by the loss of the substituent at C(5). Note that one of the steps in this series involves the loss of formaldehyde from a methoxy group.

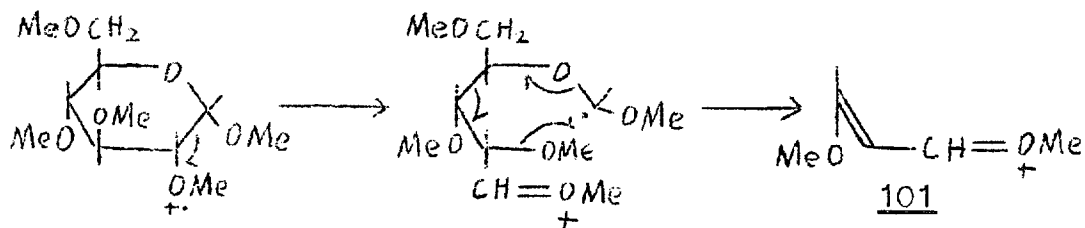


The series B fragmentation is unimportant and its place is taken by series D which gives an ion of mass 159.

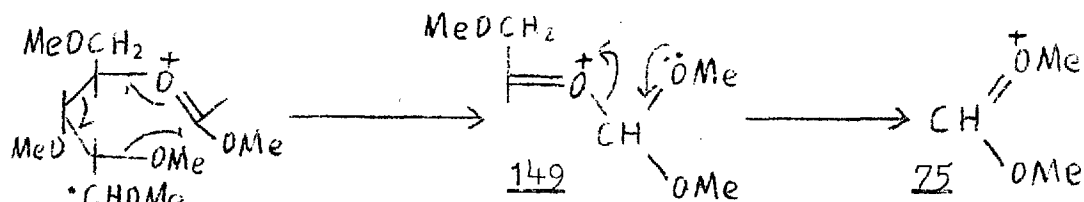


The ion of mass 190 is not observed because it immediately decomposes to give m/e 159.

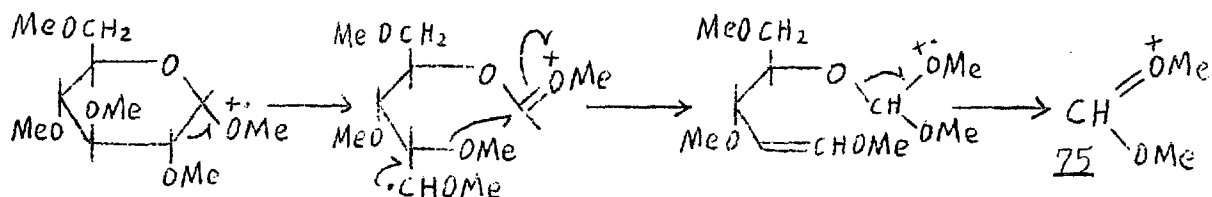
A fragmentation which is very similar to series C, is the main pathway (80%) to one of the most abundant ions in the spectrum, m/e 101.



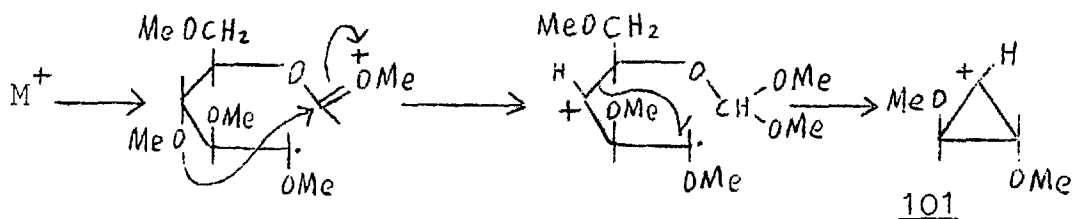
If the charge remains on the fragment during this process an ion of mass 149 is formed which can further break up to make a contribution to the abundant ion observed at m/e 75.



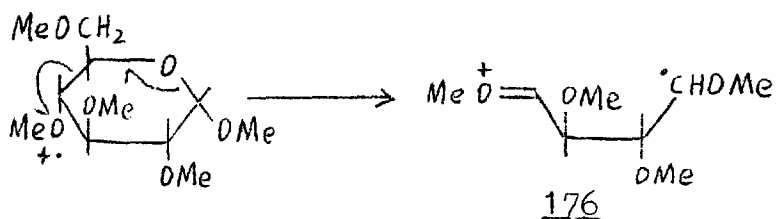
However, the authors of the papers on this subject consider that m/e 75 is mainly formed directly from the molecular ion.



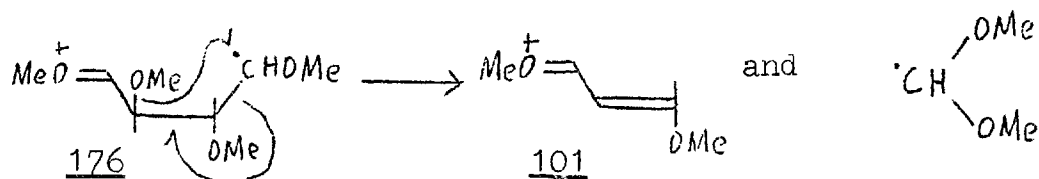
Some of the ions at m/e 101 contain the methoxy groups from C(2) and C(3) instead of those from C(2) and C(4). These former ions also originate from the fission of the C(1)-C(2) bond.



The only ion in the mass spectra of permethylated pyranoses that is not formed by a primary fission of a type undergone by peracetylated pyranoses has a mass of 176. It is formed by the loss of C(5) and the heterocyclic oxygen atom.

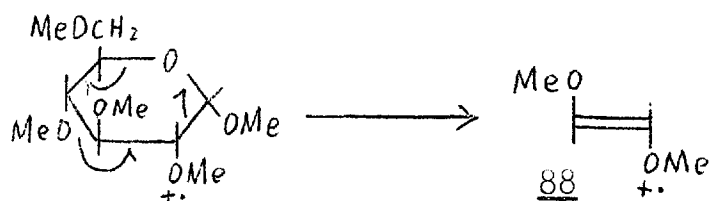


Further fission of the m/e 176 ion provides an alternative route to the m/e 101 ion.

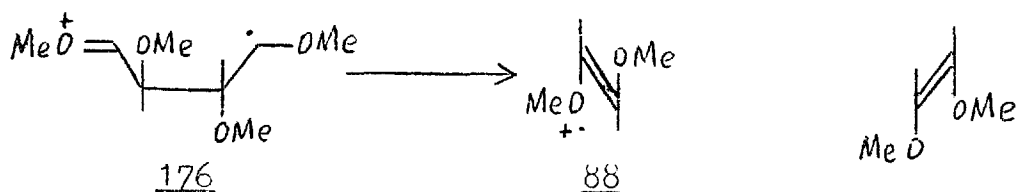


It was also stated that when the charge and free electron are in the opposite positions decomposition of m/e 176 is a pathway to the m/e 75 ion derived from the methoxy groups at C(1) and C(3). However since the m/e 176 ion is symmetrical it would be expected on this basis that some of the m/e 75 ions would contain the methoxy groups from C(2) and C(4), and this does not appear to occur.

The only other peak of high intensity in the mass spectrum of this compound is m/e 88. This contains the methoxy groups at C(2) and C(3) to the extent of 84% and can be formed from the molecular ion by a cyclic mechanism.

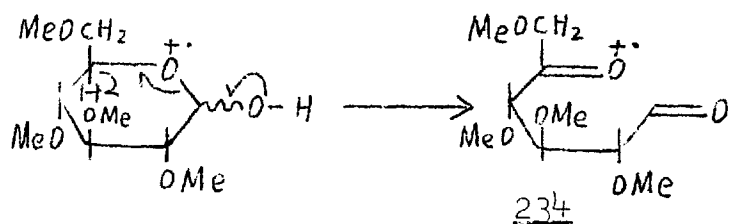


The smaller numbers of ions of this mass which carry the methoxy groups from C(1) and C(2) or C(3) and C(4) can be formed by splitting the ion of mass 176 into halves.



The mass spectra of the 2,3,4,6-tetra-O-methyl-glucopyranoses with hydroxy, methoxy and phenoxy at C(1) have been compared.⁽¹⁵⁾ The phenyl glucoside fragments according to series A, to a much greater extent than the methyl glucoside, so that m/e 219 has an intensity of 9% of the base peak and m/e 187 has an intensity of 93%. There is also an ion at m/e 218 ($M-94$) due to the loss of phenol but the source of the proton that is abstracted was not discussed.

It is interesting to note that the presence of a free hydroxy group at C(1) completely alters the fragmentation pattern. The main peaks at m/e 234, 205 and 173 are said to be due to an initial loss of H_2 from the molecular ion.



Structure Determination of Carbohydrates by Mass Spectrometry

The standard method of determining the positions of attachment of substituents to a sugar ring is by methylation of the free hydroxy groups followed by hydrolysis to give a partially methylated monosaccharide. To identify this by mass spectrometry, two methods have been suggested.

(i) Acetylate the partially methylated sugar and compare its mass spectrum with a set of reference spectra such as those published by Biemann for monomethylxyloses.⁽⁵⁾ This approach is limited because of the few reference spectra

that have been published. The spectra of several, acetylated dimethyl glucoses that appear in this thesis will assist in this approach which must be the more certain when it can be applied.

(ii) Deuteriomethylate the partially methylated sugar to form a permethylated sugar, which has a known fragmentation pattern. Then deduce the position of the deuteriomethyl groups from the shifts in m/e value of the various fragments. This is the method advocated by Kochetkov⁽¹⁶⁾ but could, in practice, be difficult to apply. This is an obvious case in which the introduction of a computer program could be of great use.

1,2 Fusicoccin

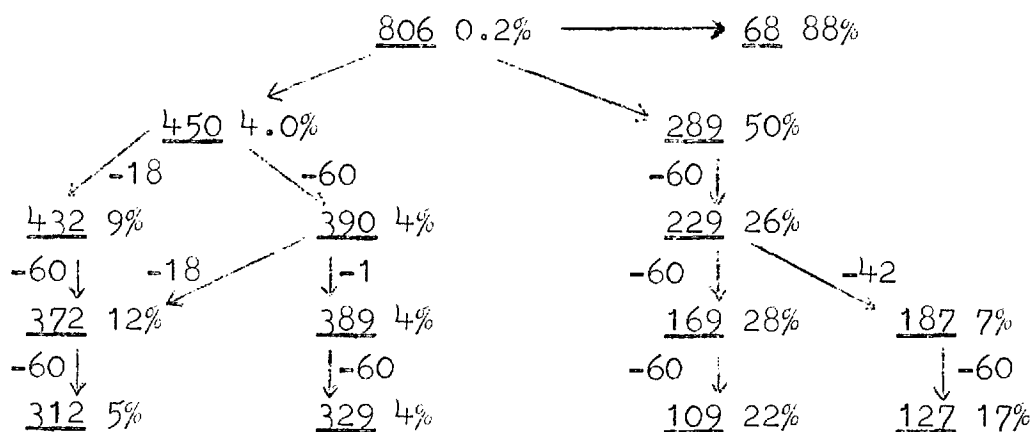
The fungus Fusicoccum Amygdali is responsible for causing a wilting disease of the almond tree. This is very common in Italy and so it was in that country that preliminary investigation of the metabolites of this fungus was carried out. It was found that culture filtrates caused the wilting symptoms, and so work ensued on the isolation of phytotoxic components. In 1964, Ballio et al reported that there were several plant pathogens present in the culture and they had isolated the major one as a crystalline compound by a lengthy series of solvent extractions followed by chromatography.⁽¹⁹⁾ This was the least polar toxin and was named fusicoccin-A, the others were named fusicoccin-A', B, C and D in order of increasing polarity.

The preliminary investigations showed that it had the formula $C_{38}H_{58}O_{13}$ but the original mass spectrum, obtained by Shannon, did not show the molecular ion at 722, only an $M^+ - H_2O$ ion at m/e 704. The n.m.r. spectrum contained a peak due to a methoxy group, and it appeared that there were signals due to two acetate methyl resonances. On catalytic hydrogenation, fusicoccin-A took up one mole of hydrogen to form dihydrofusicoccin-A which gave an highest mass peak at m/e 706 on mass spectrometry. The infra-red spectrum indicated the presence of hydroxy groups, ester groups, and some unsaturation, but this was not due to aromatic rings.

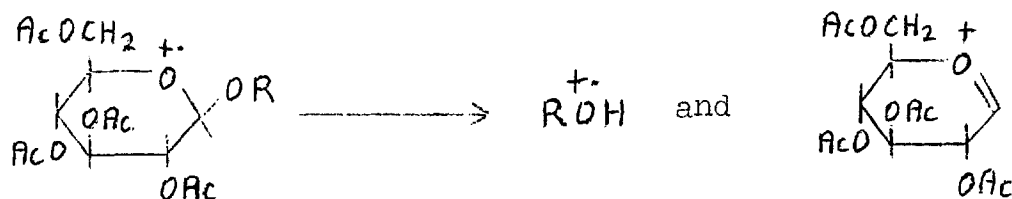
This was confirmed by the ultra-violet spectrum which only had strong end absorption. Hydrolysis of fusicoccin-A with 2N.sulphuric acid at 100°C resulted in the isolation of one mole of glucose, but no other pure products.

Work on the isolation and properties of fusicoccin has recently been continuing at Imperial College and the author has largely been responsible for the mass spectrometry of the fusicoccin derivatives described below. Most of the samples were kindly provided by Dr.R.Thomas and Dr.K.D. Barrow.

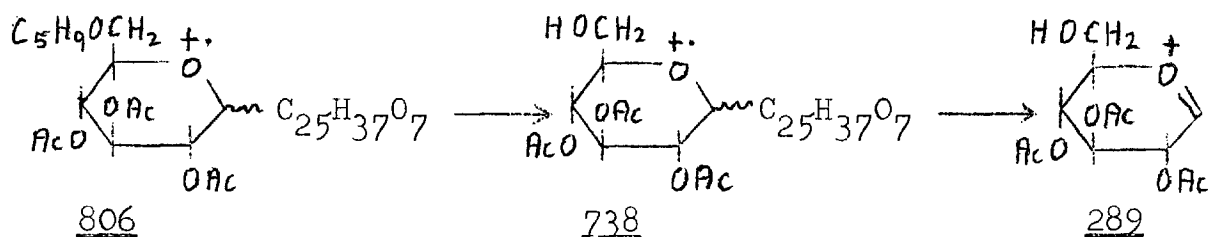
Acetylation of fusicoccin-A gives a penta-acetate $C_{42}H_{62}O_{15}$.⁽²⁰⁾ The second highest peak (after m/e 43) in the mass spectrum of this compound is at m/e 69, $C_5H_9^+$. The molecular ion is at m/e 806 and there are peaks which correspond to losses of CH_3 , H_2O , CH_3CO_2H and C_5H_8 from it. However, the main peaks are assignable to two series: namely one starting from m/e 289 and the other one starting from m/e 450.



The series from m/e 450 is much more complicated than is shown, and gives a complex series of peaks down to low mass. This mass spectrum is consistent with fusicoccin being a glucopyranoside except for the fact that the series A fragmentation of the sugar moiety would normally appear as an ion at m/e 331 and not at 289. Subsequent investigation of model compounds has shown that phenolic glucopyranosides have a similar fragmentation pattern to fusicoccin and that the primary fissions are:

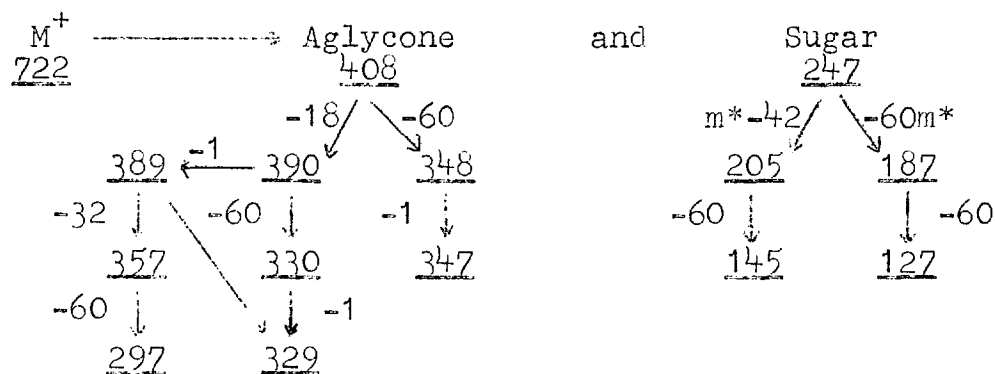


Therefore it is deduced that acetylfusicoccin has an aglycone of mass 450, $C_{25}H_{38}O_7$ and that the molecular weight should be $449+289=738$, which is 68 mass units short of the observed molecular weight. The data can be accounted for if the C_5 unit is a side chain on the sugar. This side chain could be attached to any of the four hydroxy groups, and the structure given below is one of the four possibilities for acetylfusicoccin.



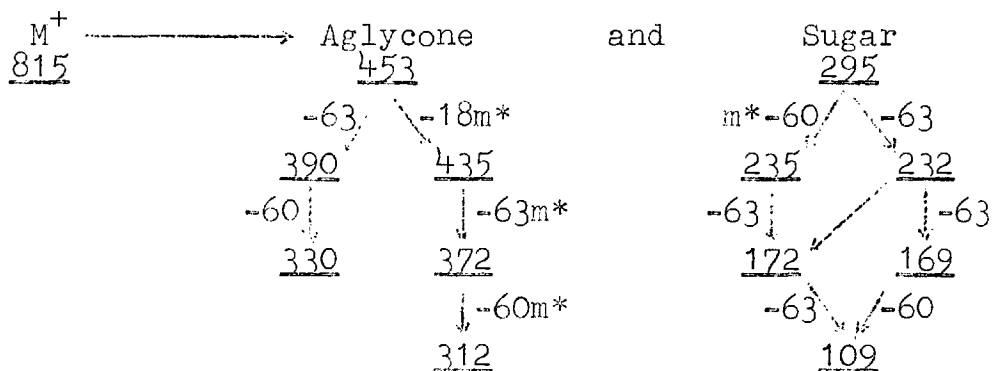
The infra-red spectrum of acetyl-fusicoccin reveals a free hydroxy group which could not be acetylated under normal conditions.⁽²¹⁾ Therefore since the aglycone ion loses one molecule of water and two of acetic acid, it must contain one hindered hydroxy group and two acetoxy groups.

The mass spectrum of fusicoccin-A (mass spectrum 1) appears to show the following fragmentations.



This indicates that there are two acetate groups on the sugar residue and one acetate group on the aglycone. The 100MHz n.m.r. spectrum shows a total of three acetate methyl resonances when the compound is crystallised from acetone, but only two acetates when the compound is vacuum dried. In an attempt to resolve this anomaly, the author has trideuterioacetylated fusicoccin-A. Reaction with either $[^2H_6]$ -acetic anhydride in pyridine or $[^2H_3]$ -acetyl chloride in pyridine gives tri- $[^2H_3]$ -acetyl-fusicoccin-A. The 100MHz n.m.r. spectrum of this compound reveals two $[^1H_3]$ -acetate groups which give signals at τ 7.88 and 8.04. The significant part of the mass spectrum of this compound, which shows the distribution of the trideuterioacetyl groups is summarised

below.

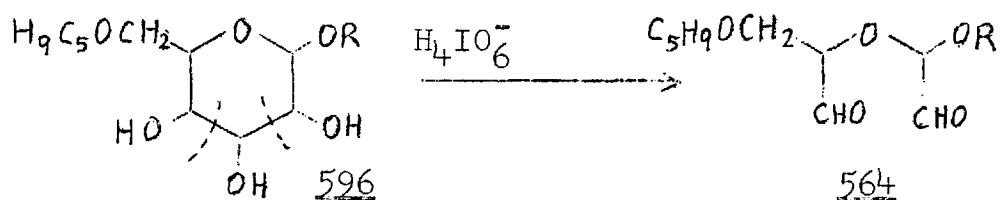


One labelled acetoxy group is on the aglycone, but two are on the sugar. Hence fusicoccin-A must have two free hydroxy groups on the sugar, and not one, as was indicated by its mass spectrum. On this basis, the molecular formula of fusicoccin-A must be amended to $C_{36}H_{56}O_{12}$ (molecular weight 680), and it contains one acetoxy group on the aglycone and one on the sugar. To explain the contradictory results obtained for the mass spectrum of fusicoccin-A, a trans-acetylation reaction must occur thermally on the probe at the temperature used to obtain the mass spectrum (250°C). In fact the true molecular ion at m/e 680 is only slightly larger than the m/e 722 peak, and also the two series A sugar peaks at m/e 205 and 247 are of comparable intensity, so that a considerable amount of trans-acetylation must take place.

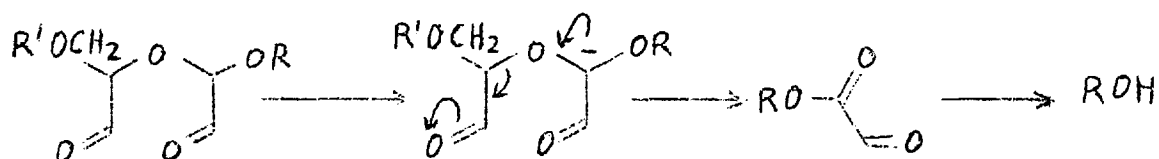
The mass spectrum of acetyl-dihydrofusicoccin-A is identical to that of fusicoccin below m/e 450, except that the peak at m/e 69 is shifted to m/e 71. Therefore the C_5 side chain on the sugar ring of fusicoccin has a double

bond which can be hydrogenated.

Controlled acid hydrolysis of fusicoccin-A only gives a very low yield of the aglycone, therefore more elegant methods of degradation of fusicoccin have been developed by Professor D.H.R. Barton and Dr.K.D. Barrow. Periodate oxidation of deacetyl-fusicoccin results in the uptake of two moles of reagent with the formation of a dialdehyde.⁽²¹⁾ The mass spectrum of this compound has the molecular ion at m/e 564, but below m/e 366, the aglycone peak, the spectrum is almost identical with that of deacetyl fusicoccin. Oxidation to give a product of molecular weight 564 can only occur if the pentenyl group is attached to C(6) of the sugar, and this fixes the position of the side chain.



Treatment of the dialdehyde with base gives the aglycone $\text{C}_{21}\text{H}_{34}\text{O}_5$.⁽²¹⁾



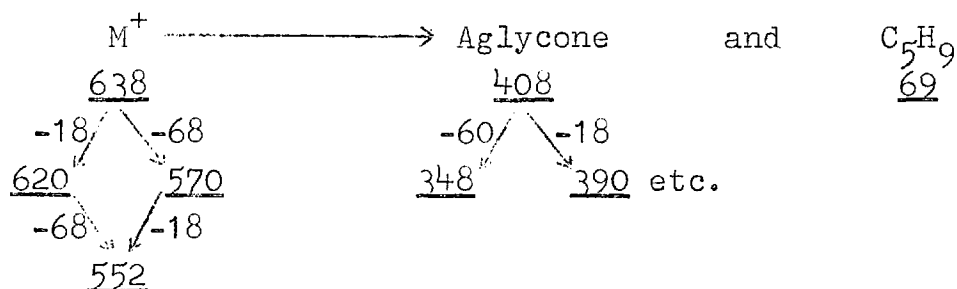
A methanolic solution of fusicoccin-A is stable at pH 2-6.5 but at higher pH it begins to hydrolyse. At pH 7-8

fusicoccin-B and fusicoccin-C are formed, whilst at pH >9 fusicoccin-D is the main product. In view of the instability of fusicoccin-A to base it was thought that fusicoccins B, C and D obtained from the fungal culture may be artifacts, and not true metabolites. By buffering the culture medium to pH 6-6.5, fusicoccin-A became more prominent in the product distribution.⁽²⁰⁾

Some knowledge about the structures of fusicoccin B, C and D has been obtained by mass spectrometry. The spectrum of fusicoccin-B was initially run with the sample on a soda-glass probe. The spectrum obtained, showed peaks at m/e 764, 722 and 680 in the high mass region, and aglycone peaks at m/e 450 and 408, suggesting that fusicoccin-B may be a mixture. However, when the mass spectrum was rerun on a ceramic probe, the m/e 764 and 450 peaks were eliminated and the molecular ion appeared to be at m/e 722. This second spectrum of fusicoccin-B was identical to that of fusicoccin-A, so it must be concluded that they are isomeric, and that the true molecular weight of fusicoccin-B is also 680. Fusicoccin-B is probably formed from fusicoccin-A by acetate migration around the sugar ring. It was noted previously that there was a compound A' which ran between A and B on thin layer chromatography. Attempts to isolate this compound have only given mixtures of A and B. Therefore A' may be the orthoester intermediate in the acetate migration.⁽²⁰⁾ The change from soda-glass probe to ceramic probe lessened the

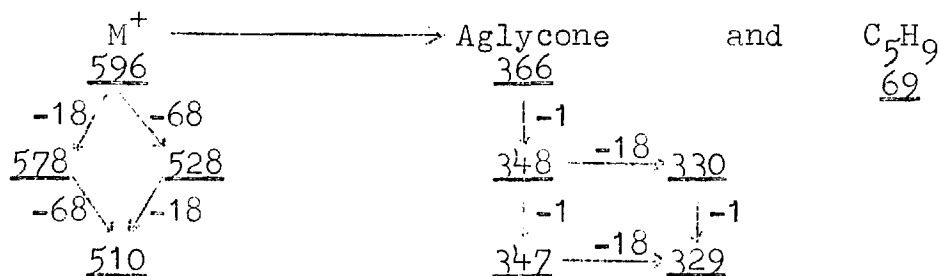
amount of trans-acetylation that occurred whilst obtaining the mass spectrum. This indicates that the reaction is base catalysed.

The mass spectral fragmentation pattern of fusicoccin-C is given below.

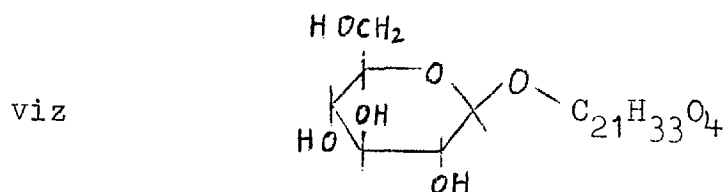


There are no peaks in the spectrum which are assignable to the sugar part of the molecule. Many unacetylated glycosides do not show any sugar peaks (see mass spectrum of phloridzin), so that this fact in conjunction with the molecular ion at m/e 638 and the aglycone peak at m/e 408, shows that fusicoccin-C has no acetate groups on the sugar. The aglycone decomposes in a manner identical to that of fusicoccin-A and hence still retains one acetate group.

Fusicoccin-D (mass spectrum 2) is identical to deacetyl-fusicoccin $C_{32}H_{52}O_{10}$ and has no acetate groups. The mass spectrum is almost identical to that of fusicoccin aglycone below m/e 366, except for the presence of the m/e 69 ion.



Acid hydrolysis of fusicoccin-A in methanol at pH 2 gives fusicoccin-E and fusicoccin-F.⁽²⁰⁾ The mass spectrum of fusicoccin-F has a small molecular ion at m/e 528 and the aglycone peaks begin at m/e 366. Below this mass, the spectrum is similar to that of fusicoccin-D except that there is no ion at m/e 69. This data is consistent with F having the same structure as D except that the C₅ side chain has been lost.



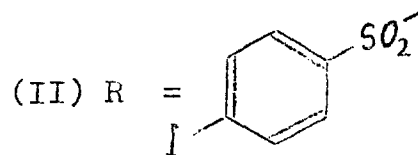
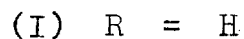
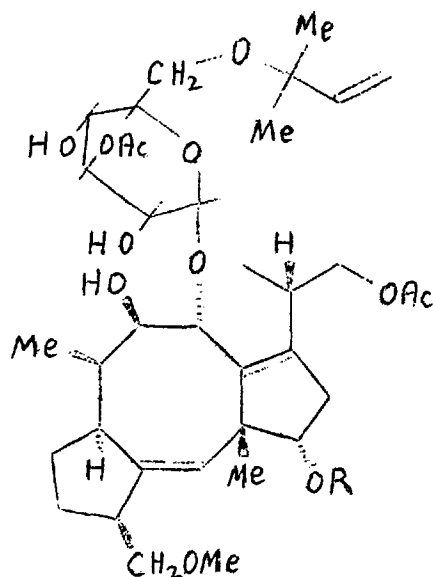
This was proved by acetylating the compound to form a hexa-acetate, which showed the following electron impact induced fragmentations.

M ⁺	Aglycone	and	Sugar
<u>780</u>	<u>450</u>		<u>331</u>
↓ -18	↓ -18		↓ -60
<u>762</u>	<u>432</u>		<u>271</u>
	↓ -60		↓ -102
	<u>372</u> etc.		<u>169</u>
			↓ -60
			<u>109</u>

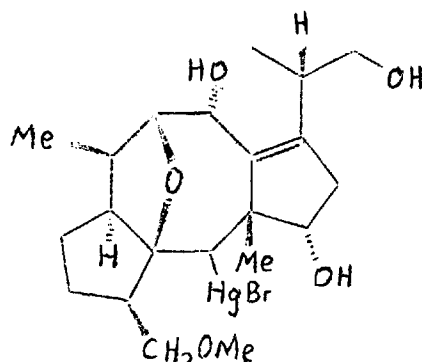
The series A ion at m/e 331 is that which is obtained from tetra-acetyl glucopyranosides and therefore confirms the structure given above. Fusicoccin-E differs from F by the presence of an acetoxy group on the aglycone.

The acid hydrolysis was carried out over a period of several days, whilst shaking the reaction mixture with carbon tetrachloride. The n.m.r. spectrum of the carbon tetrachloride solution was recorded at intervals and a signal at τ 8.78 appeared which could be due to the methyl groups of 1,1-dimethylallyl alcohol. This later collapsed and gave way to signals due to isoprene. Isoprene was also identified in the solution by gas-liquid chromatography. Additionally, the n.m.r. spectrum of fusicoccin-A reveals the presence of $-\overset{\cdot}{\underset{|}{\text{C}}}-\text{CH}=\text{CH}_2$. Therefore the side chain on the sugar ring is 2-methylbut-3-en-2-yl.⁽²⁰⁾

Since the completion of the work described above, Ballio et al⁽²²⁾ have published a structure for fusicoccin-A (I) which is based largely on the X-ray analysis of the p-iodobenzenesulphonate (II) which was prepared directly from (I).



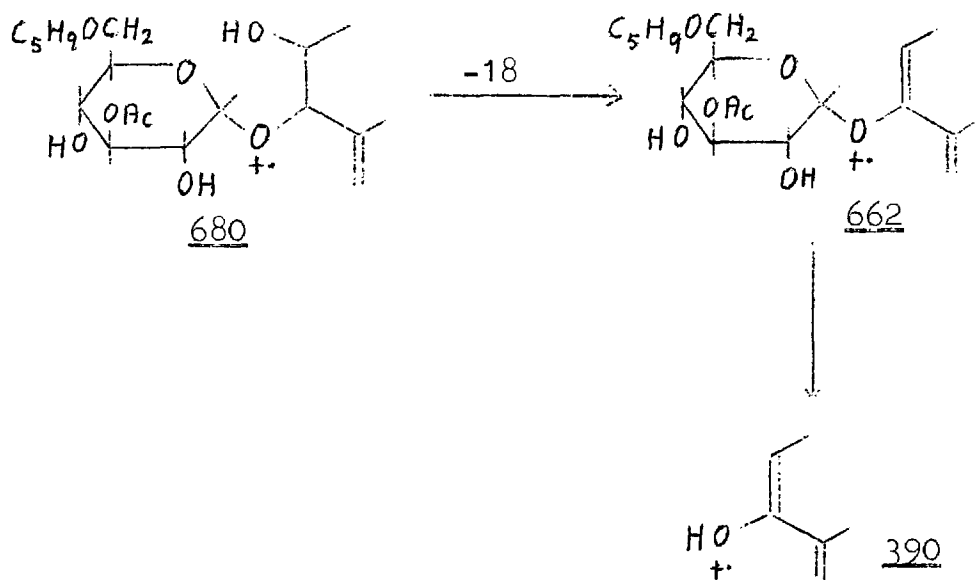
A mercury derivative which was prepared from the aglycone by U. Ohnsorge has been subjected to X-ray analysis under the direction of Professor D. Rogers and the following structure has recently been obtained for it.



This structure is obviously in agreement with that obtained by the Italian group, and is formed by a transannular reaction of fusicoccin aglycone.

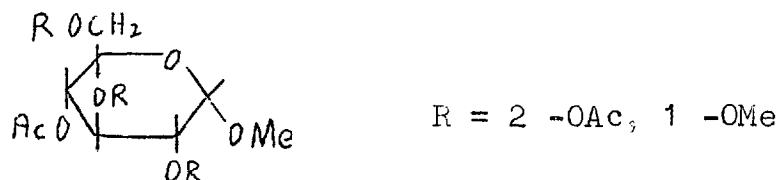
It is now necessary to try to rationalise the mass spectral fragmentations of fusicoccin with those of model compounds, that is, the aglycone forming a ROH^+ ion and the sugar fragmenting predominantly in series A. The investigations reported in sections 1,5 and 1,6 show that only those compounds which have the glycosidic oxygen atom attached to an unsaturated carbon atom, fragment in this manner. Apart from these, the only published fragmentations of this type have been observed for the iridoids (see page 24). An α hydroxy group or unsaturation in the β or γ position do not, by themselves, lead to this type of

fragmentation, but it may be that a combination of the two factors does cause it. Alternatively, most of the fragmentation of the molecular ion may take place after loss of water, which would give the aglycone as the ion at m/e 390.



1,3 Acetates of partially methylated methyl glucopyranosides

When this work was started, the position of attachment of the pentenyl group to the sugar ring of fusicoccin was not known. In an attempt to find this position using mass spectrometry it was necessary to examine some model compounds. Therefore three of the four possible methyl mono-O-methyl- α -D-glucopyranose triacetates have been prepared and their mass spectra determined.

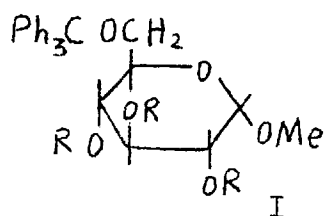


It was hoped that the electron impact induced fragmentation of the sugar ring would follow the same course as those observed for fusicoccin, and that they would be characteristic of the point of attachment of the non-glycosidic alkoxy group. When the mass spectra of these sugars were examined it was found that the simple aglycone directed the decomposition of the molecular ion along pathways that were different from those undergone by fusicoccin. Also the extra methoxy group at C(2), C(3) or C(6) was not lost at all in the manner of the pentenyl group in fusicoccin. However the mass spectra of these three glucose derivatives are distinct and could be used for determining the structure of an unidentified sugar of this type.

Preparation of methyl mono-O-methyl glucoside triacetates

The starting material for each synthesis was methyl- α -D-

glucopyranoside, and in order to obtain the desired compounds it was necessary to protect three of the hydroxy groups and methylate the fourth. It was shown by Helferich et al⁽²³⁾ that triphenylmethyl chloride reacts with the primary hydroxy groups of sugars in preference to secondary ones to form a "trityl ether". Thus methyl- α -D-glucopyranoside reacts with one mole of this reagent to form methyl 6-O-trityl- α -D-glucopyranoside (I, R=H).



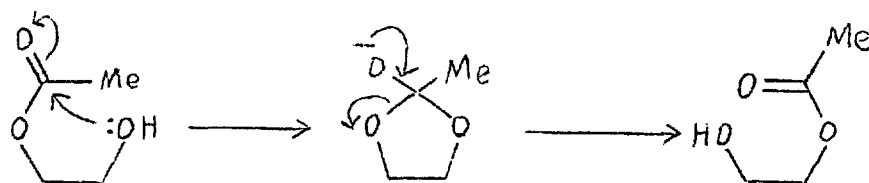
This compound has a very variable and indistinct melting point, which is caused by various degrees of solvation by the ethanol which is used for recrystallisation. Trityl ethers are particularly prone to this. For example, galactitol, which is a naturally occurring hexitol, forms a ditrityl ether which crystallises from the reaction mixture as a di-(pyridine hydrochloride) addition compound, and crystallises from ethanol with one molecule of solvent.⁽²⁴⁾

In fact trityl chloride also reacts with secondary hydroxy groups, and the rates have been obtained for the reaction of trityl chloride with three di-isopropylidene sugars which have one free hydroxy group.⁽²⁵⁾ The rate of reaction of the sugar with a free primary hydroxy group was 34 times faster than one of those with a secondary hydroxy group and 226 times faster than the other secondary

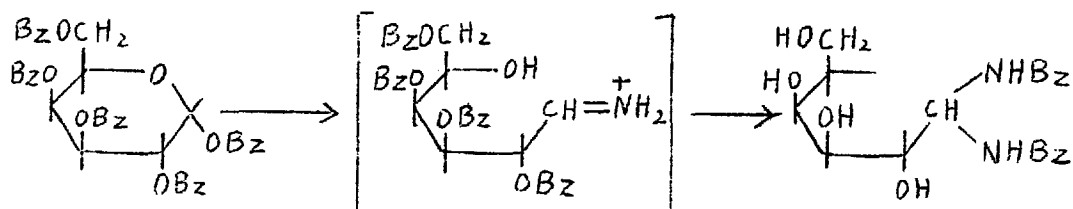
monohydroxy sugar. Therefore the selectivity shown for a primary hydroxy group is governed mainly by steric factors and it is possible that a simple diol such as propan-1,2-diol would only show a small degree of preference for trityl ether formation at the primary hydroxy group.

The ether protecting group was removed from methyl 6-O-trityl-2,3,4-tribenzoyl- α -D-glucopyranoside (I, R=PhCO) by brief treatment with hydrogen bromide in glacial acetic acid, to give methyl 2,3,4-tribenzoyl- α -D-glucopyranoside. Purdie methylation⁽²⁶⁾ followed by saponification and acetylation gave methyl 6-O-methyl-2,3,4-triacetyl- α -D-glucopyranoside.

By a similar procedure, methyl 2,3,4-triacetyl- α -D-glucopyranoside was prepared from the trityl ether. However, on Purdie methylation this gives methyl 2-O-methyl-3,4,6-triacetyl- α -D-glucopyranoside.⁽²⁷⁾ This is just one of the many examples of acetyl migration that are known. The rearrangement is sometimes catalysed by acid but more usually by mild base, in this case silver oxide, and in general the acetyl groups seem to migrate away from C(1). Thus Haworth has also observed that Purdie methylation of 1,2,3,4-tetra-acetyl- β -D-glucose gives methyl 2,3,4,6-tetra-acetyl- β -D-glucopyranoside. In this paper it was suggested that the migration goes through an ortho ester intermediate, and this is now generally accepted.⁽²⁸⁾



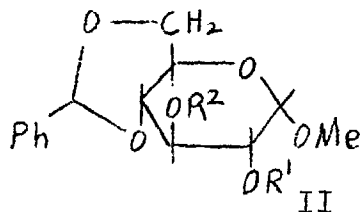
The facile migration of acetyl groups led to many false structures being given to compounds in the earlier carbohydrate literature. Fortunately benzoates do not migrate around the ring so readily and are therefore more useful intermediates. However, the migration of benzoyl groups is known and an example is provided by the treatment of penta-benzoyl- α -D-galactopyranose with methanolic ammonia.⁽²⁹⁾



Two benzoyl groups are transferred intramolecularly to C(1) in the formation of the 1,1-bis-benzamido sugar. Labelling has shown that one of them comes from C(4) and the other from C(2), C(3) and C(6).

Reaction of methyl- α -D-glucopyranoside with one mole of *p*-toluene sulphonyl chloride in pyridine gives methyl 6-O-tosyl- α -D-glucopyranoside.⁽³⁰⁾ This can be explained on the basis that this reagent reacts selectively with a primary hydroxy group in the same manner as trityl chloride. However when the 6-hydroxy group is protected by the formation of the 4,6-O-benzylidene derivative (II, $R^1=R^2=H$), then

tosylation takes place at the 2-hydroxy group to give methyl 4,6-O-benzylidene-2-O-tosyl- α -D-glucopyranoside (II, $R^1=Ts, R^2=H$).⁽³¹⁾



The increased reactivity of the 2-hydroxy group has been observed mainly in alkaline media. When the electrophile is not bulky, the 2-hydroxy group is more reactive than the primary group at C(6). Thus Wolfrom has shown that methyl α -D-glucopyranoside reacts with one mole of sodium hydroxide to form a sodium alkoxide at the 2 position.⁽³²⁾ This was explained by suggesting that the 2-hydroxy group has greater acidity because of the adjacent acetal group.

Purdie methylation of the tosylated sugar gave methyl 4,6-O-benzylidene-3-O-methyl-2-O-tosyl- α -D-glucopyranoside (II, $R^1=Ts, R^2=CH_3$), which after removal of the protecting groups followed by acetylation gave methyl 3-O-methyl-2,4,6-triacetyl- α -D-glucopyranoside.

Mass spectra of methyl mono-O-methyl-glucoside triacetates

(a) Methyl 2-O-methyl-3,4,6-triacetyl- α -D-glucopyranoside

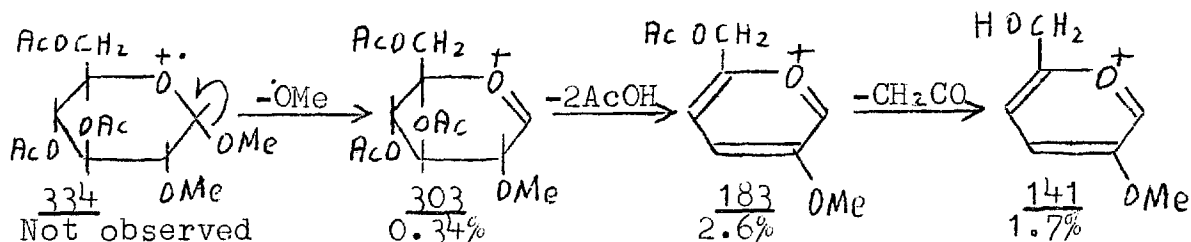
The low resolution spectrum is shown as Mass Spectrum 3 and peaks that have been mass measured are given in Table 1.

The ions of series A are relatively unimportant and give peaks at m/e 303, 183 and 141. Since acetic acid is eliminated more readily than methanol, the highly conjugated oxonium ions

Table 1.

m/e	Observed Mass	Calculated Mass	Formula
183	183.0657	183.0657	C ₉ H ₁₁ O ₄
172	172.0735	172.0736	C ₈ H ₁₂ O ₄
129	129.0552	129.0552	C ₆ H ₉ O ₃
116	116.0473	116.0473	C ₅ H ₈ O ₃
113	113.0600	113.0603	C ₆ H ₉ O ₃
112	112.0522	112.0524	C ₆ H ₈ O ₃
87	87.0448	87.0446	C ₄ H ₇ O ₂
74	74.0366	74.0368	C ₃ H ₆ O ₂

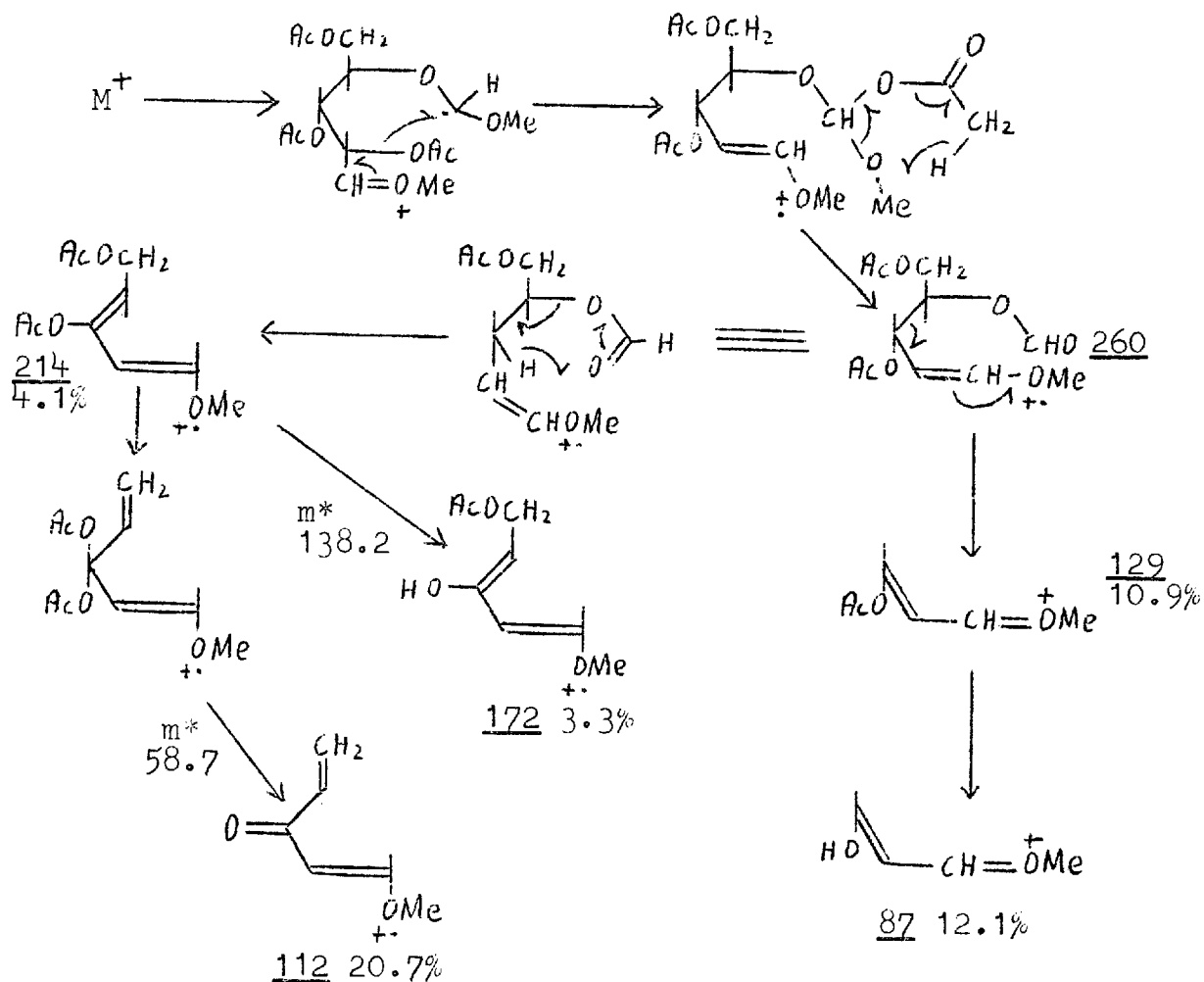
of mass 183 and 141 retain the methoxy group.



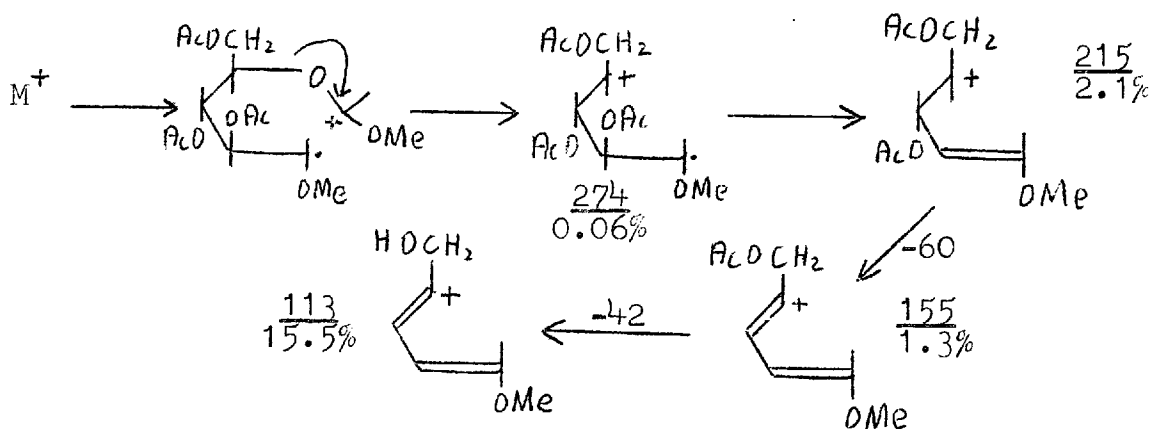
The peak at mass 261 (0.07%) is formed by loss of the side chain from C(5).

It was pointed out on page 16 that the presence of a methoxy group could stabilise the charge on the carbon atom to which it is attached. Therefore it is not surprising that the most abundant fragments are formed as a result of initial fission between C(1) and C(2). The ions of series B and C are almost certainly formed by the same mechanism as applies to peracetylated pyranoses, although no ion is observed at m/e 260. This is discussed fully in the section on alkyl tetra-acetyl glucosides. Therefore we have the

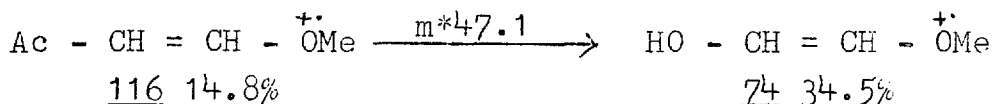
following fragmentation scheme.



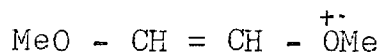
When the ionisation of the C(1)-C(2) bond takes place so that the charge is on C(1), the ions of series D are formed.



The other two abundant fragments are those containing C(2) and C(3).



There is a smaller peak at m/e 88 which is the corresponding fragment formed from C(1) and C(2).

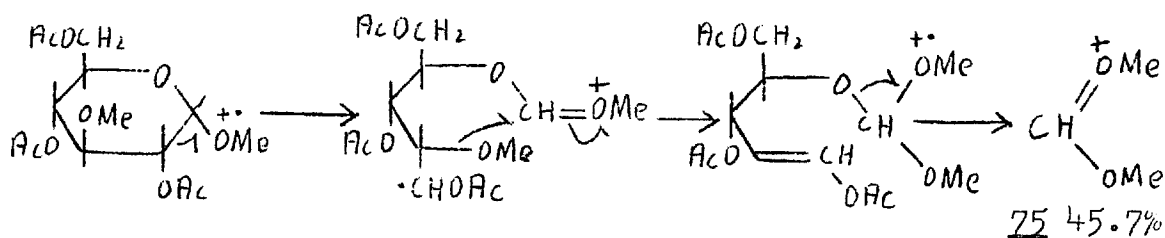


This ion can only be formed when there is a vicinal dimethoxy grouping.

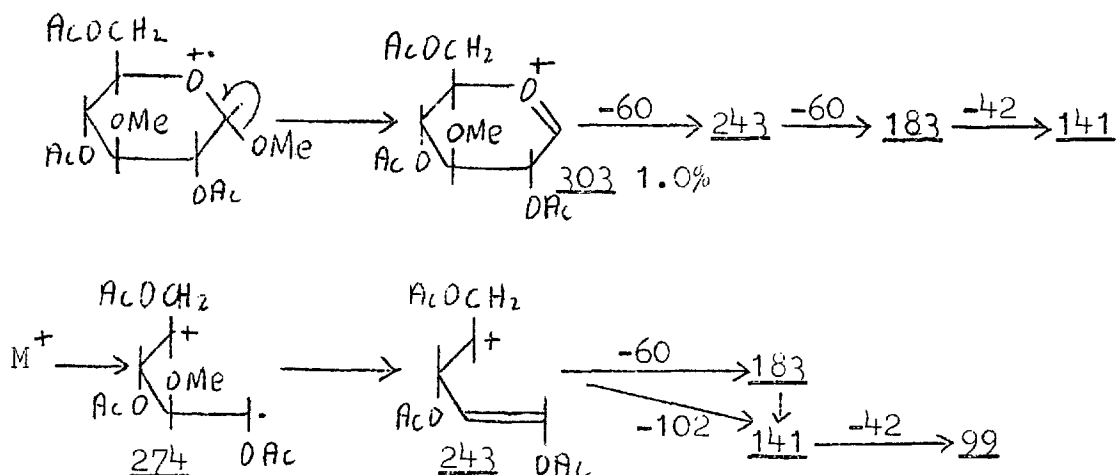
(b) Methyl 3-O-methyl-2,3,6-triacetyl- α -D-glucopyranoside

(Mass Spectrum 4)

The mass spectrum of this isomer is characterised by the very large peak at m/e 75 ($\text{C}_3\text{H}_7\text{O}_2^+$) which is not formed by either of the other isomers. This ion is formed by the migration of the methoxy group from C(3) to C(1), where the charge is stabilised by the two methoxy groups.



The ions of series B and C are present but because the substituent at C(3) is lost and the one at C(2) is retained in their formation, they are shifted 28 mass units to higher mass compared with the 2-O-methyl isomer. An intermediate of the Heyns and Müller type would have a geminal dimethoxy group which would have to lose dimethyl ether in a four membered transition state to give m/e 288.



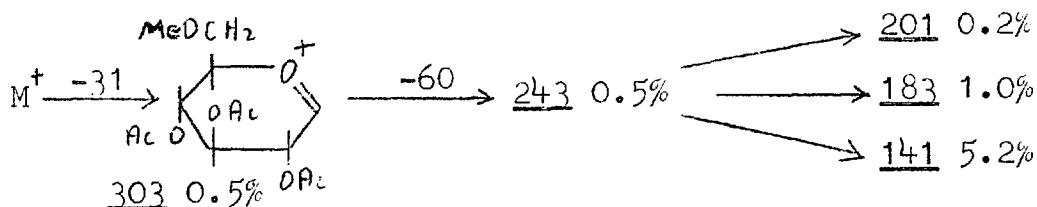
The other two abundant ions are the same as those produced by the 2-O-methyl isomer, namely m/e 116 ($\text{C}_5\text{H}_8\text{O}_3^+$), 18.3% and m/e 74 ($\text{C}_3\text{H}_6\text{O}_2^+$), 37.6%.

(c) Methyl 6-O-methyl-2,3,4-triacetyl- α -D-glucopyranoside

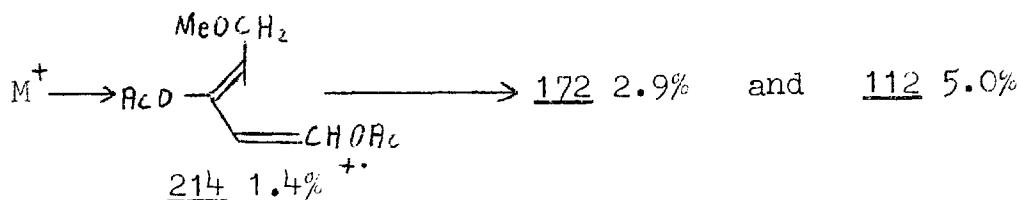
(Mass Spectrum 5)

Two-carbon fragments are of comparatively low abundance and appear at m/e 144 $\text{AcO} - \text{CH} = \text{CH} - \text{OAc}^+$ (3.3%) and m/e 102 $\text{HO} - \text{CH} = \text{CH} - \text{OAc}^+$ (6.0%). This is caused by the absence of a methoxy group at C(2) or C(3). The other four usual fragmentation series give the following peaks.

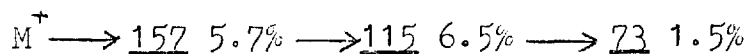
Series A



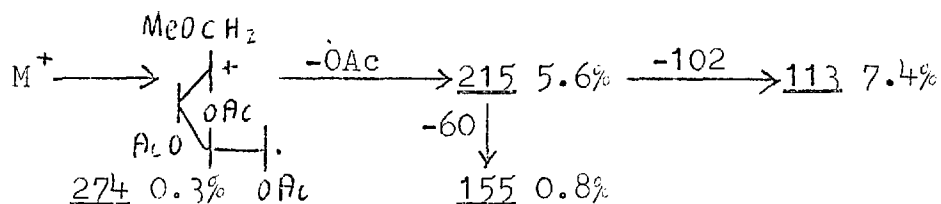
Series B



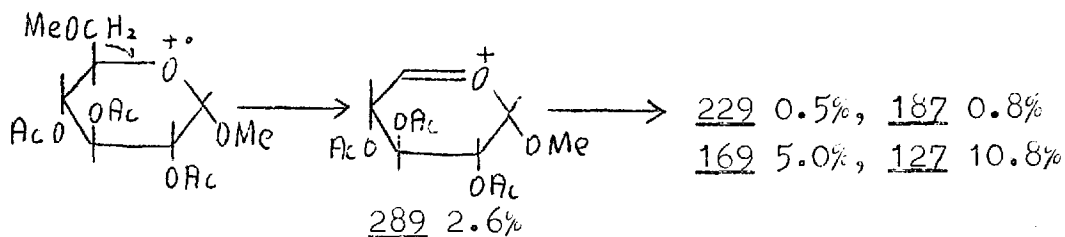
Series C



Series D



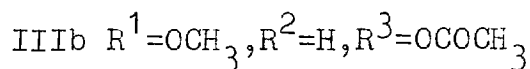
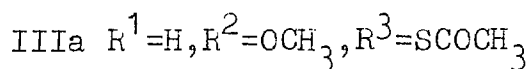
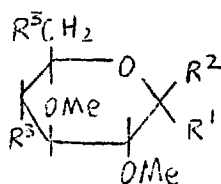
However, because there is a methoxy group at C(6) the radical $\text{MeO}-\dot{\text{C}}\text{H}_2$ and the ion $\text{Me}-\text{O}=\text{CH}_2^+$ (m/e 45, 8.1%) are stabilised and the main peaks are due to the loss of the side chain from C(5).



Methyl 2,3-di-O-methyl-4,6-diacetyl- α -D-glucopyranoside

(Mass Spectrum 6)

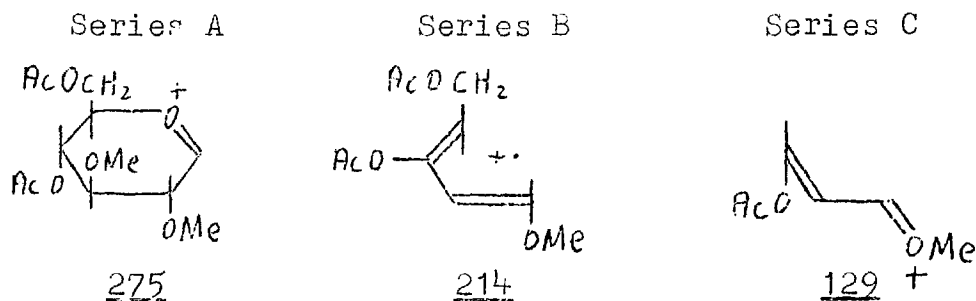
This compound was made in order to compare its mass spectrum with that of methyl 4,6-bisacetylthio-4,6-dideoxy-2,3-di-O-methyl- β -D-glucopyranoside (IIIa), which was kindly supplied by Professor L.N.Owen. There was some doubt about the structure of the dithio analogue because there was a possibility of ring contraction to a furanoside in the synthesis.⁽³⁴⁾ In addition to the chemical proof that has since been obtained, the mass spectrum is obviously that of a pyranoside.



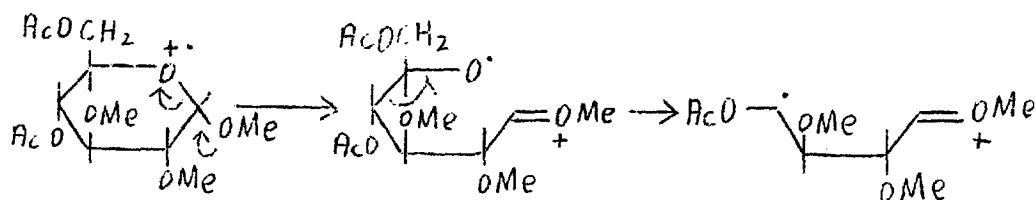
The title compound (IIIb) was prepared by methylation of methyl 4,6-O-benzylidene- α -D-glucopyranoside followed by mild acid hydrolysis and acetylation.

The main peaks in the mass spectrum of this compound (molecular weight 306) are m/e 43, 75 and 88. The peak at m/e 75 is characteristic of methoxy groups at C(1) and C(3), and the ion at m/e 88 $MeO - CH = CH - \overset{+}{O}Me$ is characteristic of methoxy groups at C(2) and C(3).

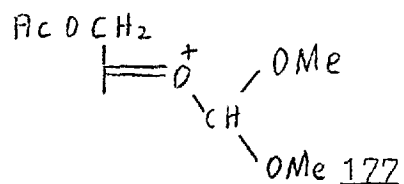
The smaller ions at the upper end of the spectrum are formed by mechanisms that have already been discussed in the cases of the other methyl glucosides.



The peak at m/e 204 is formed by a process which has only been previously observed to operate on permethylated glycosides. It is obviously becoming more important with an increasing degree of methylation.



Another fragmentation which is also found in the mass spectra of permethylated sugars gives a peak at m/e 177.



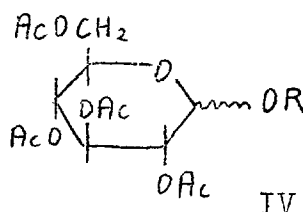
Most peaks in the mass spectrum of the dithio analogue (IIIa) are formed by the same mechanisms as discussed above. The significant ones are listed in Table 2.

Table 2

Ion in mass spec of IIIb	Intensity	Corresponding ion in spec of IIIa	Intensity
43	64%	43	96%
75	87%	75	88%
88	100%	88	100%
129	4.0%	-	-
177	2.3%	193	5.6%
204	5.8%	220	10.7%
214	2.6%	246	5.6%
275	1.1%	-	-

1,4 Methods of preparation of glycosides

It was found that the mass spectral fragmentations of the sugar ring of simple alkyl tetra-acetyl glucosides were much more complicated than the fragmentations undergone by the sugar moiety of fusicoccin. Therefore a number of tetra-acetyl glucosides (IV) have been synthesised to find out what was the influence of the aglycone R on the fragmentation of the pyranoside ring.

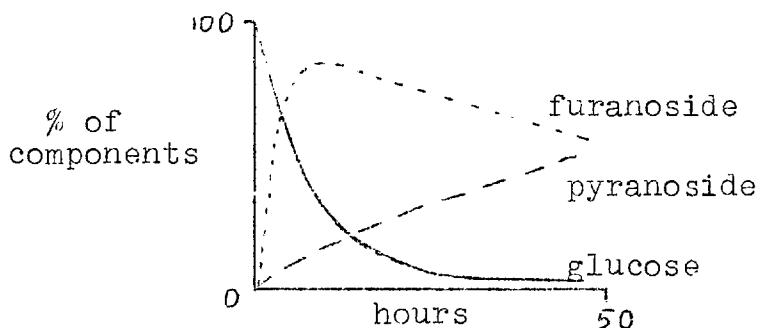


Formation of the glycoside linkage

Four basic methods were used in the preparation of the glycosides.

(i) Fischer Method. This is the only widely used method that gives the unacetylated glycoside directly from the sugar. Emil Fischer showed that when glucose was heated in methanolic hydrogen chloride for a few hours, the main product was methyl α -D-glucopyranoside.⁽³⁵⁾ However it is now known that a complex equilibrium is set up between the sugar and the alcohol. This has been studied by Levene et al⁽³⁶⁾ for various sugars. The α and β forms of both pyranosides and furanosides are present in the equilibrium mixture and the relative proportions of each are dependent upon the period of the reaction. The variation of products with time for the reaction of glucose with 0.5% hydrogen

chloride in methanol at 25°C is shown in the following diagram.

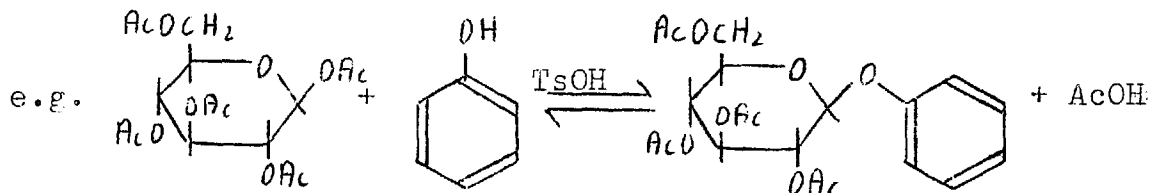


Therefore by prolonged heating of the reaction mixture the furanosides which are initially formed at a faster rate are converted to pyranosides so that methyl pyranosides are the main products.

This method of glycoside formation has only been found to be applicable to simple aliphatic alcohols, and the required product can sometimes be isolated from the reaction mixture only with difficulty.

(ii) Helferich Method. When penta-acetyl glucose and phenol are fused together in the presence of a catalyst, a mixture of the α and β anomers of phenyl 2,3,4,6-tetra-acetyl-D-glucopyranoside is formed.⁽³⁷⁾ Conditions and catalyst can be chosen in order to obtain a reaction mixture in which one isomer predominates. Thus reaction of β -penta-acetyl glucose and phenol with p-toluene-sulphonic acid as catalyst at 100°C for one hour gives mainly β -glucoside, whereas the reaction of either α or β penta-acetyl glucose using zinc chloride as catalyst at a

temperature of 125°C for three hours gives predominantly the α -glucoside.

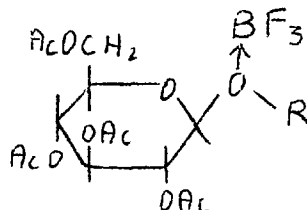


The yield in this reaction is improved by carrying it out under reduced pressure at a temperature of 100°C .⁽³⁸⁾ This forces the equilibrium to the right by distilling off the acetic acid as it is formed. It was also shown that the yield of α -isomer was improved by using penta-acetyl- α -D-glucose as the precursor.

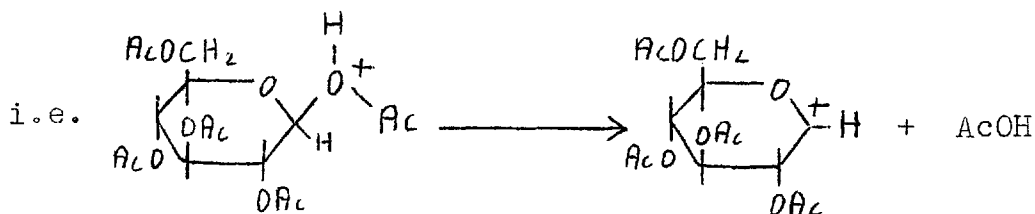
It has long been known that penta-acetyl- α -D-glucose can be prepared by reaction of glucose with acetic anhydride and zinc chloride, so this catalyst normally leads to α -isomers. This observation was used by Hudson as a basis for the isomerisation of β to α isomers. Therefore when phenyl tetra-acetyl- β -D-glucopyranoside is fused with zinc chloride and phenol the α -isomer is obtained. Similarly fusion of methyl tetra-acetyl- α -D-glucopyranoside with the same two reagents gives phenyl tetra-acetyl- α -D-glucopyranoside in a trans-glycosidation reaction.⁽³⁸⁾ This suggests that the reaction proceeds through a carbonium ion intermediate, which has lost the substituent from C(1). The method of anomerisation with zinc chloride was found useful for phenolic glycosides.

Two other methods have been used for the preparation of

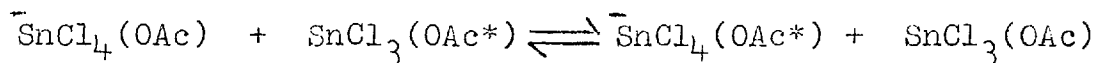
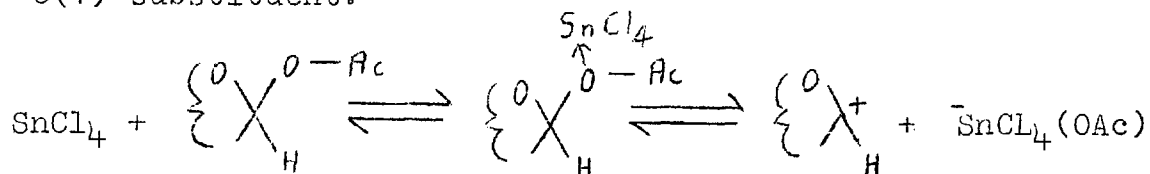
α -aliphatic glycosides from the β -isomer. The β -glucoside is treated with either titanium tetrachloride⁽³⁹⁾ or boron trifluoride⁽⁴⁰⁾ in dry chloroform. The period of reaction necessary using the latter catalyst was found to vary from $\frac{1}{2}$ hr. to 24 hrs. at room temperature. The rate was fastest when there was an electron repelling group on the aglycone. Therefore the intermediate is probably:



Since anomerisation can occur in the Helferich reaction the mechanism is probably similar to the anomerisation of glucose penta-acetates. The latter phenomenon has been fairly well studied. Painter⁽⁴¹⁾ has shown that when perchloric acid or sulphuric acid is added to a solution of either isomeric penta-acetyl glucose, mutarotation occurs and eventually an equilibrium mixture is formed, which contains 87% of the α -form and 13% of the β -form. Kinetic measurements showed that the rate determining step in this reaction is the production of a carbonium ion from protonated glucose penta-acetate.

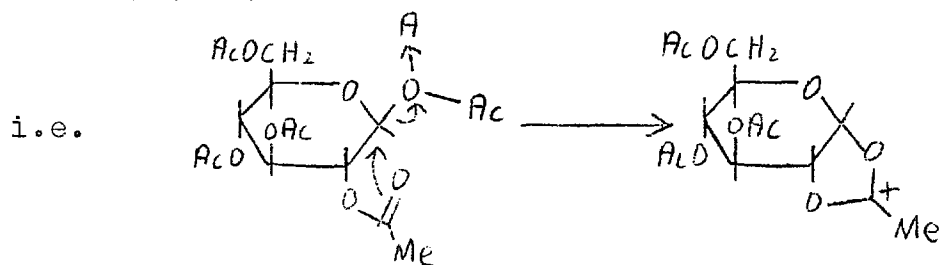


Lemieux has studied the reaction of β -glucose penta-acetate with stannic chloride in chloroform.⁽⁴²⁾ When the reaction is carried out in the presence of ^{14}C labelled $\text{SnCl}_3(\text{OAc}^*)$, labelled acetoxy group is incorporated rapidly at C(1) in the sugar. Therefore this is firm proof of an intermediate carbonium ion which lacks the C(1) substituent.



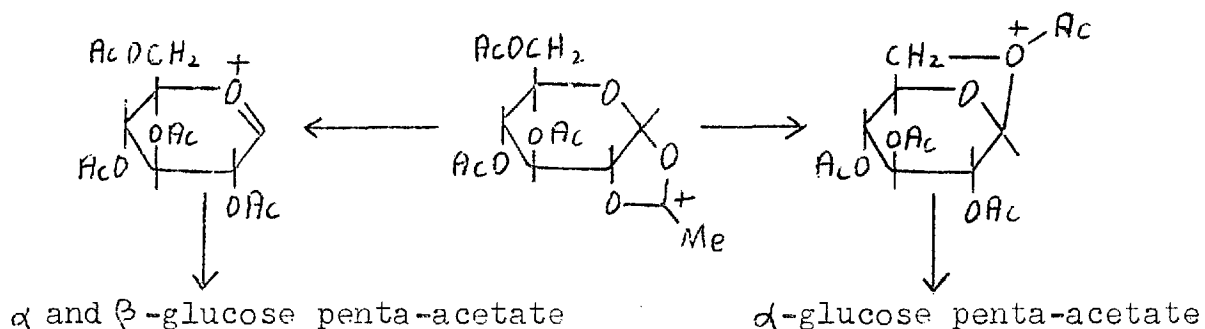
The incorporation of ^{14}C acetate into the α' -isomer is very much slower and the slowness of reaction of α -glucose penta-acetate is also manifest in its lack of reaction with phenol when *p*-toluenesulphonic acid is used as the catalyst in the Helferich reaction.⁽⁴³⁾

Therefore it is concluded that when the acetoxy group at C(2) is trans to the substituent at C(1), the loss of the substituent from C(1) can be anchimerically assisted by that acetoxy group.



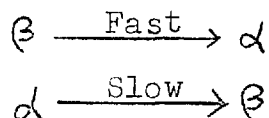
Attack of this intermediate at C(1) by an acetoxy group would once again give the β isomer. However Lemieux suggests

that there could be assistance from the C(6) acetoxy group which would lead to the α anomer. Alternatively some of the monocyclic oxonium ion may be present which could also give some of the α anomer.



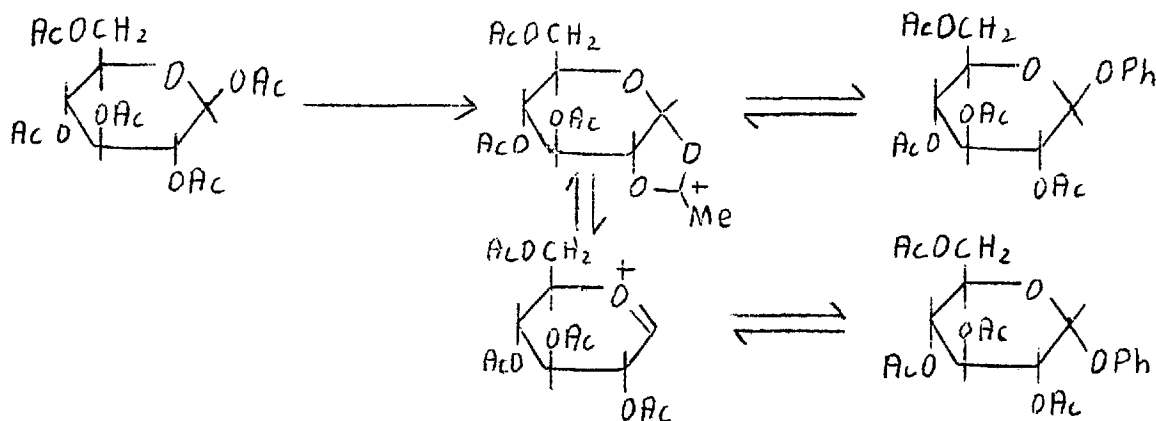
Once α -glucose penta-acetate is formed, it only reacts very slowly to form the cation because there can be no neighbouring group participation from C(2) in its formation.

Therefore because the production of a carbonium ion is the rate determining step, the two reactions are



and the α form predominates in the equilibrium mixture.

In the Helferich reaction the following processes are taking place under acid catalysis.



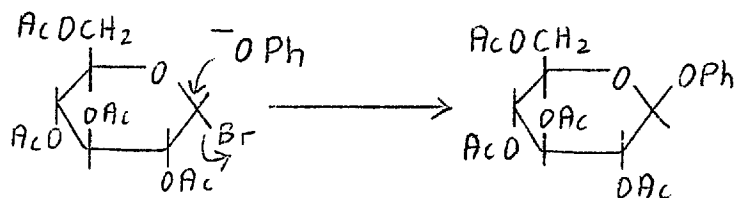
Therefore the first product to be formed is the β -glucoside but if the reaction is allowed to reach equilibrium, the α -glucoside predominates in the equilibrium mixture. It can be seen by comparison of the effects of zinc chloride and *p*-toluenesulphonic acid on the reaction of phenol and penta-acetyl- α -D-glucose, that the Lewis acid is a far better catalyst. Therefore by using short reaction times and a poor catalyst the β -glucoside is obtained, but a longer reaction period with a Lewis acid catalyst gives the α -glucoside. This conclusion is verified by the observation that reaction of penta-acetyl- β -D-glucose with methanol and stannic chloride for short periods at low temperature (1hr. at 40°C) gives a 50% yield of the β -glucoside.⁽⁴⁴⁾

In general the Helferich procedure has only been used for the preparation of phenolic glycosides. There are some limitations, however, on the phenols that can be used. The hydroxybenzaldehydes give very poor, or no yields and there is no control over whether the α or β anomer is formed.⁽⁴⁵⁾ Therefore glycosides can be made from these phenols by the Michael method instead.

(iii) Michael Method. This can only be used to make glycosides from phenols since it depends on formation of a sodium salt. The original method consisted of reaction of α -acetochloroglucose with phenol and potassium hydroxide in ethanol.⁽⁴⁶⁾ The initial product of the reaction was phenyl tetra-acetyl β -D-glucopyranoside which was deacetylated under the reaction conditions. The method was modified by Mannich⁽⁴⁷⁾ who made

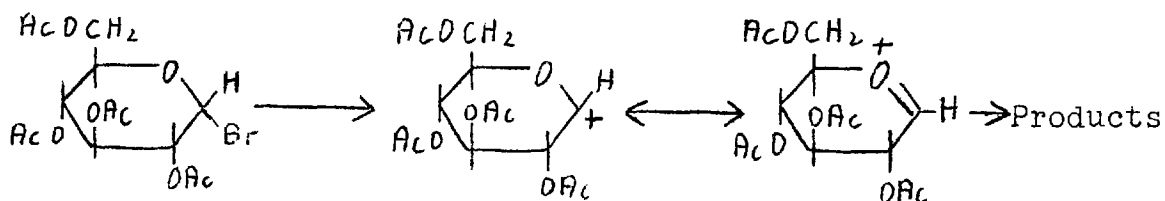
morphine tetra-acetyl- β -D-glucopyranoside by shaking a solution of morphine in aqueous sodium hydroxide with an ethereal solution of acetobromoglucose. However the most useful modification of this procedure enables it to be performed in homogeneous solution by the use of aqueous acetone as solvent.⁽⁴⁸⁾ The author has found this modification to be satisfactory, for simple phenolic glucosides. However it has been found to give rather low yields in some cases due to the competing reaction of acetobromoglucose with water.⁽⁴⁹⁾ This liberates hydrogen bromide and neutralises the phenoxide ion which is the nucleophilic species. If excess base is used, then deacetylation takes place. Therefore improved yields can be obtained by initially using 0.9 equivalents of potassium hydroxide and adding more base as the reaction proceeds to keep it at the same pH (ca pH 9).

This method of glycoside formation always results in products in which inversion at C(1) has occurred. It is therefore an S_N2 reaction.



(iv) Koenigs-Knorr Reaction. This reaction has the most general applicability of the four discussed. It can be used to prepare glycosides from alcohols, phenols and even amines. It consists of the reaction of an acetobromo sugar

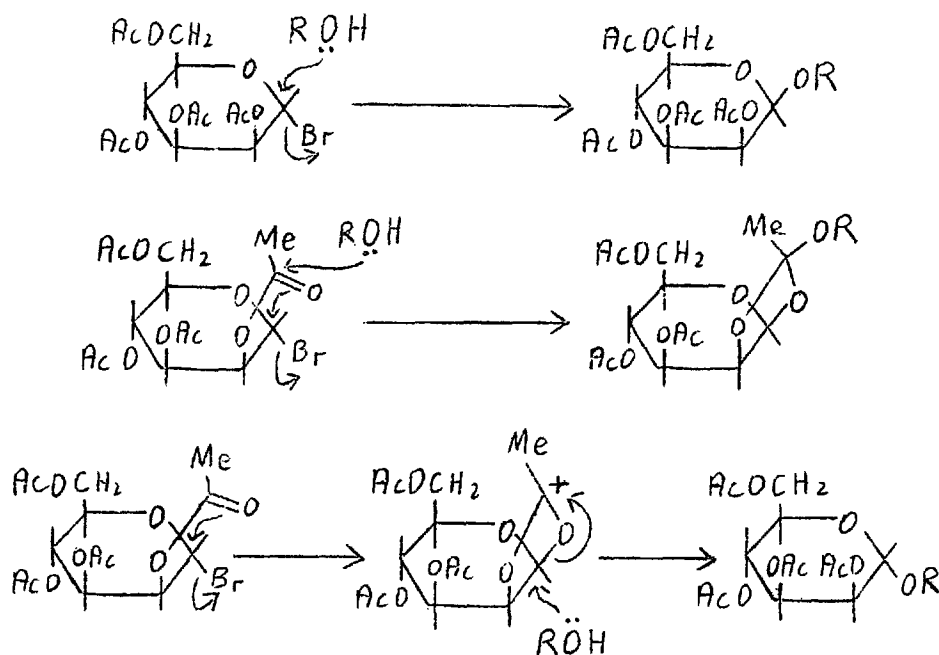
gives complicated results they used 0.02-0.06 molar solutions of the halo-sugar in methanol with no acid acceptor. At this concentration the liberated hydrogen ion causes negligible deacetylation. The solvolysis was unimolecular and therefore it was deduced that the reaction was S_N1 . The carbonium ion which is formed is stabilised by the electron releasing capacity of the heterocyclic oxygen atom.



On this basis, the Koenigs-Knorr reaction would be expected to give a mixture of α and β glycosides, and in some cases this is so. The reaction of phenol and acetobromoglucose in quinoline gives a 2:3 mixture of α and β glucosides,⁽⁵⁴⁾ but if the same conditions are used, with only the addition of silver oxide, the product is exclusively the β -glucoside with no trace of α .⁽⁵⁵⁾ It therefore appears that addition of silver oxide makes the reaction S_N2 due to the formation of some Ag^+OR^- and complete inversion occurs.

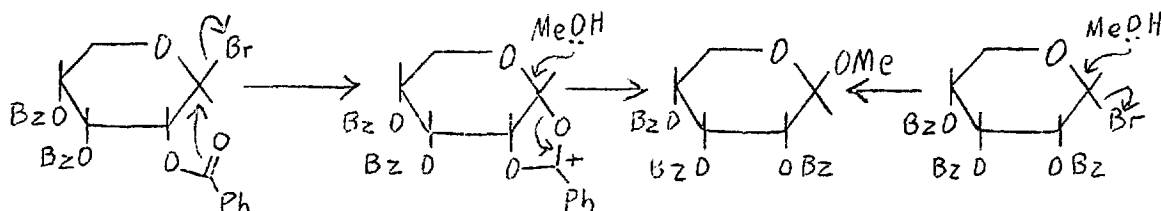
The Koenigs-Knorr reaction in the presence of silver carbonate has been extensively studied by Isbell and Frush.⁽⁵⁶⁾ They showed that there was little temperature effect in the reaction of α -acetobromoglucose with methanol and only a small difference in rate on changing from methanol to ether as solvent. Therefore they concluded that the reaction was S_N2 .

The results described above only apply when the acetoxy group at C(2) is cis to the halogen atom. When it is trans as in tetra-acetyl- α -D-mannopyranosyl bromide three products are obtained; α -glucoside, β -glucoside and a 1,2-orthoester.⁽⁵⁶⁾ This is caused by neighbouring group participation by the C(2) acetoxy group. The three competing reactions are:



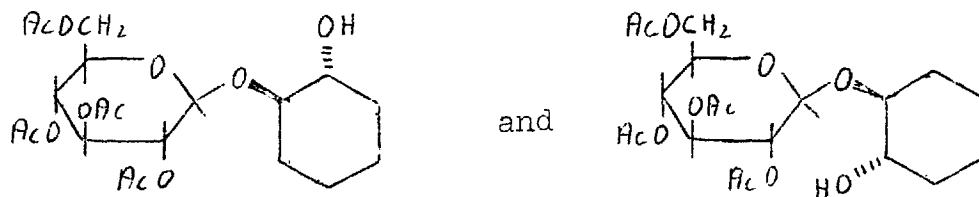
β -Acetohalogeno-sugars are generally rather unstable compounds, but the corresponding benzoylated compounds show much greater stability and are useful in some syntheses. Hudson et al⁽⁵⁷⁾ prepared the α and β forms of tribenzoyl ribopyranosyl bromide and found that under Koenigs-Knorr conditions with methanol, both isomers gave the methyl β -ribopyranoside tribenzoate. The rates of reaction of the two anomers were compared in the absence of an acid acceptor and it was found that the β -bromide reacted 19 times faster than the α -bromide. This is good evidence for anchimeric

assistance by the benzoyl group.

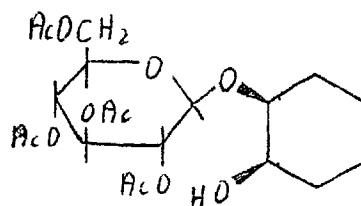
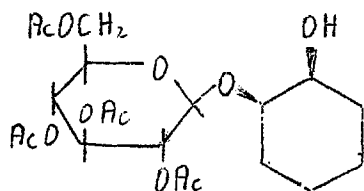


In this case there was some evidence for orthoester in the reaction mixture, but none could be isolated.

In this work the Koenigs-Knorr method has been used to synthesise glycosides from dl-cyclohex-2-en-1-ol, dl-cyclohex-3-en-1-ol and dl-trans-cyclohexan-1,2-diol. In the reaction of these racemates with a D-glucose derivative, a mixture of two diastereoisomers should be obtained. For example, the diol should give the two compounds shown below, which are not mirror images nor superimposable.



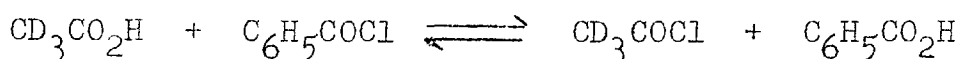
However, in each case only one glucoside could be readily isolated from the reaction mixture. Since the isolations relied on crystallisation from an ethanolic solution, this simply implies that the diastereoisomers vary greatly in their relative solubilities. A glycoside was also prepared from cis-cyclohexan-1,2-diol which is optically inactive. However since it is a meso form there are two possible diastereoisomeric mono-glucosides of which only one was isolated.



The synthetic glycoside could be acetylated to give a penta-acetate m.p. $121-124^{\circ}$ $[\alpha]_D^{22.5} -25.8^{\circ}$ (c.2.1, CHCl_3). However a natural glucoside called grandidentatin is known which is cis-2'-hydroxycyclohexyl 2-O-(p-hydroxycinnamoyl)- β -D-glucopyranoside.⁽⁵⁸⁾ This can be saponified and then acetylated to give a penta-acetate which should be identical to the synthetic glycoside. However it has m.p. $121-122^{\circ}$ and $[\alpha]_D^{30} -58.2^{\circ}$ (c.2.2, CHCl_3) and must therefore be the other diastereoisomer.

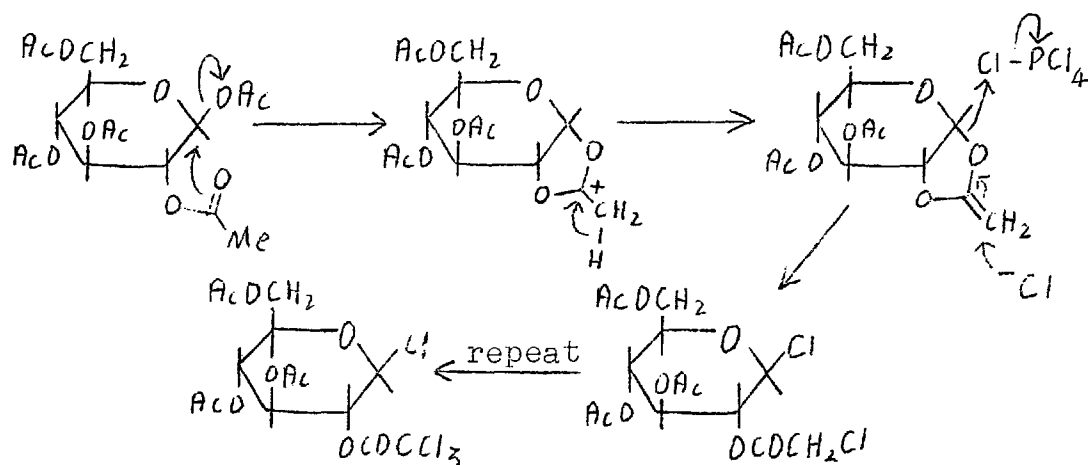
Preparation of deuteriated glycosides

Trideuterioacetates were prepared from the free glycosides by two methods. The best one was undoubtedly the reaction with $[^2\text{H}_6]$ -acetic anhydride in pyridine. Before this reagent was available, $[^2\text{H}_3]$ -acetyl chloride was made from $[^2\text{H}_3]$ -acetic acid. The use of volatile inorganic halides such as thionyl chloride in this preparation gives products which are contaminated with inorganic impurities. Therefore benzoyl chloride was used as the chlorinating agent.⁽⁵⁹⁾ When it is mixed with $[^2\text{H}_3]$ -acetic acid an equilibrium is set up.



The acetyl chloride is distilled from the reaction mixture as it is formed, and the equilibrium is forced to the right, giving the required product in high yield.

In order to follow the sequence of losses of ketene and acetic acid in the mass spectra of tetra-acetyl phenolic glycosides, the preparation of this type of compound with only one trideuterioacetoxy group has been attempted. *p*-Nitrophenyl-3,4,6-tri- $[^1\text{H}_3]$ -acetyl-2- $[^2\text{H}_3]$ -acetyl- β -D-glucopyranoside(VII) was prepared by the following series of reactions. Penta-acetyl- β -D-glucopyranose was heated with phosphorus pentachloride to give 3,4,6-triacetyl-2-trichloroacetyl- β -D-glucopyranosyl chloride.⁽⁶⁰⁾ This reaction must first involve chlorination at C(1) followed by chlorination of the acetoxy group at C(2). Abramovitch has tried to explain this selective chlorination by a combination of the inductive effect of the halogen atom at C(1) and the configuration of the acetoxy group.⁽⁶¹⁾ However, the most plausible mechanism has been suggested by Lemieux⁽⁶²⁾ and involves neighbouring group participation.

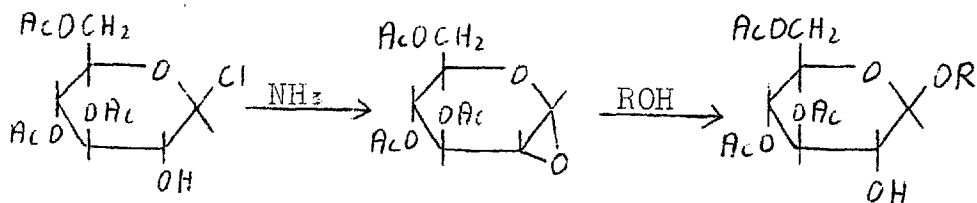


The inductive effect of the trichloromethyl group allows the selective removal of the ester group at C(2) by brief treatment with ethereal ammonia and 3,4,6-triacetyl-

β -D-glucopyranosyl chloride is obtained. These two glucosyl chlorides are the only reasonably stable ones that are known in the β series and have therefore been used for the preparation of α -glucosides.⁽⁶³⁾

The triacetyl glucosyl chloride was used in the Koenigs-Knorr synthesis with p-nitrophenol to give crystalline p-nitrophenyl-3,4,6-triacetyl- α -D-glucopyranoside in low yield. Trideuterioacetylation of this with either reagent gives a mixture of α and β glucosides which was separable by preparative thin layer chromatography. A similar anomerisation of this triacetyl glucoside has also been observed on deacetylation.⁽⁶⁴⁾

Unfortunately the attempted Koenigs-Knorr reaction with phenol was unsuccessful. Therefore Brigl's anhydride was prepared as an alternative reagent.⁽⁶⁰⁾ This is the 1,2-anhydro sugar formed on prolonged treatment of 3,4,6-triacetyl- β -D-glucopyranosyl chloride with dry ammonia.



It has been reported that fusion of aliphatic alcohols with the anhydride gives glucosides.⁽⁶³⁾ However, no glucoside was isolated on fusion with phenol.

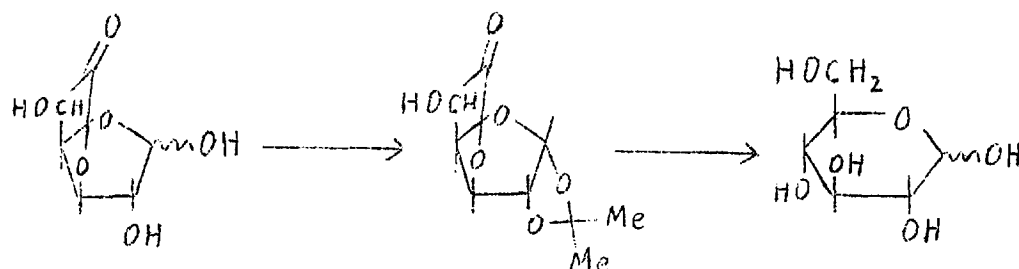
Apart from the method of synthesis, there is no definite proof that the trideuterioacetate is attached at C(2) in the p-nitrophenyl glucoside but the 100 MHz n.m.r. spectrum

of the β isomer shows only three acetate methyl resonances at ca 8 τ . This means that the trideuterioacetate is confined to a single position. Also it was shown on page 45 that acetate groups generally migrate away from C(1), therefore it is fairly certain that the trideuterioacetate is located at C(2).

Trideuterioacetylation of syrupy methyl 2,3,4-triacetyl- α -D-glucopyranoside (page 45) gave a deuteriated tetra-acetyl compound. The 100 MHz n.m.r.spectrum of this compound showed acetate resonances at τ 7.90, 7.92, 7.97 and 7.99 in the approximate ratio 1:3:2:3. This showed that acetate migration had taken place towards C(6) either on detritylation or trideuterioacetylation. The migration of an acetoxy group from C(4) to C(6) is extremely facile under basic conditions and has even occurred because of catalysis by a soft glass container.⁽⁶⁵⁾ Therefore it seems that it would be difficult to prepare tetra-acetyl glycosides that had been selectively trideuterioacetylated at any position except C(2).

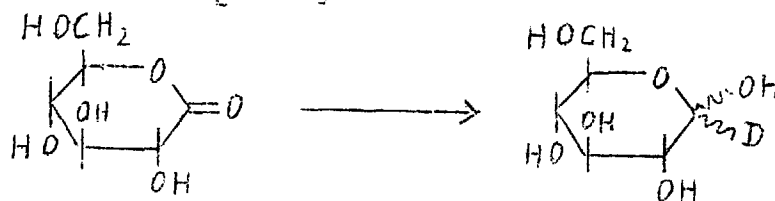
Three tetra-acetyl glycosides have been prepared which have deuterium directly attached to the sugar chain. Glucose, containing tritium at the C(6) position has been prepared from glucuronolactone,⁽⁶⁶⁾ so this lactone was chosen as the starting material for the synthesis of $[6,6\text{-}^2\text{H}_2]$ -glycosides. The hemiacetal group was protected by forming 1,2-O-isopropylidene - α -D-glucuronolactone⁽⁶⁷⁾ which served the additional purpose of blocking one of the hydroxy groups

which would otherwise have decomposed some of the metal hydride.



The lactone group was reduced with sodium borodeuteride in water, and acid work up gave $[6,6-^2\text{H}_2]$ -D-glucose which was converted into methyl glucoside and phenyl tetra-acetyl glucoside (IX).

Gluconolactone was the starting material for obtaining $[1-^2\text{H}_1]$ -D-glucose. A metal hydride reduction would have taken the lactone through to the hexitol so an alternative reducing agent was used. Thus sodium amalgam in deuterium oxide⁽⁶⁸⁾ gave deuteriated glucose but only with 79% incorporation at C(1). This compound was used to prepare phenyl tetra-acetyl- $[1-^2\text{H}_1]$ - β -D-glucopyranoside (VIII).



The 100 MHz n.m.r. spectrum of phenyl tetra-acetyl- β -D-glucopyranoside (V) is shown in figure 1 and those of the deuteriated isomers VIII and IX are shown in figure 2.

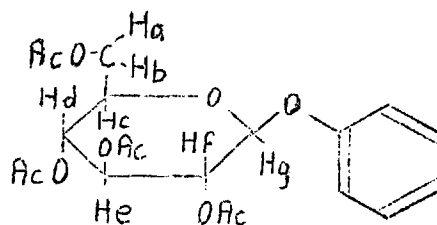


Figure 1.

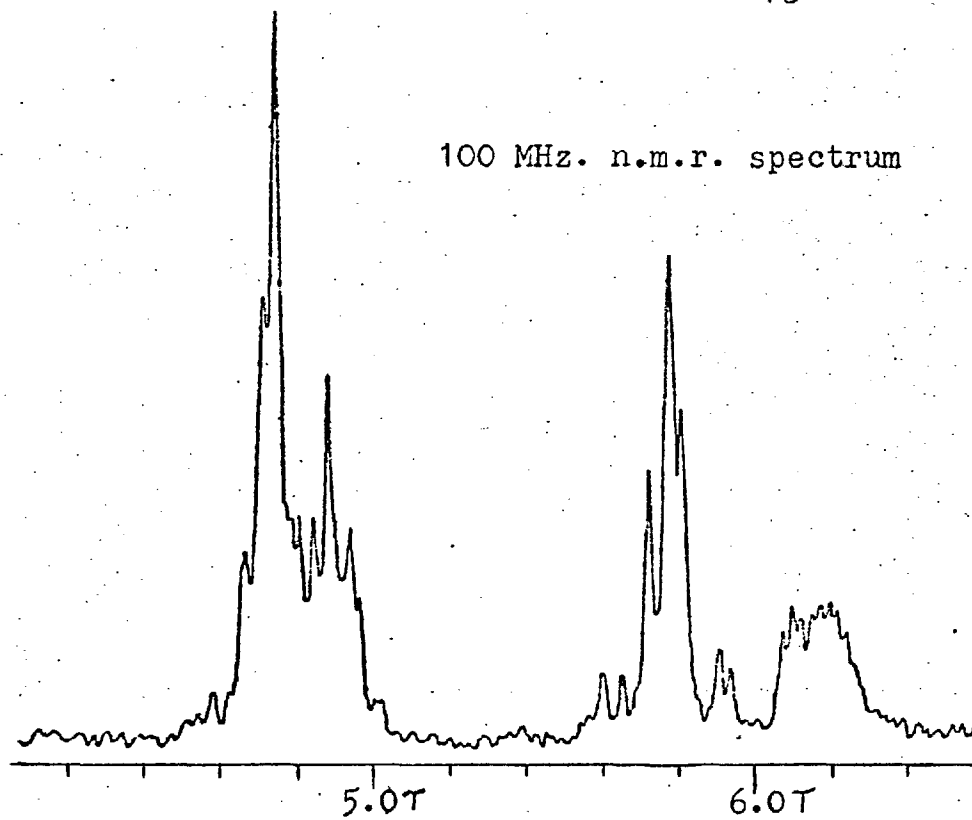
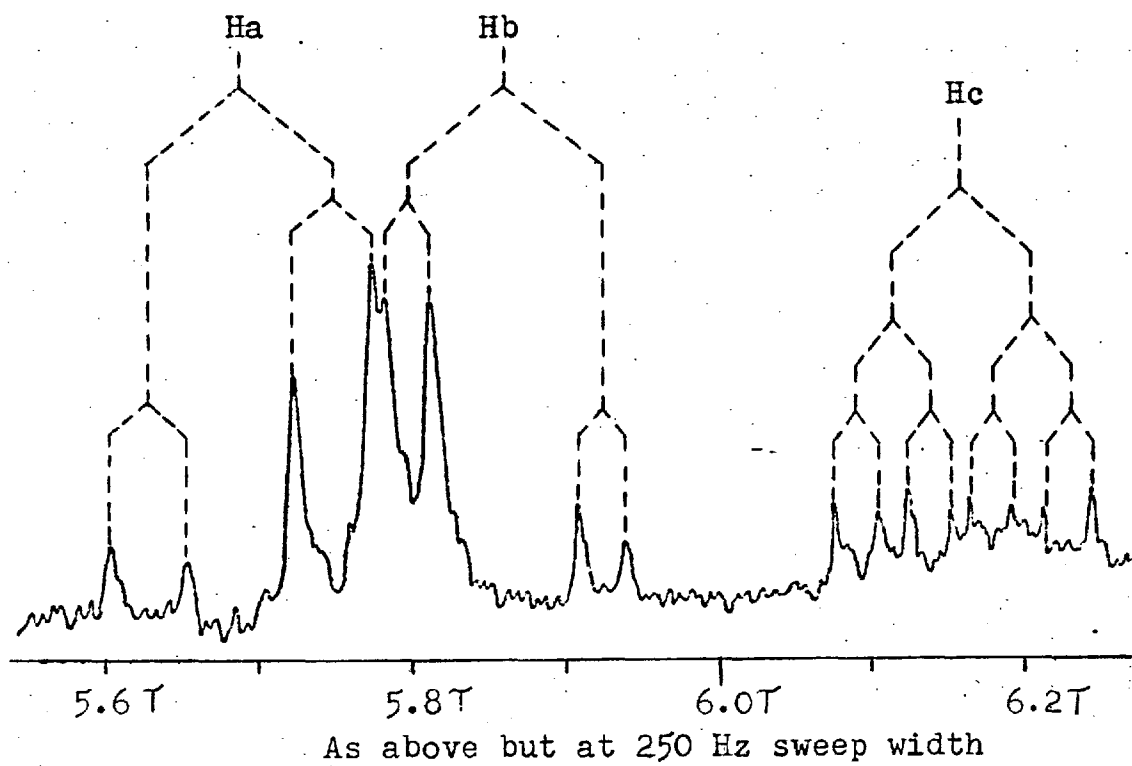
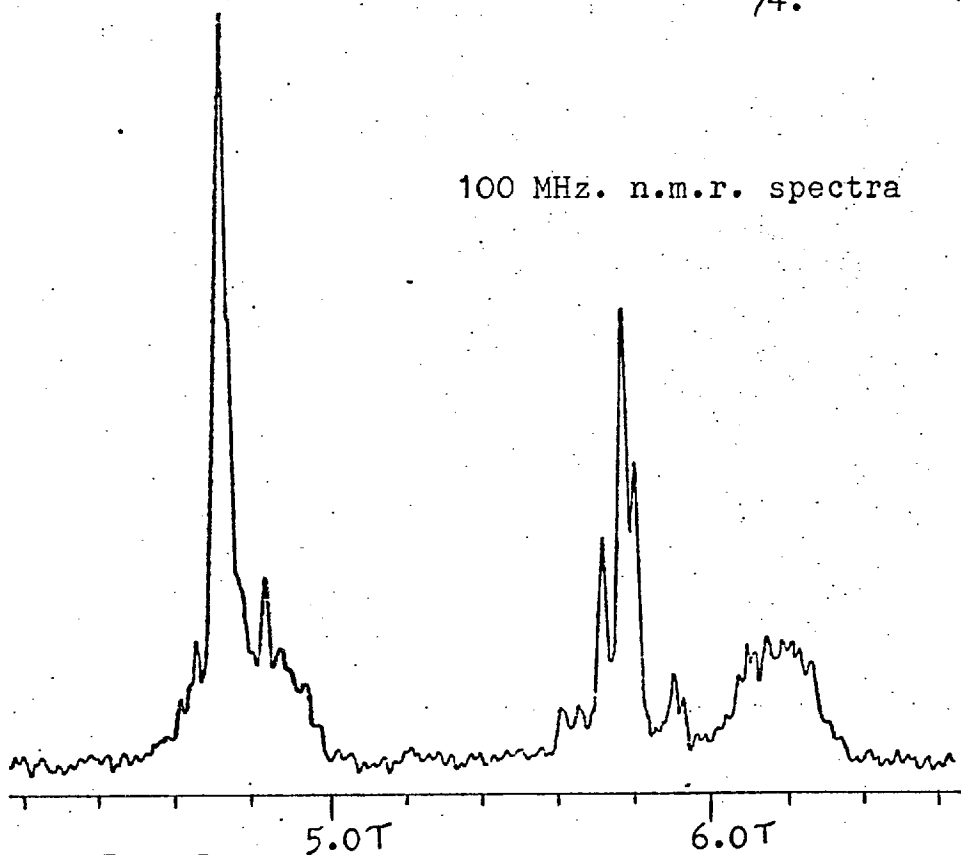
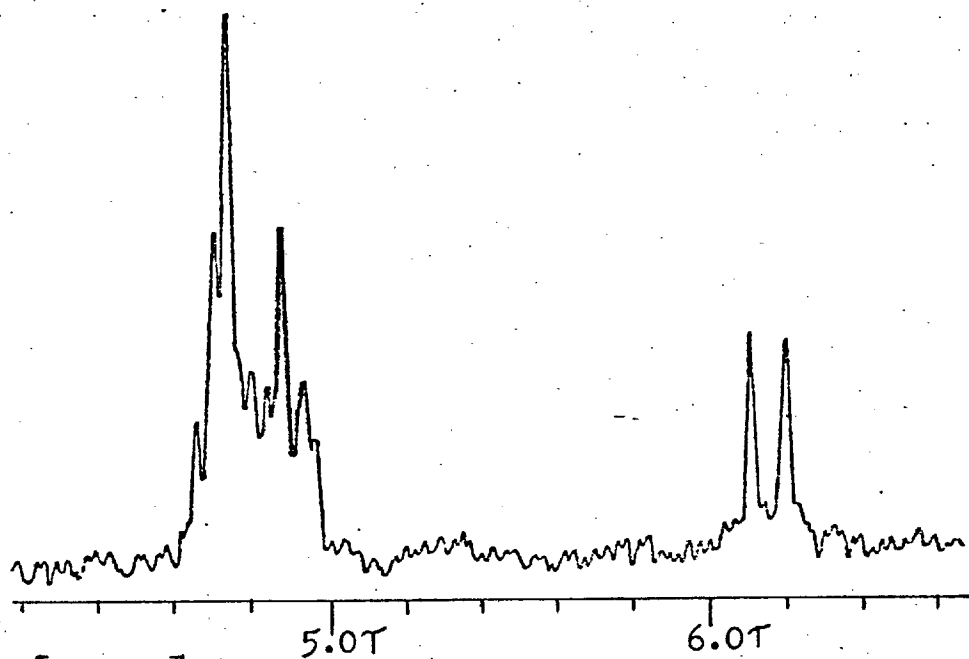
(V) Phenyl β -D-glucopyranoside tetra-acetate

Figure 2.

100 MHz. n.m.r. spectra

(VIII) Phenyl $[1-^2\text{H}_1]$ - β -D-glucopyranoside tetra-acetate(IX) Phenyl $[6,6-^2\text{H}_2]$ - β -D-glucopyranoside tetra-acetate

The two protons at C(6) might be expected to occur as a doublet coupled to the proton at C(5), but in actuality these three protons form an ABC system, because the CH_2 group is attached to an asymmetric centre, so that H_a and H_b become magnetically non equivalent. This system can be analysed as a first order one and the chemical shifts and coupling constants (without sign) are given in table 3.

Table 3

Proton	τ value	Coupling constant	Magnitude
H_a	5.69	J_{ab}	12.5 Hz
H_b	5.86	J_{ac}	5 Hz
H_c	6.16	J_{bc}	3 Hz
		J_{cd}	9 Hz

The remaining protons, $\text{H}_d, \text{H}_e, \text{H}_f$ and H_g are in the group at 4.6 - 5.1 τ and could only be resolved by spin decoupling. The integral trace over this multiplet shows that it is reduced from 4 protons to 3 protons in the spectrum of the $[1-^2\text{H}_1]$ isomer (VIII).

1,5 Mass Spectra of Phenolic Glycosides

These glycosides will be discussed first because the mass spectra are relatively simple, and have been investigated in some detail. Phenyl β -D-glucopyranoside tetra-acetate (V) (mass spectrum 7) is a typical example. The strongest peak, as in every case studied, is at m/e 43 (CH_3CO^+) and there are the two associated oxonium ions at m/e 103 and 145. A weak molecular ion is found at m/e 424 (0.004%) and the other peaks at the high mass end of the spectrum are due to the loss of an acetoxy group at m/e 365 (M-59, 0.03%) and the loss of the side chain from C(5) at m/e 351 (M-73, 0.04%).

The remaining peaks can be divided into two groups; those derived from the acetylated sugar moiety and the remainder arising from the aglycone. When the aglycone is a substituted phenol, the sugar peaks remain at the same positions in the mass spectrum and the peaks corresponding to the new aglycone (ROH) are superimposed upon them.

Peaks arising from the sugar moiety

These remain of constant relative intensities irrespective of the nature of the phenol that forms the aglycone. Some small differences in relative intensities are observed when glycosides contain different isomeric sugars and these will be discussed later.

There are three fragmentation sequences for the sugar moiety which correspond to series A and C, discussed previously, and to a new series which will be designated E.

Series A. This is the series of pyronium ions beginning with m/e 331, which decays to give peaks at m/e 271, 229, 211, 187, 169, 127, 109 and 81.

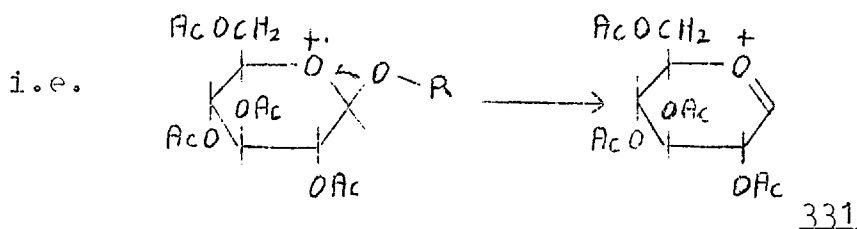


Table 4 shows the shifts that are observed for these peaks in the mass spectra of phenyl β -D-glucopyranoside tetra- $[\text{}^2\text{H}_3]$ -acetate (VI), *p*-nitrophenyl β -D-glucopyranoside 3,4,6-tri- $[\text{}^1\text{H}_3]$ -acetate-2- $[\text{}^2\text{H}_3]$ -acetate (VII), phenyl $[1\text{-}^2\text{H}_1]$ - β -D-glucopyranoside tetra-acetate (VIII) and phenyl $[6,6\text{-}^2\text{H}_2]$ - β -D-glucopyranoside tetra-acetate (IX). The deuterium content of these is greater than 98% at the specific sites except for compound VIII which has only 79% incorporation. The figures given in the table are corrected for the 21% ^1H at C(1). The metastable peaks given by compounds V, VI and VII are shown in table 5.

It is obvious from these figures that the fragmentation pathways are very complex. The deuterium labelling results can not be rationalised on the basis of simple losses of ketene and acetic acid as postulated by Biemann et al^(4,5) for methyl tetra-O-acetyl glycosides. In fact the results differ from those of Biemann in one important aspect. He found that the m/e 109 peak was split in the mass spectrum of the tetra- $[\text{}^2\text{H}_3]$ -acetyl compound to give peaks at m/e 109 and 110 of equal intensity. However the spectra

Table 4. The ions of series A

Compound				
V	VI	VII	VIII	IX
<u>331</u> 12.7	<u>343</u> 12.7	<u>334</u> 10.9	<u>332</u> 15.8	<u>333</u> 19.9
<u>271</u> 1.1	<u>280</u> 0.83	<u>274</u> 1.09	<u>272</u> 1.00	<u>273</u> 3.14
<u>229</u> 1.1	<u>235</u> 0.69	<u>229</u> 0.69	<u>230</u> 1.17	<u>231</u> 2.15
	<u>236</u> 0.26	<u>230</u> 0.21		
		<u>232</u> 0.57		
<u>211</u> 0.9	<u>217</u> 0.85	<u>211</u> 0.49	<u>212</u> 0.89	<u>213</u> 2.94
		<u>214</u> 0.73		
<u>187</u> 1.1	<u>191</u> 0.33	<u>187</u> 0.61	<u>188</u> 1.1	<u>189</u> 2.20
	<u>192</u> 0.46	<u>188</u> 0.67		
<u>169</u> 36.0	<u>172</u> 27.6	<u>169</u> 38.8	<u>170</u> 38.4	<u>171</u> 72.8
	<u>173</u> 12.6	<u>170</u> 10.9		
		<u>172</u> 3.5		
<u>127</u> 11.1	<u>128</u> 5.2	<u>127</u> 6.8	<u>128</u> 11.4	<u>129</u> 18.3
	<u>129</u> 4.2	<u>128</u> 6.9		
<u>109</u> 31.4	<u>109</u> 34.6	<u>109</u> 40.6	<u>110</u> 31.6	<u>111</u> 54.5
<u>81</u> 4.0	<u>81</u> 3.4	<u>81</u> 4.7	<u>82</u> 4.0	<u>83</u> 5.2

Intensities of ions are given as percentage of height of the base peak which is m/e 43, except for VI when it is shifted to m/e 46.

Table 5. Metastable peaks in the mass spectrum of phenyl β -D-glucopyranoside tetra-acetate and two trideuterioacetyl analogues.

Compound V

Metastable	Transition	Height*
70.3	169 $\longrightarrow$ 109	L
73.1	145 $\longrightarrow$ 103	M
86.3	187 $\longrightarrow$ 127	L
95.4	169 $\longrightarrow$ 127	L
105.4	271 $\longrightarrow$ 169	M
124.8	229 $\longrightarrow$ 169	S
135.4	211 $\longrightarrow$ 169	L
152.8	229 $\longrightarrow$ 187	S
158.4	331 $\longrightarrow$ 229	S
164.1	271 $\longrightarrow$ 211	S
222.0	331 $\longrightarrow$ 271	L

Compound VI

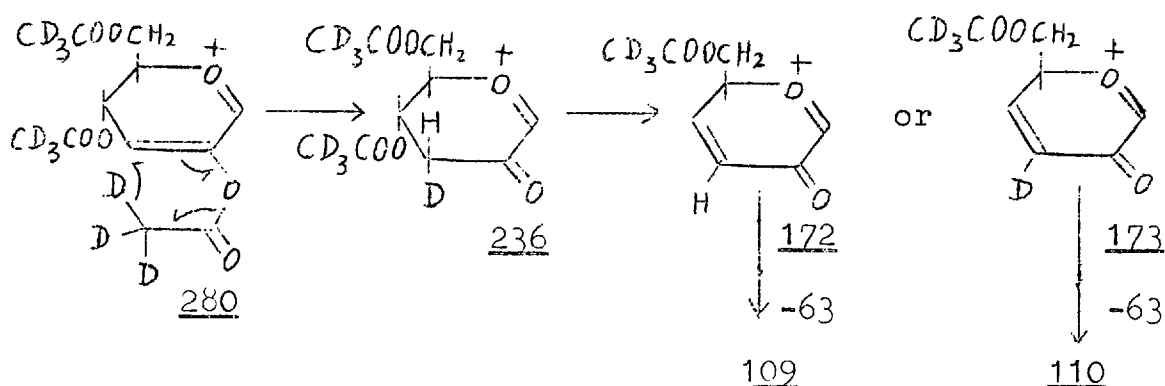
69.1	172 $\longrightarrow$ 109	L
85.9	191 $\longrightarrow$ 128	M
86.8	192 $\longrightarrow$ 129	S
95.2	172 $\longrightarrow$ 128	M
96.1	173 $\longrightarrow$ 129	M
105.8	280 $\longrightarrow$ 172	M
138.0	217 $\longrightarrow$ 173	L
228.2	343 $\longrightarrow$ 280	L

Compound VII

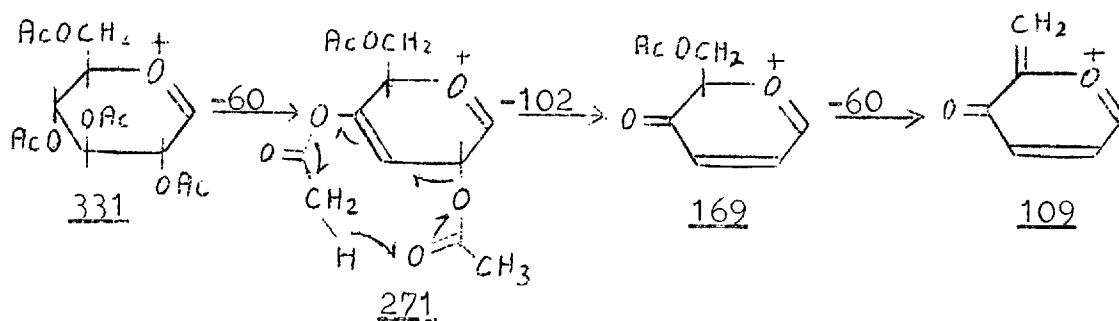
70.2	169 $\longrightarrow$ 109	L
95.4	169 $\longrightarrow$ 127	M
96.4	170 $\longrightarrow$ 128	M
104.2	274 $\longrightarrow$ 169	M
224.5	334 $\longrightarrow$ 274	L

* S=small, M=medium, L=large

of the trideuterioacetylated compounds which are described in this work show that m/e 109 ($C_6H_5O_2^+$) is not shifted, even partially to m/e 110. The shift to m/e 110 was said to be caused by the transfer of a deuterium atom to the sugar ring when ketene was expelled. The subsequent elimination of acetic acid resulted in either abstraction of an hydrogen atom or a deuterium atom.



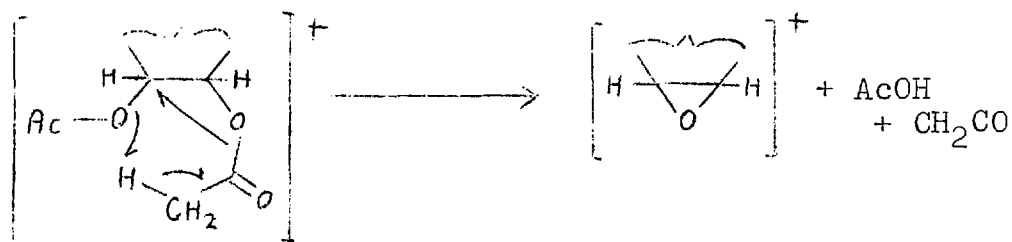
To explain the results presented in this thesis, ketene must be expelled without transfer of a proton to the sugar ring. The only other place that it can be transferred to is an acetoxy group in a concerted loss of acetic acid and ketene. A metastable ion at m/e 105.4 corresponds to a loss of 102 mass units from m/e 271 in the undeuteriated compound. Therefore the following mechanism has been proposed as the route to m/e 109.⁽⁹⁾



Molecular models show that such a mechanism is possible.

Subsequently Heyns and Müller observed a similar concerted loss of 102 mass units in the mass spectrum of penta-acetyl glucose,⁽⁸⁾ and the slightly more elegant mechanism for the loss which is described on page 21 was postulated.

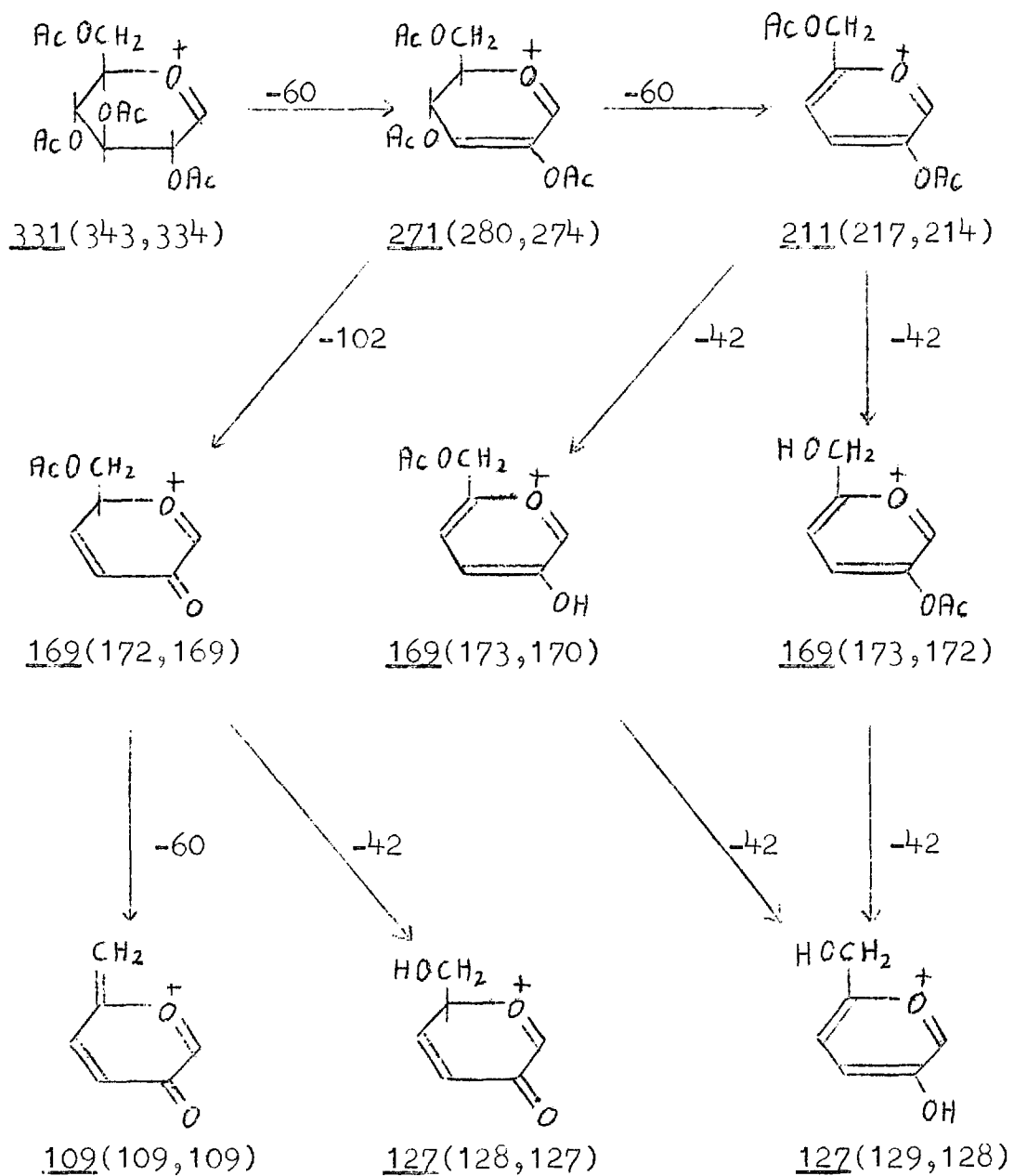
A second ion which must be formed, or partially formed, by a one step expulsion of 102 mass units is that at m/e 229. In the trideuterioacetate (VI) this is shifted mainly to m/e 235, whereas elimination of acetic acid and ketene in discrete steps would result in a shift to m/e 236. In addition, there is a weak metastable peak at m/e 158.4 in the mass spectrum of the undeuteriated compound, corresponding to the transition $331 \rightarrow 229$. In this case there can be no allylic rearrangement and the following mechanism for the process is therefore postulated.



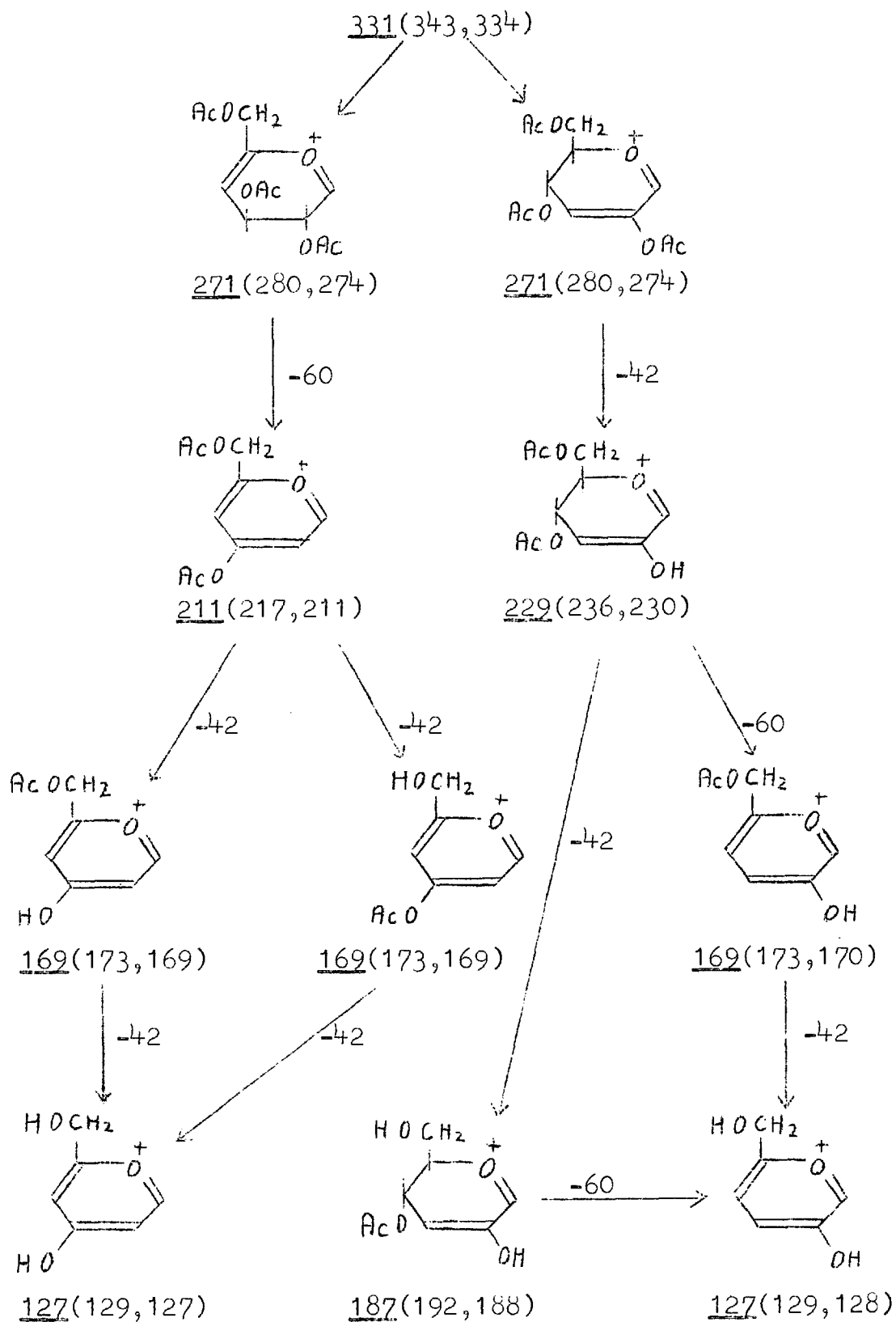
Scheme 2 shows a fragmentation sequence which accommodates all of the information gained from the deuterium labelling experiments. The main route is that which gives the ion at m/e 109 and is the same as that proposed by Heyns and Müller (see left hand pathway of scheme on page 20) for the decomposition of m/e 331. However, their second route (right hand pathway on page 20) does not occur because it would

Scheme 2.

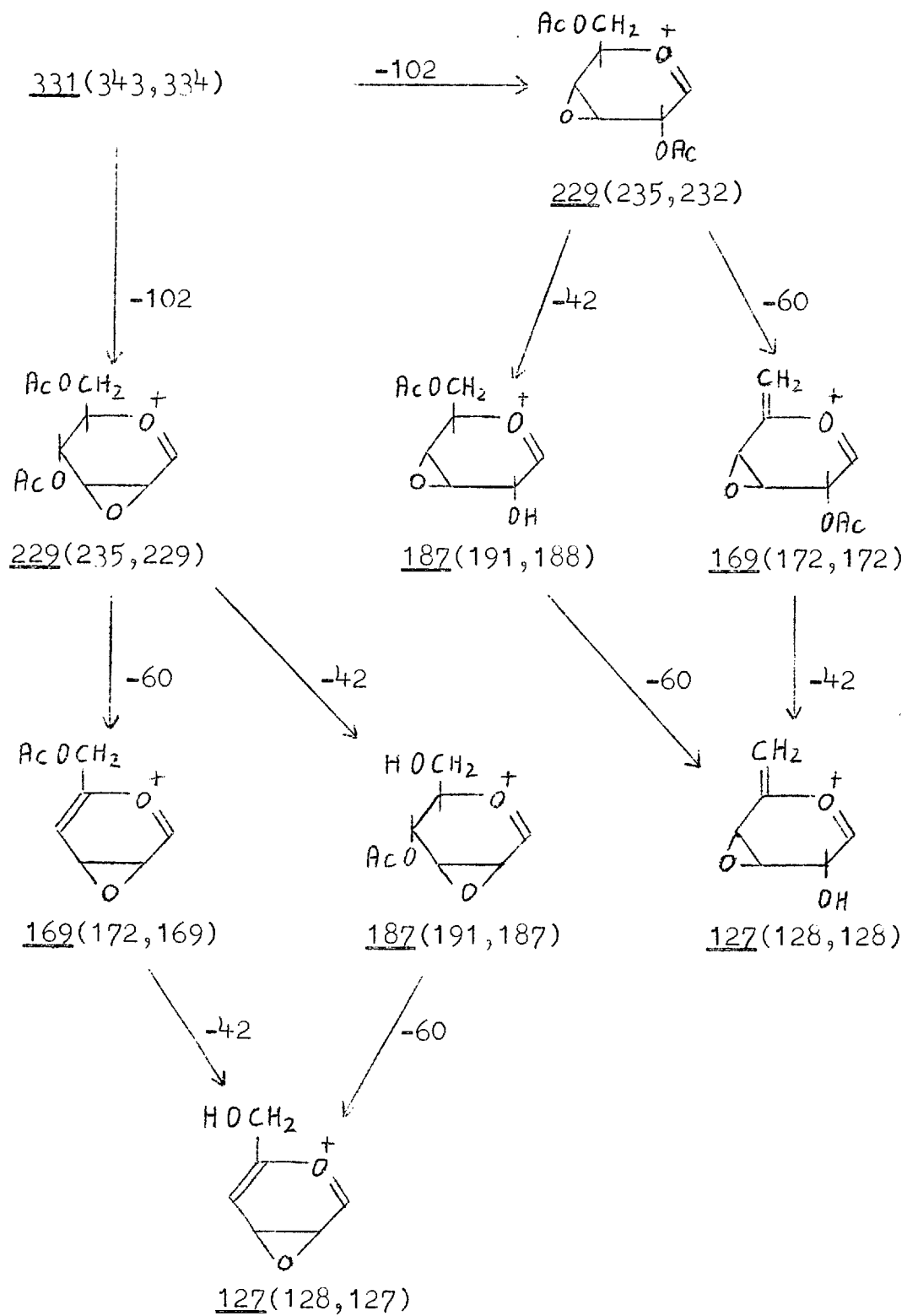
The m/e values are for compounds V, VI and VII respectively.



Scheme 2 (Cont.).

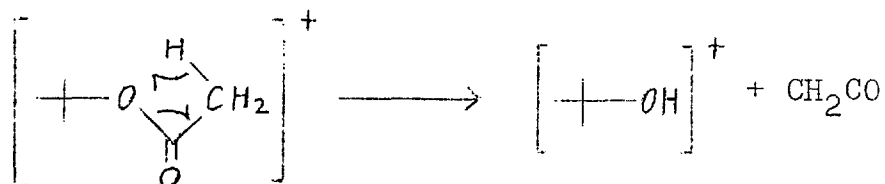


Scheme 2 (Cont.).

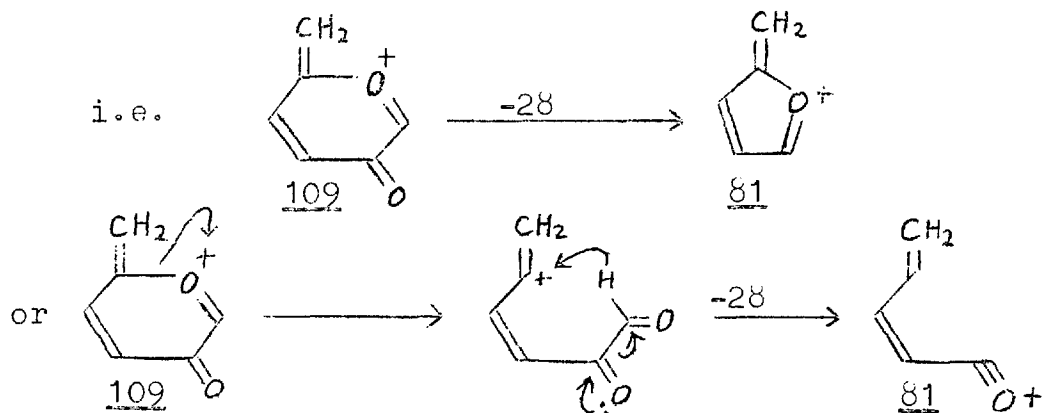


require compound VII to give an ion at m/e 271 and alternative schemes involving loss of the acetoxy group from C(4) are postulated as leading to m/e 211. It should be noted that ions of mass 173 formed by compound VI can not lose acetic acid without prior rearrangement, whereas ions of mass 172 can lose acetic acid readily to form m/e 109.

In addition to the ions which are formed according to scheme 2, there are also ions at m/e 289 and 81 which belong to series A. Loss of ketene from m/e 331 gives a very small peak at m/e 289 and since there is no vinyl acetate group in m/e 331, this must be by a four membered transition state.



There is no metastable peak due to the formation of m/e 81 ($\text{C}_5\text{H}_5\text{O}^+$) but its elemental composition and deuterium labelling results indicate that it is most probably formed by loss of carbon monoxide from m/e 109.

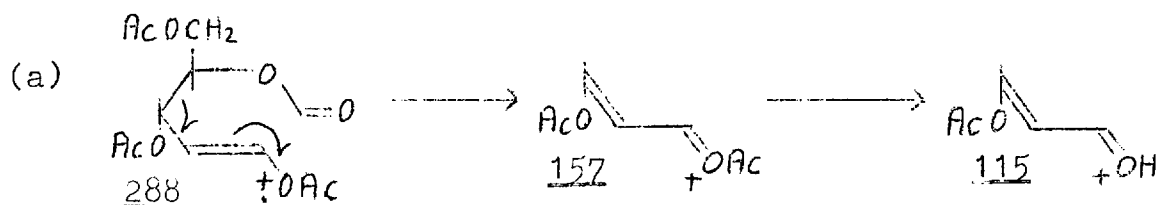


Series C. In the mass spectra of tetra-acetyl phenolic glycosides the only peaks observed in this series are at m/e 73 and 115. The m/e 157 peak is never present. The masses of the series C ions formed by the four deuteriated glycosides are shown in table 6.

Table 6.

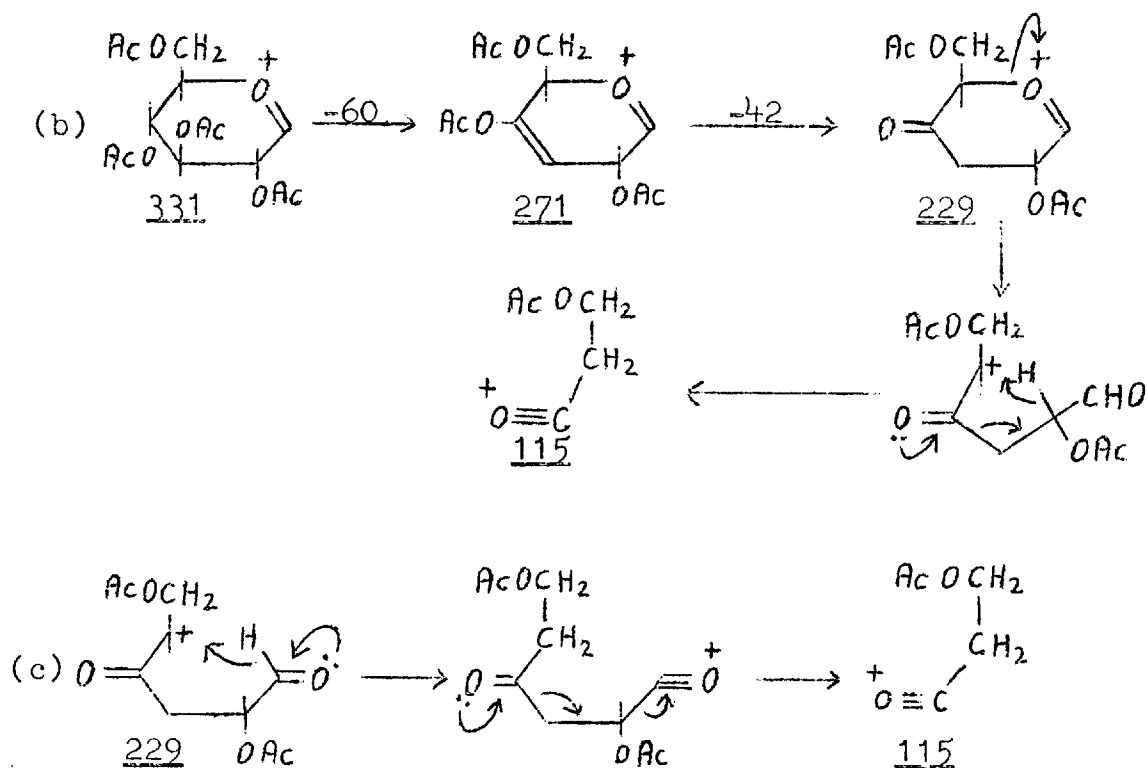
V	VI	VII	VIII	IX
<u>115</u> 2.9	<u>118</u> 1.6	<u>115</u> 3.2	<u>115</u> 2.4	<u>115</u> 1.3
	<u>119</u> 1.0	<u>116</u> 1.2	<u>116</u> 1.3	<u>117</u> 2.6
<u>73</u> 1.2	-	-	<u>73</u> 0.9	-
<u>139</u> 3.1	<u>142</u> 2.8	<u>139</u> 3.5	<u>140</u> 3.3	<u>139</u> 3.7
<u>97</u> 3.7	<u>98</u> 3.1	<u>97</u> 3.9	<u>98</u> 4.0	<u>97</u> 4.5

The mechanism of Heyns and Müller (page 22) can only be operating to form, at the most, 30% of the ions at m/e 115 because this is the extent to which m/e 115 is shifted to m/e 116 in the mass spectrum of VII and remains at m/e 115 in the spectrum of IX.



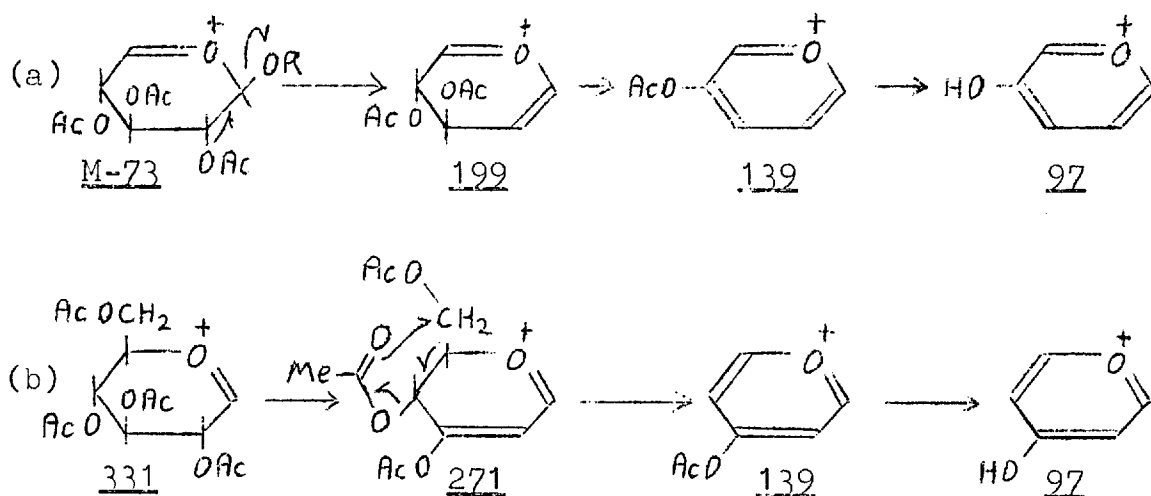
Any mechanism which involves formation of m/e 115 by loss of ketene from m/e 157 will give a shift of the ion m/e 115 to m/e 119 in the mass spectrum of VI and to m/e 116 in the spectrum of VII. Therefore the remaining ions of mass 115 must be formed from a different precursor and

must contain the two protons attached at C(6). Also, approximately half of the remaining ions must contain a proton that was attached to C(1). The following two mechanisms, each resulting in about 35% of the total number of ions of mass 115, explain the deuteration results.



The preferred primary fission of penta-acetyl glucose is between C(1) and C(2) so that fragmentation series A is not present to a large extent and the ion m/e 115 is mainly formed from the m/e 288 ion by mechanism (a). The primary fission of a phenyl tetra-acetyl glucoside is mainly of the C(1) - O Aglycone bond leading to fragmentation series A. Therefore the m/e 115 ions arise mainly as degradation products of the m/e 331 ion by mechanisms (b) and (c).

Series E. This consists of the two peaks at m/e 139 ($C_7H_7O_3^+$) and m/e 97 ($C_5H_5O_2^+$) and the shifts of these peaks in the mass spectra of the four deuteriated compounds are shown in table 6. The series E ions have lost the side chain from C(5) and the aglycone from C(1) and the acetate group from C(2). Therefore the following two mechanisms are possible.



Effect of stereochemistry of sugar. The abundance of ions of m/e 109, 127, 169 and 331 for some tetra-acetyl glucopyranosides and tetra-acetyl galactopyranosides is shown in table 7. Note that the intensities are expressed as a percentage of the total peak heights. The ion at m/e 331 is normally much stronger for galactosides. The acetoxy group that is lost first is the one at C(3) and in galactosides only the proton at C(2) is suitably orientated for abstraction whereas glucosides have protons at C(2) and C(4) which are in a cis relationship to the acetoxy group.

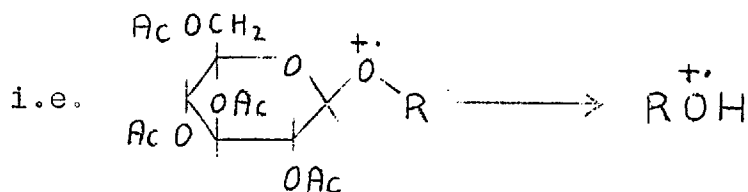
Table 7.

Tetra-acetyl- β -galactopyranosides	% $\sum_{40}^{M^+}$			
Aglycone	109	127	169	331
4-methyl-7-hydroxycoumarin	9.7	4.6	14.6	13.1
<u>p</u> -nitrophenol	9.2	4.7	13.1	10.7
<u>o</u> -nitrophenol	8.6	4.4	11.2	6.1
6-bromo-2-naphthol	8.8	4.9	14.1	11.6
Tetra-acetyl- β -glucopyranosides				
<u>o</u> -nitrophenol	12.7	4.5	17.0	3.3
<u>o</u> -hydroxybenzyl acetate	13.5	4.3	17.7	5.4
phenol	12.3	4.4	14.1	5.0
<u>p</u> -bromophenol	13.9	4.7	17.2	8.4
2-naphthol	12.7	5.9	17.9	5.6

Therefore the ion at m/e 331 formed by glucosides can decompose more readily than the ion of same mass formed by galactosides. Conversely, the m/e 109 ion and the m/e 169 ion are more abundant in the mass spectra of glucosides than in the mass spectra of galactosides.

The Origin of the Phenol Ion; ROH^+

The second most important fragmentation of phenolic tetra-acetyl glycosides is that which gives a phenol ion corresponding to the aglycone.



The interesting point about this process is the origin of the hydrogen atom in ROH^+ . Consideration of the mass spectrum of phenyl β -D-glucopyranoside tetra-acetate (V) and its three deuteriated analogues has helped to resolve this point. For the undeuteriated compound the phenol ion is found at m/e 94 ($\text{C}_6\text{H}_6\text{O}^{+}$) and shifts of m/e of this ion in the spectra of the deuteriated compounds are given in table 8.

Table 8.

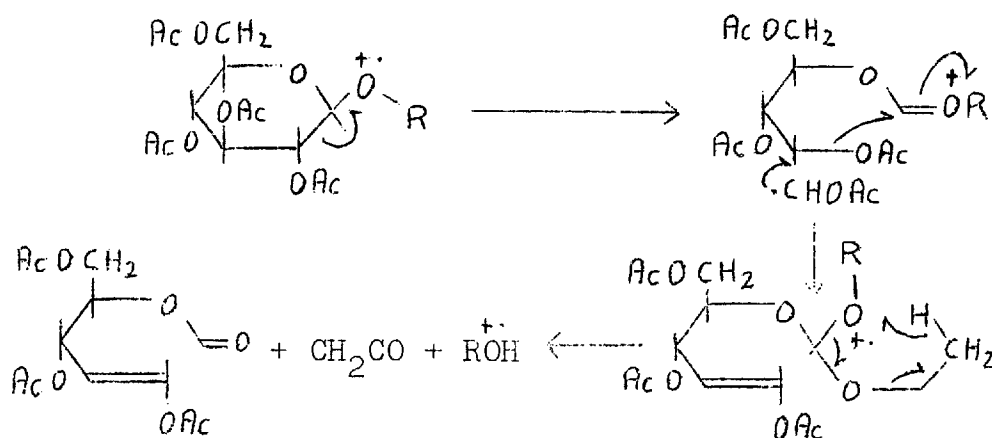
	Intensity as % of m/e 43*		% shift to m/e 95	Calculated shift for random abstraction
Compound	m/e 94, m/e 95			
V	9.6	0	0	0
VI	1.7	6.4	79%	63%
VIII	10.0	0.5	5%	5%
IX	11.7	0.4	3%	11%

* Corrected for ^1H at site of deuteriation and for ^{13}C isotope peaks.

The figure of 79% for the shift of the phenol ion to m/e 95 in the spectrum of the trideuterioacetylated compound (VI) is rather larger than would be expected if the hydrogen atom is abstracted randomly from the sugar moiety. Also the small shifts observed for the other two compounds VIII and IX are the same as, or below the figure for random abstraction. The last two results are within experimental error for zero abstraction and therefore the proton most probably comes from an acetate methyl group.

Unfortunately, *p*-nitrophenyl β -D-glucopyranoside 3,4,6-tri- $[^1\text{H}_3]$ -acetate-2- $[^2\text{H}_3]$ -acetate (VII) does not give any phenol ions and is therefore of no help in finding out which acetate group donates the hydrogen atom.

Heyns and Müller⁽⁸⁾ in their study of penta-acetyl glucose showed that the migration of an acetoxy group from C(3) to C(1) readily occurred. If it is assumed that this takes place in the electron impact induced fragmentation of phenyl tetra-acetyl glucosides, then the following mechanism for the formation of the phenol ion is attractive.

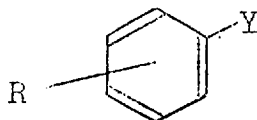


Effect of the Substituent on the Aglycone

A weak molecular ion is detected for all of the phenolic tetra-acetyl glycosides studied, except those derived from nitrophenols, when the highest mass peak detected corresponds to loss of NO from the molecular ion. The nitrophenyl glycosides are also peculiar because there is no ROH peak in the mass spectrum and no other peaks derived from the aglycone. All of the other glycosides have an ROH peak and peaks due to the further fragmentation of ROH. Table 9 shows

the ion current carried by ions arising from the aglycone as a percentage of the total ion current.

It will be noted that the total percentage fraction (P) of ion current carried by ions derived from the aglycone varies according to the compound. Therefore an attempt has been made to correlate P with the effect of the of the substituent attached to the phenol. In 1937, L.P.Hammett formulated an equation (later known as the Hammett equation) which related the reactivity of the side chain (Y) attached to a benzene ring to the substituent (R) on the ring.⁽⁶⁹⁾



$$\log k - \log k_0 = \rho \sigma$$

Where σ is a constant for the substituent R,

ρ is a constant for the reaction under consideration,

k is rate (or equilibrium) constant for the aromatic compound substituted with R,

k_0 is rate (or equilibrium) constant for the aromatic compound when $R = H$.

This is fundamentally an empirical relationship although attempts have been made to put it on a theoretical basis. It was originally used to relate the acid dissociation constants of substituted benzoic acids, for which the reaction constant ρ was taken as unity for ionisation in water at 25°C. Later, its use was extended to give linear relationships

Table 9. Ions arising from the aglycone.

(a) Tetra-acetyl glucosides

Aglycone	Peaks and intensities as $\% \sum_{40}^M$	P
Phenol	<u>95</u> 0.4; <u>94</u> 3.4; <u>77</u> 0.9, <u>66</u> 0.2, <u>65</u> 0.4	5.3
<i>o</i> -cresol	<u>109</u> 0.3; <u>108</u> 3.6; <u>107</u> 1.0; <u>91</u> 1.0 <u>77</u> 0.5	6.4
<i>p</i> -cresol	<u>109</u> 0.4; <u>108</u> 5.1; <u>107</u> 1.6; <u>91</u> 0.7 <u>77</u> 0.5	8.3
<i>p</i> -bromophenol	<u>174</u> 1.8; <u>172</u> 1.8	3.6
<i>p</i> -methoxyphenol	<u>125</u> 0.8; <u>124</u> 7.5; <u>123</u> 1.3; <u>95</u> 0.3	9.9
<i>m</i> -acetoxyphenol	<u>152</u> 0.7; <u>111</u> 0.8; <u>110</u> 3.5; <u>82</u> 0.3	5.3
<i>p</i> -acetoxyphenol	<u>152</u> 0.9; <u>111</u> 0.8; <u>110</u> 6.4	8.1
<i>m</i> -hydroxyacetophenone	<u>136</u> 0.8; <u>121</u> 1.8; <u>93</u> 0.5	3.1
<i>p</i> -hydroxybenzaldehyde	<u>123</u> 0.9; <u>122</u> 1.0, <u>121</u> 1.4	3.3
<i>o</i> -hydroxybenzyl acetate	<u>166</u> 0.6; <u>135</u> 0.3; <u>107</u> 2.2; <u>106</u> 2.4 <u>91</u> 0.3; <u>78</u> 1.1, <u>77</u> 0.5	7.4
5-hydroxyindane	<u>135</u> 0.9; <u>134</u> 6.7; <u>133</u> 3.1; <u>117</u> 0.6 <u>116</u> 0.4; <u>105</u> 0.6; <u>91</u> 0.5	12.8
1-naphthol	<u>145</u> 0.8; <u>144</u> 7.2; <u>116</u> 1.1; <u>115</u> 2.6	11.7
2-naphthol	<u>145</u> 0.8; <u>144</u> 8.4; <u>116</u> 0.6; <u>115</u> 1.7	11.5
9-hydroxyanthracene	<u>195</u> 1.6; <u>194</u> 9.4; <u>193</u> 3.3; <u>178</u> 0.4 <u>177</u> 0.4; <u>176</u> 0.4; <u>166</u> 0.6; <u>165</u> 3.7	19.8
6-hydroxy-7-methoxy- coumarin	<u>193</u> 1.2; <u>192</u> 4.7; <u>191</u> 0.8; <u>178</u> 0.7 <u>177</u> 0.5; <u>164</u> 1.1; <u>149</u> 1.4	10.4

(b) Tetra-acetyl galactosides

4-methyl-7-hydroxy- coumarin	<u>178</u> 0.3; <u>177</u> 2.7; <u>176</u> 2.6; <u>148</u> 1.7 <u>147</u> 0.6; <u>119</u> 0.2; <u>105</u> 0.3; <u>91</u> 0.4	8.8
6-bromo-2-naphthol	<u>224</u> 3.3; <u>222</u> 3.7; <u>195</u> 0.4; <u>193</u> 0.4 <u>143</u> 0.7; <u>126</u> 0.9; <u>114</u> 0.6	10.0

for the effect of R on many reactions of the side chain Y and equilibria involving Y. These reactions and equilibria have been reviewed by Jaffé⁽⁷⁰⁾ who also tabulated the constant σ for a wide range of substituents. The Hammett σ is a summation of all the electronic effects of the substituent and has been derived experimentally as the mean result from a large number of reactions.⁽⁷⁰⁾ The Hammett relationship only holds for meta and para substituents. With an ortho substituent there is always a possibility of participation at the reacting site and an increase in reaction rate.

The reaction constant ρ can be either positive or negative. A negative ρ implies that the reaction is favoured by high electron density at the reacting side chain and vice versa.

It has been shown that the Hammett equation can be applied to certain physical phenomena as well as solution chemistry. Thus the carbonyl stretching frequencies in the infra-red spectra of some meta and para substituted acetophenones have been successfully related to the σ constants.⁽⁷¹⁾ Furthermore it has been shown that the ionisation potentials of substituted benzenes can be correlated with σ^+ values.⁽⁷²⁾ When the substituent has an unshared electron pair, the ionisation potential could be due to loss of an electron from the substituent or from the π molecular orbital. In these cases the ionisation potential is normally lower than that of a non-aromatic with

the same substituent so that the electron almost definitely is removed from the π orbital. It is interesting that a phenyl substituent has a greater influence on the ionisation potential than is reflected by the σ^+ value. Therefore because the Hammett equation can be applied to ionisation, which is a mass spectrometric process, it is not unreasonable to suppose that it can be extended to cover the effects of substituents on subsequent fragmentation of the ionised species.

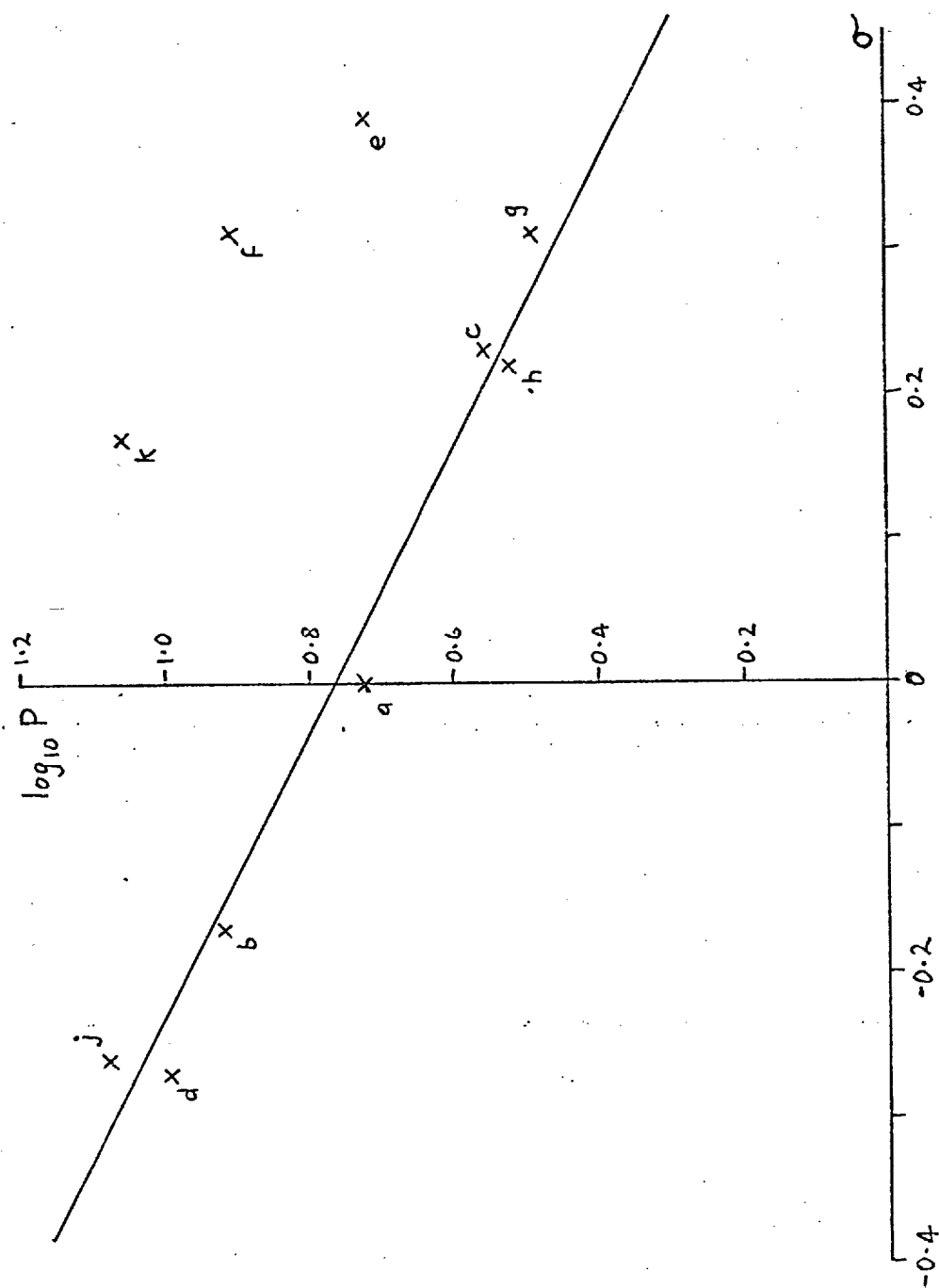
The percentage fraction (P) of the total ion current carried by ions derived from the aglycone in the mass spectra of phenyl tetra-acetyl glucopyranosides is related to σ constants in table 10. The results are presented in figure 3 as the graph of $\log_{10}P$ against σ according to the equation:

$$\log_{10}P - \log_{10}P_0 = \rho \sigma$$

There is a good correlation except for naphthyl and acetoxypheⁿyl glucosides. The σ constants for the meta and para acetox^y groups are obtained from a different source from the remainder.⁽⁷³⁾ They were only calculated from one chemical process, i.e. from the apparent dissociation constants of the substituted benzoic acids in 50% aqueous ethanol, and the generality of the values is open to doubt. Although the electron donating capacity of an acetox^y group is much less than that of a methox^y group, one would expect the σ constant to be slightly negative.

The Hammett equation does not hold for the polycyclic

Figure 3. Hammett plot for substituted phenolic tetra-acetyl glucosides.



Aglucone	P	$\log_{10} P$	σ^*	point on graph
phenol	5.3	0.724	0	a
p-cresol	8.3	0.919	-0.17	b
p-bromophenol	3.6	0.556	0.23	c
p-methoxyphenol	9.9	0.996	-0.27	d
m-acetoxyphenol	5.3	0.724	0.39	e
p-acetoxyphenol	8.1	0.909	0.31	f
m-hydroxyacetophenone	3.1	0.491	0.31	g
p-hydroxybenzaldehyde	3.3	0.519	0.22	h
5-hydroxyindane	12.8	1.079	-0.26	j
m-nitrophenol	0	-	0.71	-
2-naphthol	11.5	1.061	0.17	k

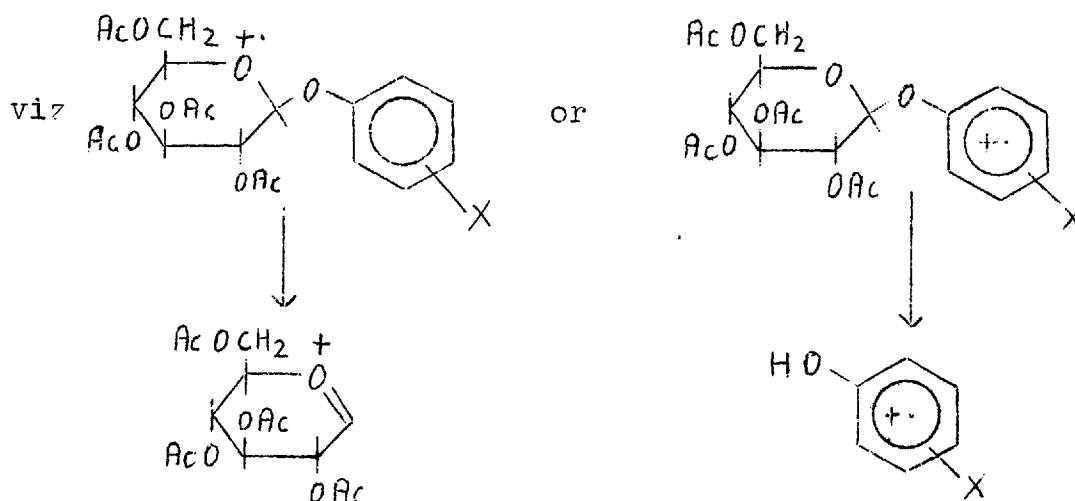
* σ values obtained from ref.70, except for e and f which come from ref.73.

aromatic glucosides that have been studied. As the number of rings increases from one, to two, to three, the value of P gets much larger (see table 9). However P is smaller when there is a bromo substituent on a naphthyl ring, than when it is unsubstituted, therefore an Hammett type relationship may apply for a series of substituted naphthalenes. The fact that a fused ring system has a larger effect on P than would be expected from the σ value is probably related to the greater influence of phenyl substituents on ionisation potential.⁽⁷²⁾

The effect of the substituent on P can be explained by considering the charge distribution over the glycoside molecule. For simplicity the charge will be considered as

being localised on the sugar heteroatom or on the benzene ring π orbital.

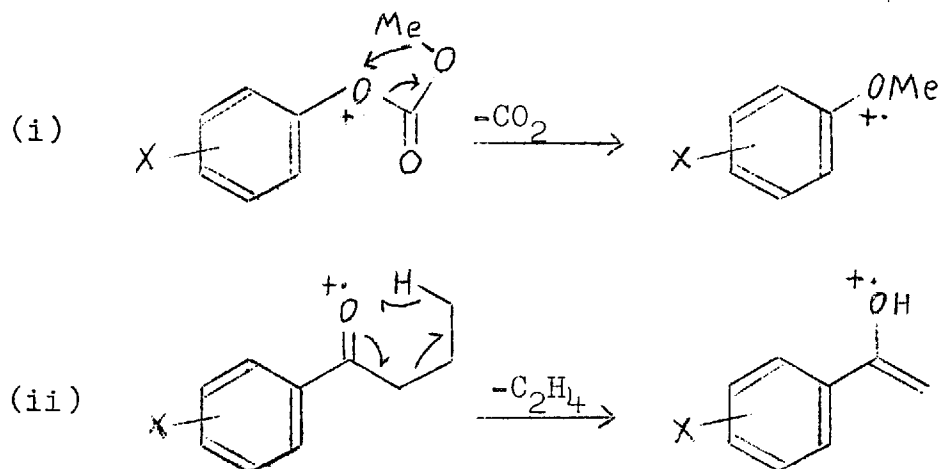
98.



Therefore any factor that promotes localisation of the charge on the benzene ring will tend to give a larger value for P . The negative slope of the graph in figure 3 is indicative of a negative reaction constant ρ and from this it can be deduced that P is larger when there is a higher electron density at the reacting site. In this case the reaction is ionisation which occurs in a region of high electron density so that it is possible to understand why the Hammett equation accounts for the observed values of P .

It is now possible to explain why nitrophenyl glycosides give no ions corresponding to the aglycone. The nitro group is strongly electron withdrawing so that the π molecular orbital of the benzene ring may be regarded as carrying a δ^+ charge. Thus the charge which is formed on the molecule by electron impact, is only localised on the sugar heteroatom and the molecule fragments accordingly.

More recently, several groups of workers have been applying Hammett plots to mass spectral processes in order to obtain information on the reaction centre. The loss of carbon dioxide from substituted methyl phenyl carbonates (i)⁽⁷⁴⁾ and the McLafferty rearrangement of substituted butyrophenones (ii)⁽⁷⁵⁾ both give reasonable correlations when σ is plotted against a function based on ion abundances.

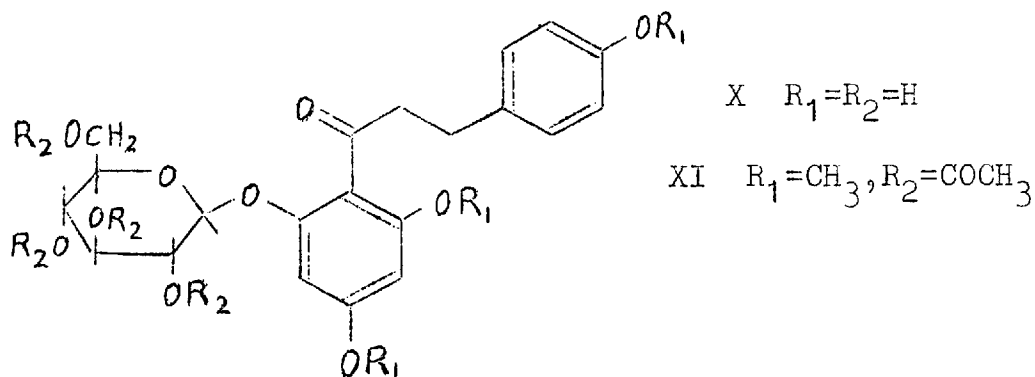


In both cases the reaction constant ρ is positive, showing that the rearrangement is facilitated by a lower electron density at the atom to which the group migrates. However, different conclusions were reached as to the significance of the positive ρ value in the two rearrangements. Thus Djerassi stated that in (i) the methyl migrated with the pair of bonding electrons,⁽⁷⁴⁾ whereas McLafferty postulates migration of an hydrogen atom in reaction (ii).⁽⁷⁵⁾

Johnstone believes that for a significant Hammett correlation, the σ function should be plotted against the

energy of activation of the reaction, i.e. the difference between the ionisation potential of the molecule and the appearance potential of the fragment ion.⁽⁷⁶⁾

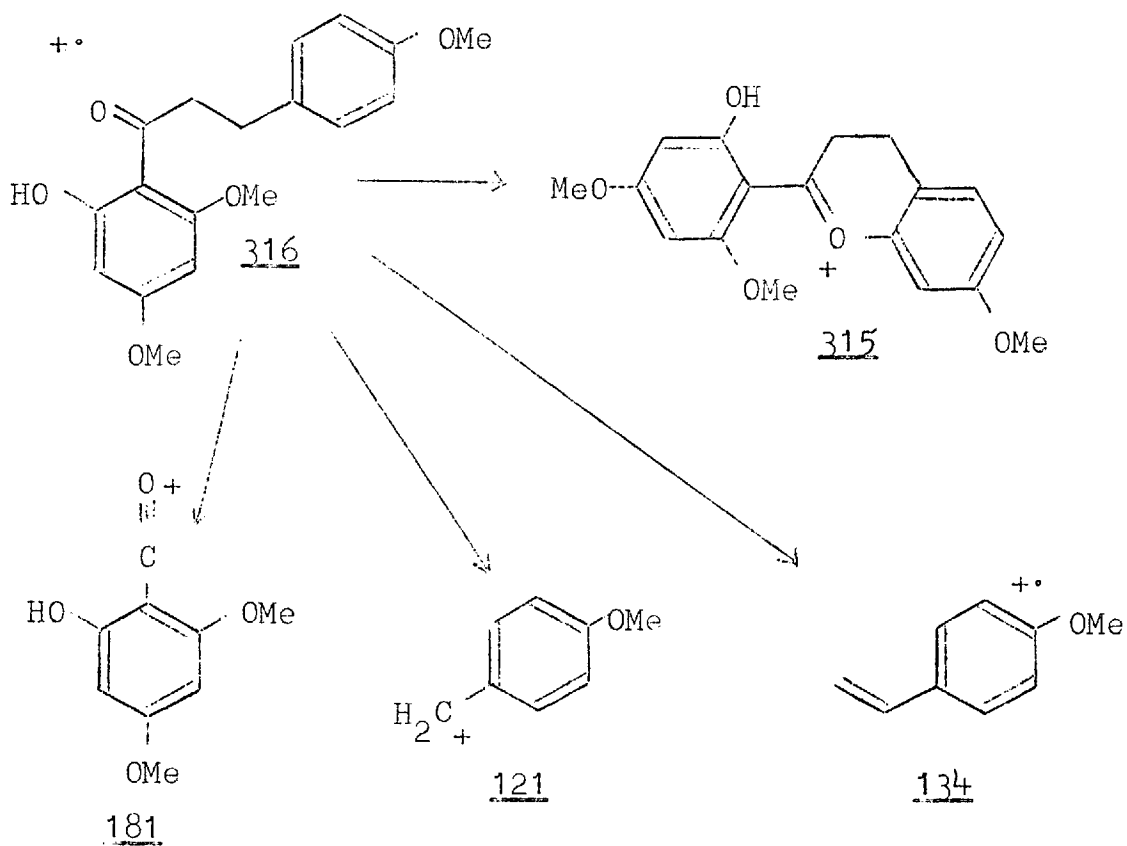
The mechanisms of fragmentation that have been elucidated for simple phenolic glycosides are applicable to more complicated glycosides of this general type. Phloridzin (X), which is isolated from apple tree root bark, will be considered as an example.



The mass spectrum of tri-O-methyl-tetra-O-acetyl-phloridzin (XI) is shown in mass spectrum 8. It consists of the peaks due to the sugar moiety with the spectrum of the aglycone, phloretin, superimposed upon them. The main fragmentations of the aglycone are shown in scheme 3.

When the mass spectrum of phloridzin itself, is obtained (mass spectrum 9), there are very few peaks which are assignable to the sugar residue. The spectrum largely contains peaks due to the aglycone, and the main ones correspond to ions in scheme 3 with appropriate shifts due to the lack of the O-methyl groups. In fact this provides

Scheme 3.



an excellent method for obtaining the mass spectrum of the aglycone without the need for hydrolysis.

1,6 Mass Spectra of Aliphatic Glycosides

This section deals with tetra-acetyl glucopyranosides derived from alcohols, as distinct from those derived from phenols. The fragmentation of phenolic glycosides follows the same pattern as fusicoccin but since fusicoccin does not have any characteristic ultra-violet absorption it can not have a structure of this type. Therefore the mass spectra of the glycosides listed in table 11 have been recorded to find out what structural features are necessary to produce a phenolic type of fragmentation.

Most of the peaks in the spectra are due to fragmentation of the sugar moiety, and eight separate series are discernible. These have been given code letters A to H and where applicable, are the same as those used for similar schemes at other places in this thesis. The percentage of the total ion current carried by ions of each series has been calculated and the results are given in table 12. From this it will be seen that the abundances of ions of certain series, particularly series E, varies considerably, according to the aglycone. With the aid of the high resolution measurements shown in table 13, the mechanisms of formation of the ions have been deduced.

Series A. This is the series of pyronium ions which was fully discussed in the section on phenolic glycosides, namely m/e 331, 271, 229, 211, 187, 169, 127, 109 and 81.

Table 11. List of aliphatic glucosides.

XII	Methyl α -D-glucopyranoside tetra-acetate (mass spectrum 10)
XIII	Methyl $[6,6-^2\text{H}_2]$ - α -D-glucopyranoside tetra-acetate
XIV	Ethyl β -D-glucopyranoside tetra-acetate
XV	Cyclohexyl β -D-glucopyranoside tetra-acetate
XVI	<u>Tert</u> -butyl β -D-glucopyranoside tetra-acetate (mass spectrum 11)
XVII	5,5-Dimethyl-3-oxocyclohex-1-en-1-yl β -D-glucopyranoside tetra-acetate
XVIII	Allyl β -D-glucopyranoside tetra-acetate
XIX	Cyclohex-2-en-1-yl β -D-glucopyranoside tetra-acetate
XX	Benzyl β -D-glucopyranoside tetra-acetate (mass spectrum 12)
XXI	Cholesteryl β -D-glucopyranoside tetra-acetate
XXII	Cyclohex-3-en-1-yl β -D-glucopyranoside tetra-acetate (mass spectrum 13)
XXIII	2-Hydroxyethyl β -D-glucopyranoside tetra-acetate
XXIV	1,3-dihydroxyprop-2-yl β -D-glucopyranoside tetra-acetate
XXV	<u>cis</u> -2-hydroxycyclohexyl β -D-glucopyranoside tetra-acetate (mass spectrum 14)
XXVI	<u>trans</u> -2-hydroxycyclohexyl β -D-glucopyranoside tetra-acetate
XXVII	<u>cis</u> -2-acetoxycyclohexyl β -D-glucopyranoside tetra-acetate
XXVIII	<u>trans</u> -2-acetoxycyclohexyl β -D-glucopyranoside tetra-acetate

Table 12. Total ion current carried by each series of ions formed by aliphatic glucosides.

Compound	% of total ion current carried by each series							
	A	B	C	D	E	F	G	H
XII	7.8	10.1	8.1	5.2	1.1	8.6	4.9	-
XV	7.0	19.4	14.4	2.9	2.1	1.0	2.1	-
XVI	8.7	18.6	14.0	2.3	3.1	0.6	1.2	-
XVII	40.6	1.7	1.9	2.0	3.4	-	-	-
XVIII	7.3	6.8	6.1	4.3	3.3	3.6	4.2	-
XIX	14.5	5.1	6.2	-	19.7	-	-	1.2
XX	4.7	5.5	6.1	-	14.3	-	-	5.4
XXI	6.0	4.5	7.7	-	-	-	-	-
XXII	25.0	5.1	5.7	1.3	9.3	0.4	0.4	-
XXIII	7.5	6.0	6.4	3.5	2.5	3.7	3.5	-
XXIV	14.5	4.2	4.8	3.2	1.7	1.8	2.2	-
XXV	23.0	10.0	9.8	1.8	4.3	0.5	1.7	-
XXVI	16.8	11.7	9.8	2.1	3.8	0.6	2.0	-
XXVII	16.1	16.1	10.3	6.3	3.3	1.7	3.6	-
XXVIII	14.7	10.1	6.7	3.1	2.6	0.6	2.4	-

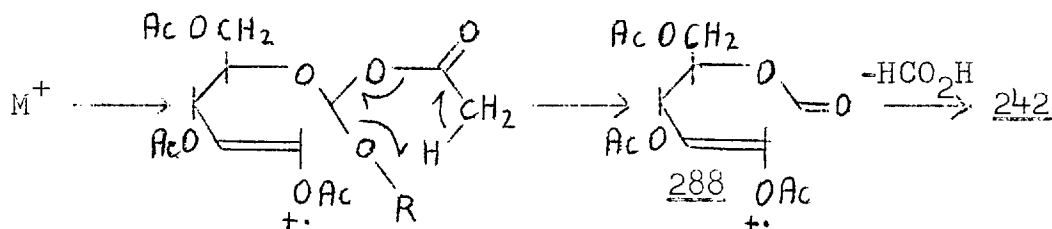
Series B. This consists of the ions at m/e 242, 200, 158, 140 and 98, for which two mechanisms of formation have been proposed in the literature. Heyns and Müller have suggested that m/e 242 is formed from m/e 288 according to the scheme shown on page 21, whereas Biemann believed that it was

Table 13. Mass measurements of ions in the mass spectra of aliphatic glucosides.

	m/e	Observed Mass	Calculated Mass	Formula
XII	144	144.0422	144.0423	$C_6H_8O_4$
	116	116.0471	116.0473	$C_5H_8O_3$
	112	112.0522	112.0524	$C_6H_8O_2$
	102	102.0317	102.0317	$C_4H_6O_3$
	74	74.0367	74.0368	$C_3H_6O_2$
	70	70.0419	70.0419	C_4H_6O
XX	152	152.0468	152.0473	$C_8H_8O_3$
	110	110.0365	110.0368	$C_6H_6O_2$
XIX	259	259.0819	259.0818	$C_{11}H_{15}O_7$
	245	245.0660	245.0661	$C_{10}H_{13}O_7$
	139	139.0395	139.0395	$C_7H_7O_3$

produced by loss of acetic acid from an ion of mass 302 according to the mechanism shown on page 15.

A peak is found at m/e 302 in the mass spectra of XII, XIV and XV but there are no metastable ions due to its decomposition and it is probably the precursor of series D. On the other hand, a peak is present in the mass spectra of XIV, XV and XVI at m/e 288 and there is a metastable peak at m/e 203.3 due to the fragmentation $288 \longrightarrow 242$. Therefore the mechanism of formation of m/e 242 is as follows.



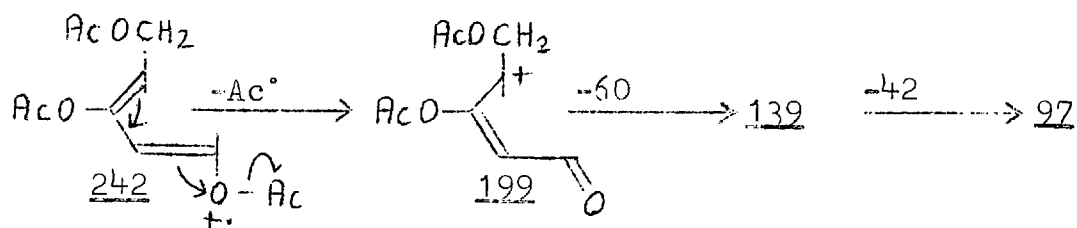
The metastable peak was only observed for these three compounds because ions of series B are much more abundant in the mass spectra of the saturated glycosides XIV, XV and XVI than the other glycosides.

Series C. The ions at m/e 157, 115 and 73 are formed by two competing mechanisms. The deuteriated glycoside XIII shows no shift of the m/e 157 ion to higher mass, but m/e 115 is shifted to m/e 117 to an extent of 30%. Therefore for the methyl glucoside, most ions of series C are formed from m/e 288 according to the scheme on page 22, but the alternative mechanisms described on page 87 are responsible for the formation of 30% of the ions at m/e 115 from m/e 229. It seems probable that the percentage of ions formed by each mechanism depends on which primary fragmentation is preferred. If it is the breaking of the C(1)-C(2) bond then most series C ions are formed from m/e 288, but if it is the loss of the aglycone then most of the series C ions will be formed from m/e 229. Thus the dimedone glucoside (XVII) does not have a m/e 157 ion and probably forms most of the m/e 115 ions by decomposition of m/e 229.

Series D. The peaks at m/e 243, 201, 183, 141 and 99 in the mass spectra of these glucosides are formed by the

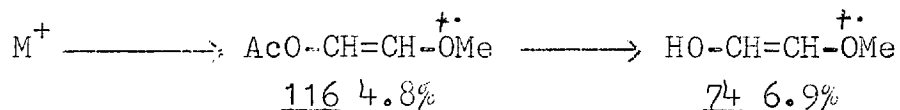
fragmentation of the m/e 302 ion as described on page 16.

Series E. With the exception of the mass spectra of compounds XIX and XX, the peaks of this series at m/e 199, 139 and 97 are not of very high intensity. There are three possible mechanisms that could lead to these ions, of which one has already been described on page 88 in connection with phenyl glucosides. There is a metastable peak at m/e 163.7 in the mass spectra of several of the glucosides and could be due to the formation of m/e 199 from m/e 242.



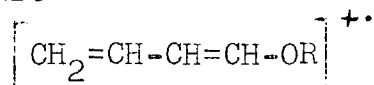
The third route for the formation of ions of series E is from m/e 347. This ion is weak in the mass spectra of most of the glycosides, e.g. XV and XVI, but for the two compounds which have very abundant ions of series E, i.e. XIX and XX, this ion becomes very prominent. Compounds XIX and XX also give ions of mass 259 and 245, and there are metastable peaks showing that these result from the decomposition of m/e 347. Therefore for glucosides XIX and XX the main pathway leading to m/e 139 and 97 is that shown in scheme 4.

In the initial step the aglycone is lost as a radical, so that this series occurs to a large extent in the mass spectra of those glycosides which can form a very stable

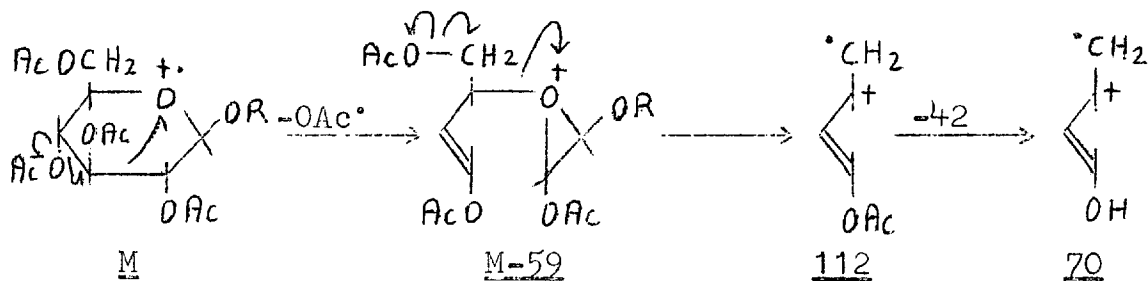


The ethyl glucoside (XIV) has a much smaller peak in its mass spectrum at m/e 88, $HO-\overset{+}{CH}=CH-OEt$, but none of the other glucosides give a corresponding peak.

Series G. This series is represented by ions at m/e 112 and 70, which are shifted to m/e 114 and 72 in the mass spectrum of the $[6,6-^2H_2]$ -glucoside (XIII). The elementary composition of these ions indicates that they probably have the structure:

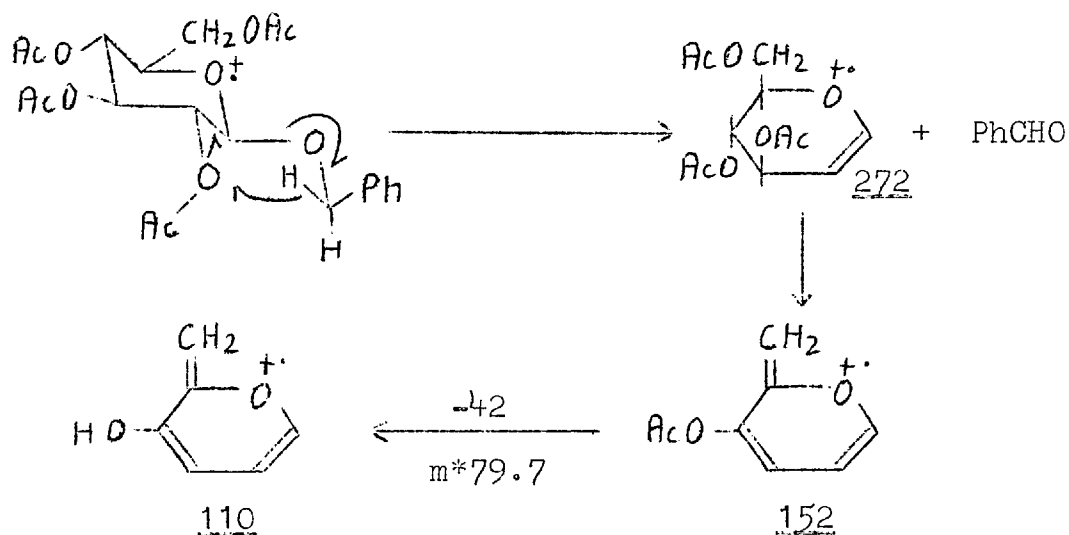


The CH_2 group comes from C(6), therefore the carbon chain is that which is present at C(3) to C(6) in the sugar ring. A satisfactory formal mechanism for the formation of these ions is rather difficult, but the following one does fit the experimental findings.



Series H. Ions of this series are only present in the mass spectra of the allylic glucoside XIX and the benzylic glucoside XX. Therefore allylic activation must be one of the factors responsible for promoting fragmentation of the glycosides to give peaks at m/e 272, 152 and 110.

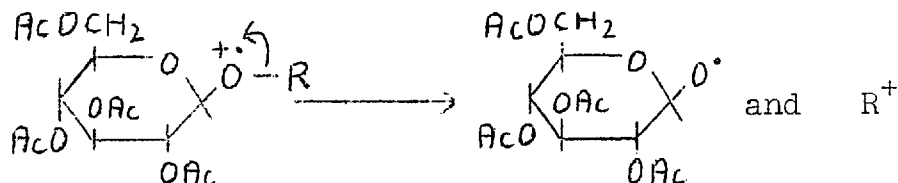
These ions are more abundant in the spectrum of XX and a possible mechanism for their formation is shown below.



There is also an ion at m/e 105, $\text{PhC}\equiv\text{O}^+$ (5.8% of $\sum_{40}^M$) in the mass spectrum of XX. When the charge is on the aglycone instead of the sugar moiety, a benzaldehyde ion would be formed by the above mechanism. Loss of an hydrogen atom from this would give the m/e 105 ion.

Fragmentations of the aglycone

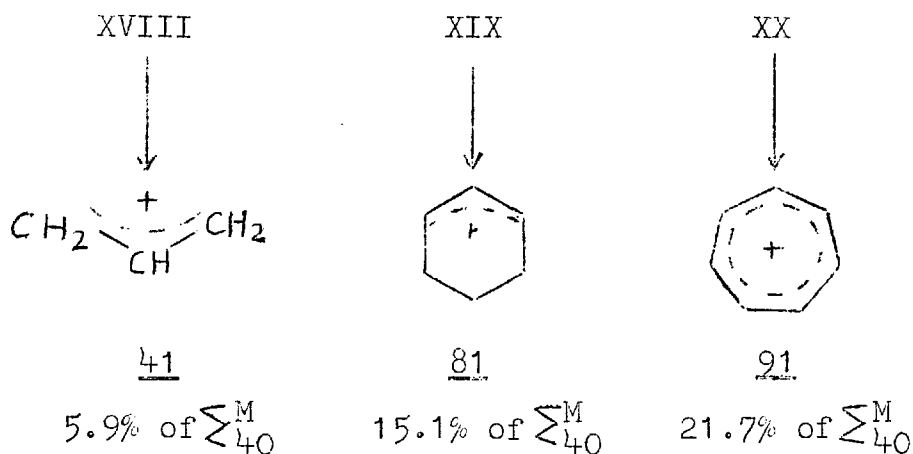
In the mass spectra of all of the aliphatic glucosides except XVII, the aglycone appears as the carbonium ion R^+ .



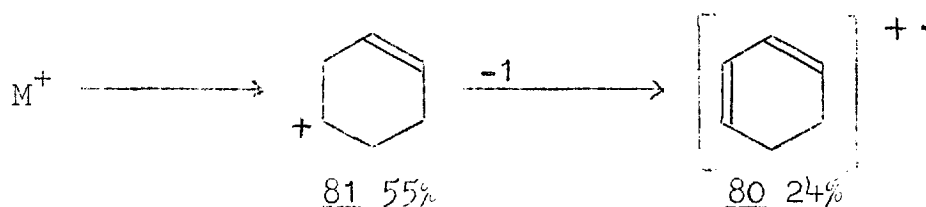
In the simple cases where there is little subsequent fragmentation of R^+ , the intensity of this ion can be

related to its stability. Thus the primary alkyl glucosides XII and XIV do not form an ion corresponding to R^+ , but the cyclohexyl glucoside XV, which is secondary, gives an ion at $m/e 83, C_6H_{11}^+$ which carries 3.2% of the total ion current. The tertiary glucoside XVI has a still more abundant ion in its mass spectrum at an m/e value of 57 ($C_4H_9^+$) which carries 9.4% of the total ion current.

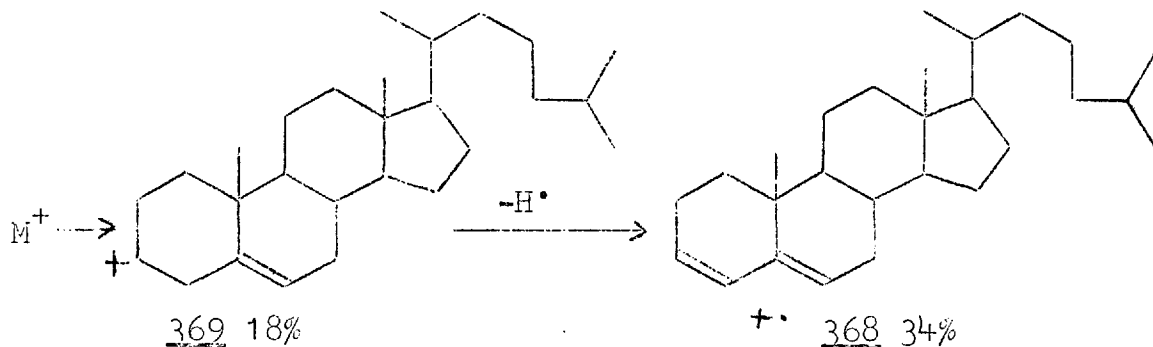
The R^+ ions formed from allylic and benzylic glucosides would be expected to be more stable and therefore more abundant than the aglycone ions formed from saturated glycosides. This proves to be so, except that the allyl carbonium ion is not so abundant as the tert-butyl ion.



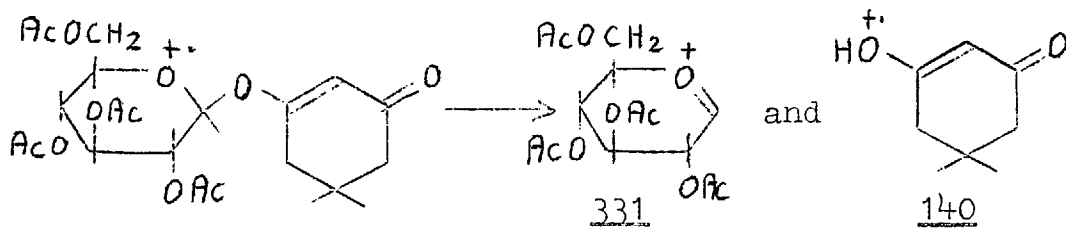
The Δ^3 -glucoside XXII forms a carbonium ion which readily loses one mass unit, giving ions at m/e 81 and 80.



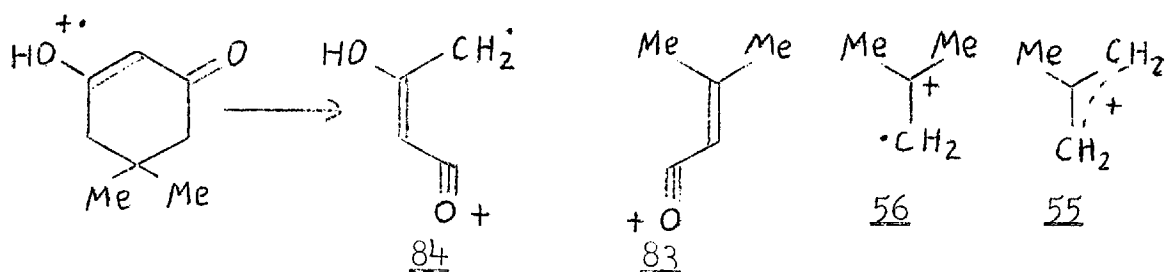
This tendency is more pronounced in the case of cholesteryl glucoside XXI, because the most abundant aglycone ion is at m/e 368. Fragmentation of this aglycone ion is then very complex and the fragment ions carry a large amount of the total ion current.



The enol glucoside XVII that is readily available from dimedone gives a mass spectrum which is very similar to those of phenolic tetra-acetyl glucosides. Thus most of the sugar ions belong to series A and the aglycone forms an ROH^+ ion.

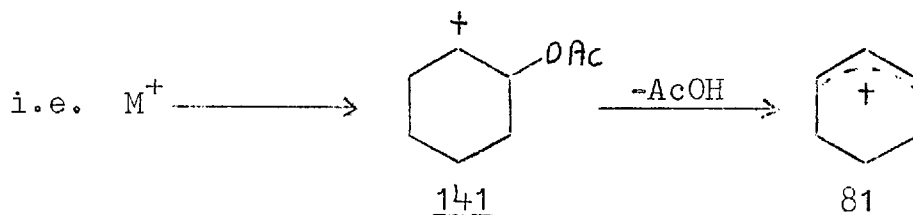


The decomposition of the ion m/e 140 is similar to that for the molecular ion of dimedone, giving ions at m/e 83, 84, 55 and 56.⁽⁷⁷⁾



It should be noted that of the O-glycosides studied, only those in which the glycosidic oxygen atom is attached to an unsaturated centre give an aglycone ion of the form ROH^+ .

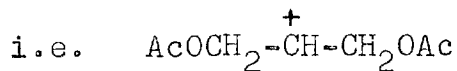
The glycosides derived from α -glycols give some aglycone ions that are of special interest. The fully acetylated compounds XXVII and XXVIII behave normally because they form a carbonium ion m/e 141 which then loses acetic acid to give m/e 81.



The abundances of these two ions in the spectra of the two isomers varies considerably. In the mass spectrum of the trans isomer (XXVIII) m/e 141 is the base peak, carrying 26% of the total ion current, and m/e 81 carries 9%. In the spectrum of the cis isomer (XXVII) m/e 43 is the base peak and the ions m/e 141 and 81 carry 5% and 7% of the of the total ion current, respectively.

The mass spectral fragmentations of 2-O- α -D-galacto-

pyranosyl-glycerol hexa-acetate have been published.⁽⁷⁸⁾ This compound gives the expected aglycone peak at m/e 159.

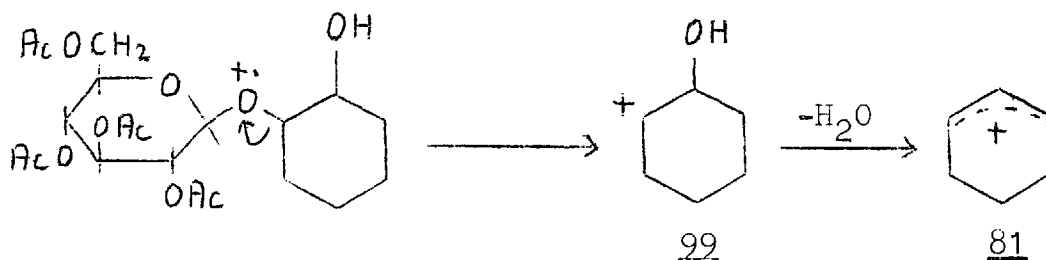


The main aglycone peaks in the mass spectra of the four glycosides with a free hydroxy group are given in Table 14.

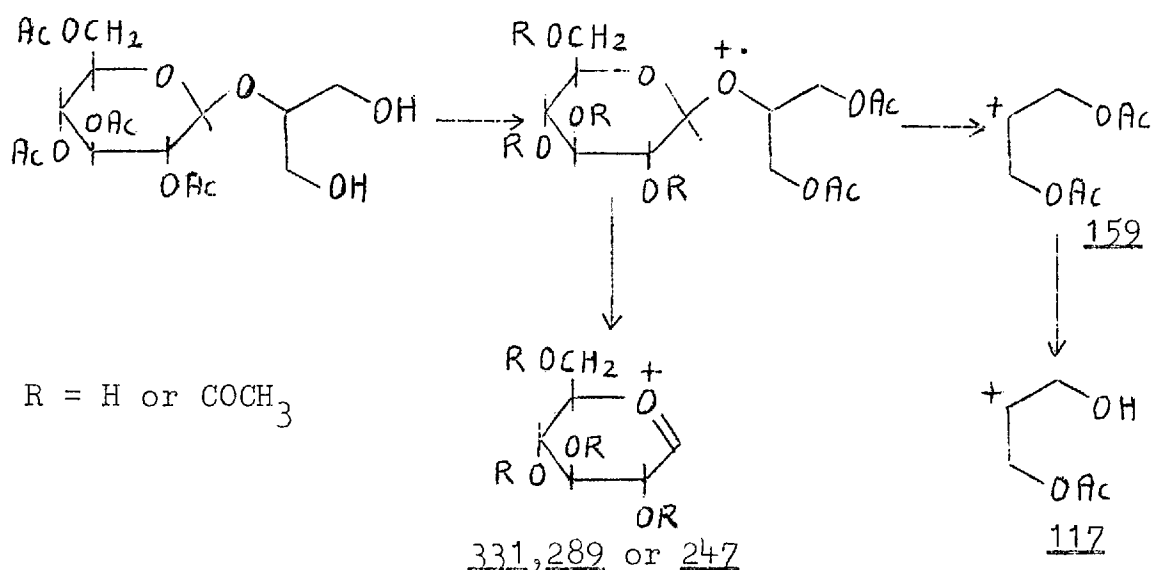
Compound		R-18	R	R+28	R+42	R+70	R+84
XXIII	m/e	-	45	73	87	-	-
	%*	-	1.4	13.3	2.5	-	-
XXIV	m/e	-	75	103	117	145	159
	%*	-	0	0.9	3.7	6.2	11.0
XXV	m/e	81	99	127	141	-	-
	%*	3.0	4.5	12.7	1.0	-	-
XXVI	m/e	81	99	127	141	-	-
	%*	4.4	1.8	3.8	1.2	-	-

* These percentages are $\% \sum_{40}^M$ and are corrected for any sugar ions at the same mass number.

The m/e 99 and 81 peaks in the mass spectra of the cyclohexanediol derivatives are formed by the usual mechanism.



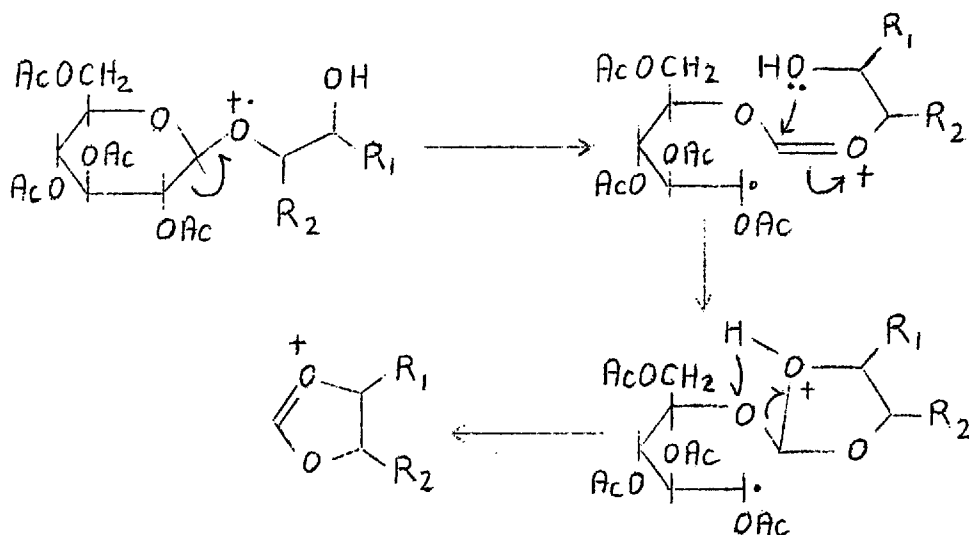
The $R+42$ and $R+84$ peaks have been mass measured for XXIV and the elementary formulae are $C_5H_9O_3$ and $C_7H_{11}O_4$. To explain this it is necessary to postulate an acetate migration from the sugar to the free hydroxy groups on the aglycone. It is not known whether this occurs before or after ionisation, or whether it is intermolecular or intramolecular.



If this mechanism is taking place, the parent ions of series A should be found that are deficient of acetoxy groups. This is the case, because the mass spectrum of XXIV has peaks at m/e 331, 2.0%; m/e 289, 1.1% and m/e 247, 1.9%, of which the latter two ions are not normal decomposition products of m/e 331. These are therefore primary fragments formed by tetra-acetyl, triacetyl and diacetyl glucopyranosides respectively.

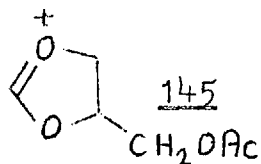
Any mechanism which explains the formation of the $R+28$

peaks must involve the α -hydroxy group, so that the following mechanism is suggested.



The initial step of C(1)-C(2) fission is a common primary fragmentation, but instead of migrating an acetoxy group to C(1) the hydroxy group is in a suitable position to attack C(1). The cis-hydroxy glucoside XXV gives a much larger R+28 peak than the trans glycoside XXVI because there is less strain inherent in a five membered ring cis fused to a six membered ring than there is in one which is trans fused.

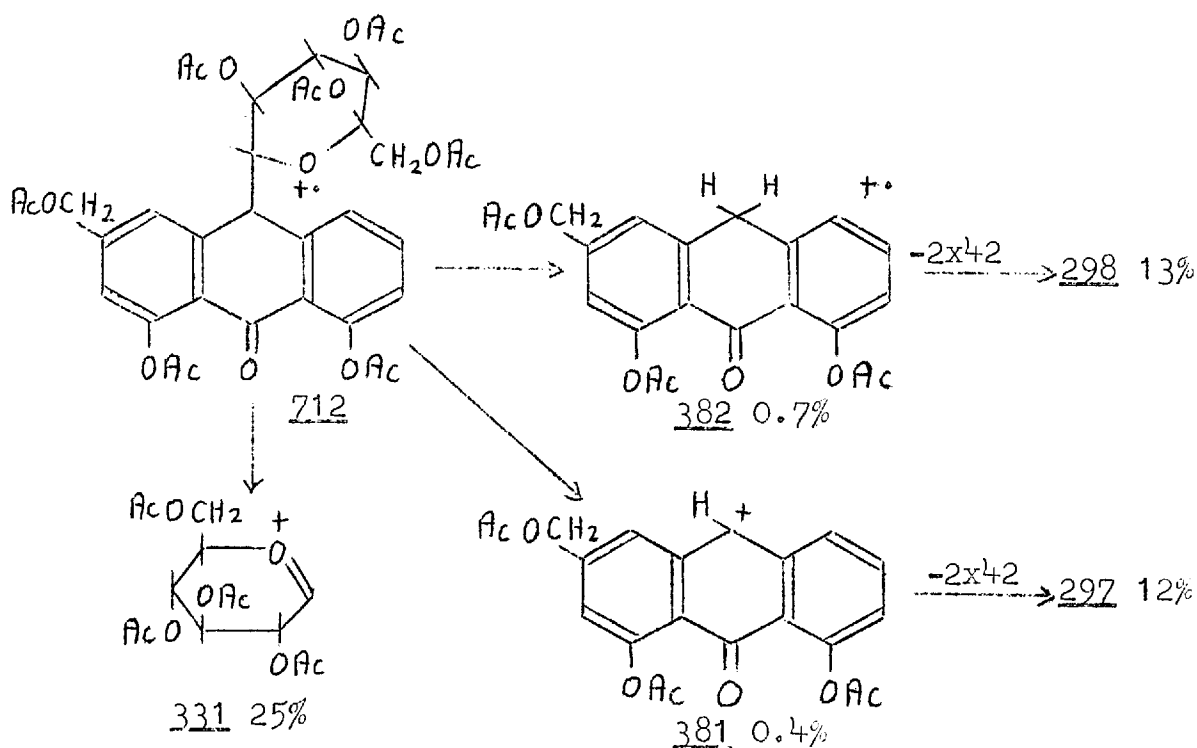
The R+70 ion in the mass spectrum of the glyceryl glucoside XXIV is obtained by a combination of both of the mechanisms outlined above, and has the structure:



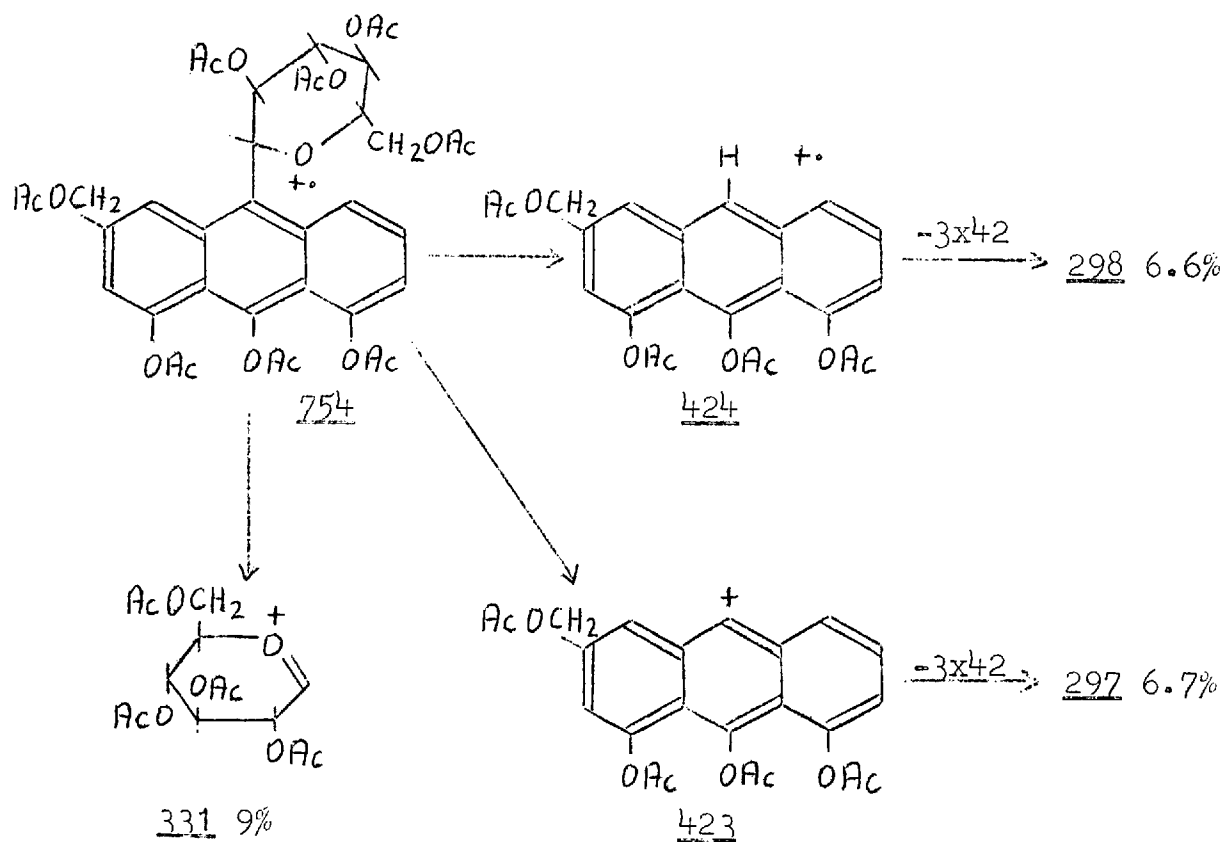
1,7 C-Glycosides

The mass spectra of some unacetylated C-glycosides⁽⁷⁹⁾ and a permethylated C-glycoside⁽⁸⁰⁾ have been published but the fragmentations of peracetates have not been studied. Barbaloin is a C-glycoside which is found in some species of aloe, in particular Aloe Ferox, and has found use as a purgative. Its structure was determined by Evelyn Hay and Haynes⁽⁸¹⁾ but there is a certain amount of confusion in the literature about the products of acetylation.

Acetylation of barbaloin with acetic anhydride and zinc chloride gives a mixture from which an amorphous hepta-acetate was isolated as the main product. It is not fluorescent and the infra-red spectrum has $\nu_{\max}(\text{CCl}_4)$ 1740, 1680, 1625 and 1610 cm^{-1} showing that the anthrone nucleus is preserved. The mass spectrum of this hepta-acetate has M^+ at 712 (0.05%) and there are no peaks of high intensity at high mass. The molecular ion decomposes in a similar way to those of phenolic O-glycosides giving sugar ions belonging mainly to series A. The aglycone appears as two ions at m/e 382 and 381, of which the latter is caused by simple benzylic fission. However the m/e 382 ion must involve transfer of an hydrogen atom from the sugar to the aglycone and this probably comes from an acetate methyl group in the same way as for O-glycosides. Therefore the mass spectrum of this compound could perhaps be easily mistaken for that of a phenolic O-glycoside.



Acetylation of barbaloin with acetic anhydride in pyridine gives a second amorphous product which is an octa-acetate. It has a strong blue fluorescence and $\nu_{\max}(\text{CCl}_4)$ 1750, 1640 and 1625 cm^{-1} , showing that it has an anthracene nucleus. The mass spectrum of the octa-acetate is quite different because the vinylic bond from the aromatic system to the sugar is not easily cleaved. Thus the molecular ion at m/e 754 (1.3%) is more intense and there are strong peaks at M-42-60 (m/e 652, 12%) and M-42-42-42 (m/e 628, 10%). No peaks of comparable intensity are observed at the high mass end of any of the other glycosides that have been studied. Splitting of the glycosidic bond occurs in the same manner as observed for the hepta-acetate.



1,8 4,6-O-Benzylidene Acetals of Glucosides

Cyclic acetals and ketals are very useful compounds for the carbohydrate chemist⁽⁸²⁾ and are important for two main purposes. Firstly they are useful protecting groups which are reasonably stable to alkali and secondly, the presence of another ring fused to the sugar ring holds it in a fixed conformation so that the reactivities of the groups can be related to their spatial arrangement.

The mass spectrometric behaviour of a number of isopropylidene derivatives of sugars has been studied and reviews of this have been published^(1,2) but little work has been reported on benzylidene acetals. Therefore as examples, the electron impact induced fragmentations of methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside and the phenyl β -glucoside analogue are discussed in this section.

Preparation

In general, a cyclic acetal fused to a six membered ring can be formed from a 1,3-glycol or a cis-1,2-glycol but not from a trans-1,2-glycol. The distance between the two hydroxy groups in a cyclohexane cis and trans-1,2-diol is almost the same, therefore this does not account for the difference in reactivity. Angyal and MacDonald⁽⁸³⁾ have suggested that the difference in stability of the transition state is responsible. To form the five membered dioxolane system, the two hydroxy groups must become almost

planar, and in the cis diol this results in a flattening of the six membered ring and a separation of the polar groups. If the hydroxy groups in a trans diol become planar then the cyclohexane ring becomes more puckered and the polar groups come closer together, increasing non-bonded interactions.

For α -glucopyranose the two sites most suitably orientated for acetal formation are the 1,2 and 4,6 positions, so that reaction of glucose with benzaldehyde and zinc chloride gives firstly 4,6-O-benzylidene-D-glucose and on further reaction it goes to 1,2:4,6-di-O-benzylidene- α -D-glucose.⁽⁸⁴⁾ The first stage in the reaction is the formation of a benzaldehyde-zinc chloride complex, but there is doubt as to whether, in solution, this is in the ratio 1:1 or 2:1. A crystalline 1:1 complex has been isolated and this is immediately decomposed by water.

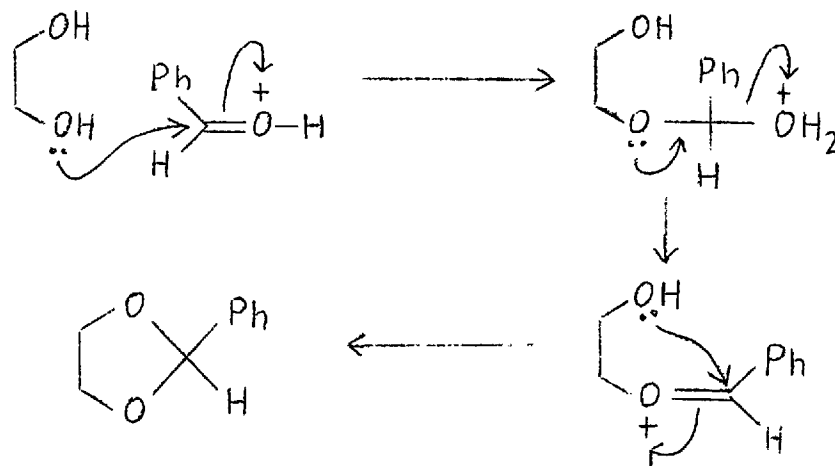


Therefore exclusion of water from the reaction is necessary so that the benzaldehyde complex is formed and can react with the hydroxy groups.

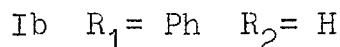
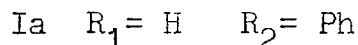
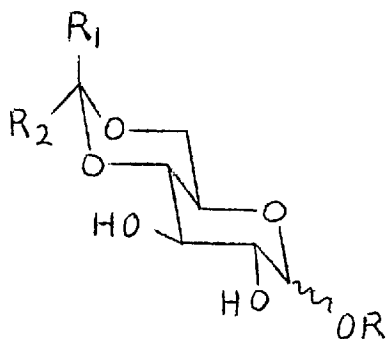
Buchanan and Saunders⁽⁸⁵⁾ have shown that there is a difference in the reactivity of the complex when using zinc chloride catalyst or mineral acid catalyst. The conjugate acid of a carbonyl compound $\text{>}\overset{+}{\text{O}}\text{-H}$ is much smaller than the zinc chloride complex $\text{>}\text{O} \rightarrow \text{ZnCl}_2$. Therefore the zinc chloride complex should have greater steric requirements

and may show a preference for primary hydroxy groups. The reaction with mineral acid catalyst is reversible but it was suggested that reaction using zinc chloride is essentially irreversible because the zinc chloride also acts as a dehydrating agent, and does not reform a benzaldehyde complex once it is hydrated.

The mechanism of acid catalysed formation of cyclic benzyldene acetals has been studied by n.m.r. techniques using a nitromethane solution of the reactants.⁽⁸⁶⁾ The following mechanism was thus proposed.



When the C(1) hydroxy group of glucose is protected by formation of a glucoside, acid catalysed reaction with benzaldehyde gives only a mono-4,6-O-benzyldene derivative. There are two possible structures for this compound with the phenyl group either equatorial (Ia) or axial (Ib).



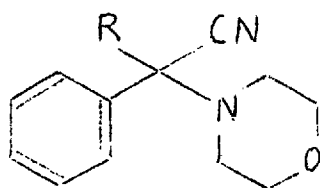
When the benzylidene derivative of methyl α -D-glucoside was first made, it was thought that it was across the 5,6-positions of a furanoside and two isomers were isolated.⁽⁸⁷⁾ One of these was actually Ia but the identity of the second one is very doubtful, although the authors stated that it had the opposite configuration of the phenyl group. Other examples of pairs of isomers of cyclic benzylidene derivatives of sugars have been published, but it has been proved that these are only dimorphs of the compound with the phenyl group equatorial.⁽⁸⁸⁾

The configuration of the phenyl group in 4,6-O-benzylidene-D-glucose has been rigorously established as equatorial by Foster et al by degradation of the glucose ring to a known 1,3-dioxane derivative.⁽⁸⁹⁾

Recently a fully authenticated preparation of the axial form of methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside has been reported.⁽⁹⁰⁾ Reaction of methyl 2,3-di-O-methyl- α -D-glucopyranoside with benzal dibromide, potassium tert-butoxide and tert-butanol in refluxing toluene gives a 50:50 mixture of two benzylidene derivatives.

One is identical to the product of acid catalysed condensation and the other has been given the axial phenyl configuration. A solution of hydrogen chloride in carbon tetrachloride rapidly and completely isomerises the **axial** form to the thermodynamically more stable equatorial form. The n.m.r.evidence for the conformation of the axial isomer indicates that the dioxane ring may exist in a boat form, although this is not certain. The 4,6-O-benzylidene-2,3-di-O-methyl-D-glucopyranosides which are described in this section were made by Lewis acid catalysed condensation and therefore have the phenyl group equatorial.

Two types of labelling have been adopted to elucidate the mass spectral fragmentations of the alkyl and aryl benzylidene glucosides. Firstly, several homologues have been prepared, such as the benzylidene acetals of methyl and ethyl glucosides, and phenyl and tolyl glucosides. Also benzylidene and p-tolylidene derivatives have been investigated. Several deuterium labelled compounds have been prepared from precursors that are described in sections 1,3 and 1,4 or from $[\alpha\text{-}^2\text{H}_1]$ -benzaldehyde. The deuteriated benzaldehyde, which had 90% incorporation at the α -position, was made according to the method of Bennett et al⁽⁹¹⁾ as follows. Reaction of benzaldehyde with morpholine perchlorate, morpholine and sodium cyanide gives 2-phenyl-2-morpholino-acetonitrile (IIa).



IIa R=H

IIb R=D

The α -hydrogen atom in this derivative is fairly acidic so that reaction with sodium hydride in dry dimethylformamide generates the anion at the α position. This can be quenched with deuterium oxide to form the deuteriated derivative IIb. Compound IIb was hydrolysed with hydrochloric acid, but the choice of solvent for this was important. Hydrolysis in aqueous ethanol gives $[\alpha\text{-}^2\text{H}_1]$ -benzaldehyde mixed with ethanol, and these are difficult to separate because they form an azeotrope. Therefore the hydrolysis is best done in water only.

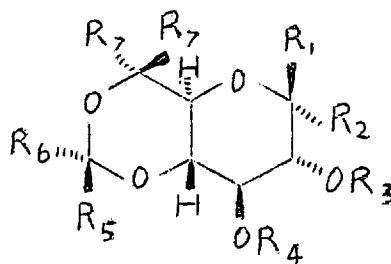
Mass Spectrometry.

A list of the benzylidene derivatives that have been subjected to mass spectrometry is given in table 15, and the results will be discussed separately for methyl and phenyl glucoside derivatives.

(i) Methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside. (mass spectrum 15)

The fragmentation modes for this sugar have been worked out from the shifts in mass number of the ions in the spectra of related alkyl glucosides (table 16) and from high resolution measurements of accurate masses of ions (table 17). The ions are formed as a result of several

Table 15.



comp.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
III	H	CH ₃ O	CH ₃	CH ₃	H	C ₆ H ₅	H
IV	C ₂ H ₅ O	H	CH ₃	CH ₃	H	C ₆ H ₅	H
V	H	CH ₃ O	CH ₃	CH ₃	H	<i>p</i> -CH ₃ C ₆ H ₄	H
VI	H	CH ₃ O	CD ₃	CH ₃	H	C ₆ H ₅	H
VII	H	CH ₃ O	CD ₃	CD ₃	H	C ₆ H ₅	H
VIII	H	CH ₃ O	CH ₃	CH ₃	D	C ₆ H ₅	H
IX	H	CH ₃ O	CH ₃	CH ₃	H	C ₆ H ₅	D
X	C ₆ H ₅ O	H	CH ₃	CH ₃	H	C ₆ H ₅	H
XI	<i>o</i> -CH ₃ C ₆ H ₄ O	H	CH ₃	CH ₃	H	C ₆ H ₅	H
XII	C ₆ H ₅ O	H	CD ₃	CD ₃	H	C ₆ H ₅	H
XIII	C ₆ H ₅ O	H	CH ₃	CH ₃	D	C ₆ H ₅	H
XIV	C ₆ H ₅ O	H	CH ₃	CH ₃	H	C ₆ H ₅	D
XV	C ₆ H ₅ O	D	CH ₃	CH ₃	H	C ₆ H ₅	H

Table 16. Ions in the mass spectra of benzylidene derivatives of alkyl glucosides.

III	IV	V	VI	VII	VIII	IX
310	324	324	313	316	311	312
279	279	293	282	285	280	281
267	281	281	270	273	268	267
247	247	261	250	250	248	249
			247			
235	235	249	238	238	236	237
224	238	238	224	227	225	226
219	219	233	222	222	220	221
218	218	232	221	221	219	220
181	195	195	181	184	182	181
173	173	173	176	179	173	175
161	175	161	164	167	161	161
149	149	163	149	149	150	151
145	145	159	145	145	145	147
121	121	135	124	124	122	121
			121			
113	113	113	116	116	113	<u>115</u>
						114
106	106	120	106	106	107	106
105	105	119	105	105	105	105
101	115	101	104	<u>107</u>	101	101
	<u>101</u>		101	104		
88	102	88	91	<u>94</u>	88	88
	<u>88</u>			91		
75	89	75	75	78	75	75
45	45	45	48	48	45	45
			45			

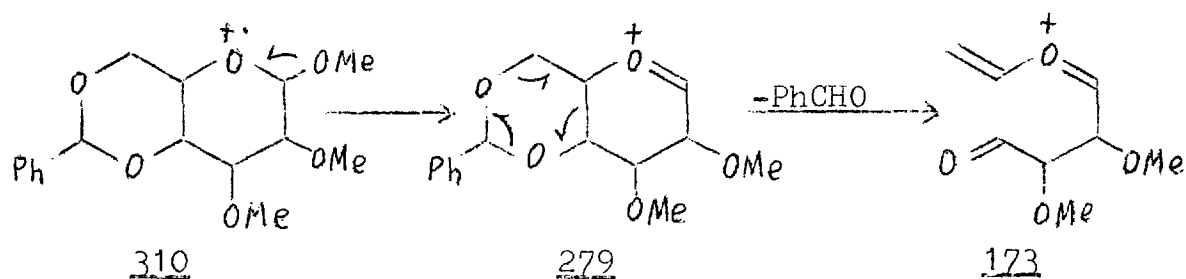
Where a peak is split into two in the spectra of the labelled compounds, the more intense one is underlined. Where they are of similar intensity, neither is underlined.

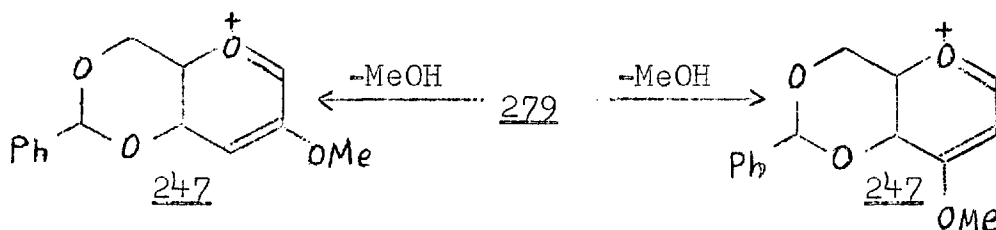
Table 17. Mass measurements on methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside (III).

m/e	Observed Mass	Calculated Mass	Formula
181	181.0866	181.0865	C ₁₀ H ₁₃ O ₃
161	161.0813	161.0814	C ₇ H ₁₃ O ₄
149	149.0612	149.0606	C ₉ H ₉ O ₂
145	145.0654	145.0653	C ₁₀ H ₉ O
	145.0865	145.0865	C ₇ H ₁₃ O ₃
121	121.0649	121.0653	C ₈ H ₉ O
113	113.0594	113.0603	C ₆ H ₉ O ₂
106	106.0412	106.0419	C ₇ H ₆ O
105	105.0333	105.0340	C ₇ H ₅ O
101	101.0605	101.0603	C ₅ H ₉ O ₂

primary fissions.

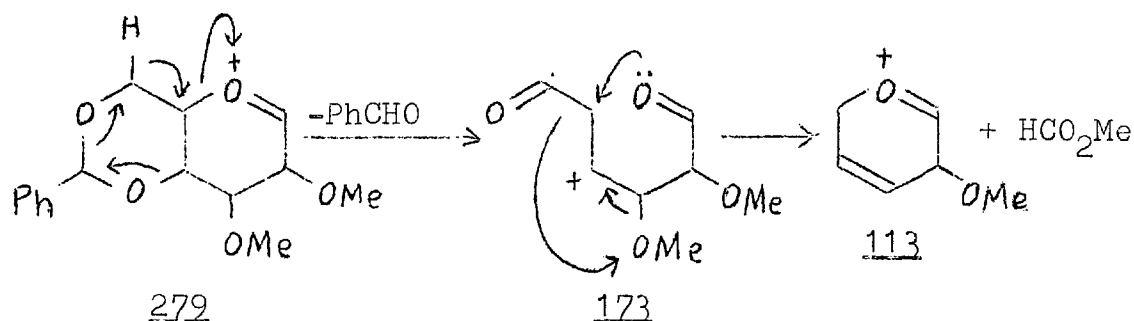
(a) Breaking of the C(1)-Aglycone bond gives a pyronium ion of mass 279, which further fragments along two different pathways. Loss of either methoxy group as methanol gives m/e 247, whereas loss of benzaldehyde from the acetal ring gives m/e 173.



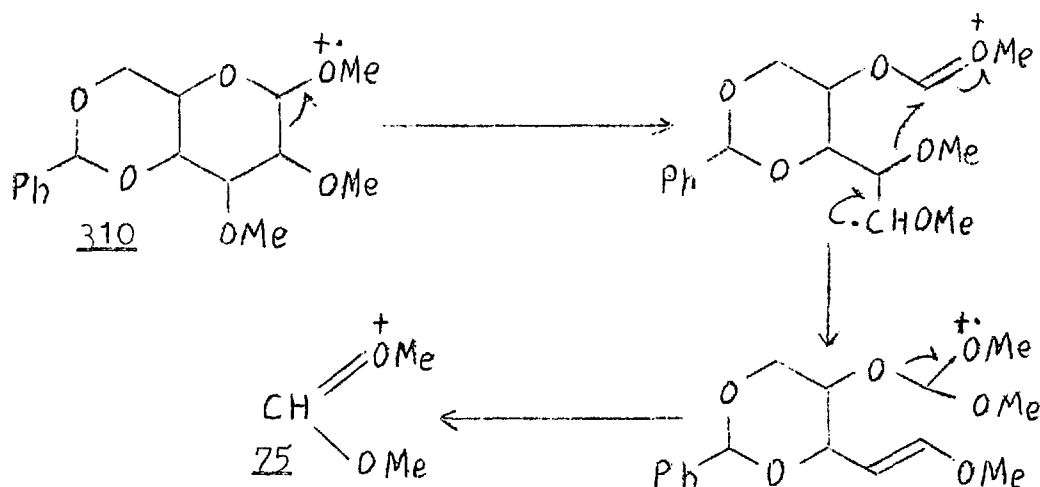


The loss of methanol is represented as a 1,2-elimination for convenience only, since the proton may well be removed from positions further from the methoxy group. It has been shown that an acyclic ether, such as ethyl n-hexyl ether, loses ethanol to give an ion m/e 84 which has lost an hydrogen atom from C(4) to an extent of 33%, but the amount of hydrogen abstracted from the other positions is not known.⁽⁹²⁾ Similarly, 91% of the elimination of water from n-hexanol is 1,4 with the remaining 9% probably 1,3.⁽⁹³⁾ The absence of any 1,2-elimination of water from cyclohexanol has also been demonstrated, but in this case the hydrogen atom is abstracted equally from the C(4) position and the C(3)+C(5) positions.⁽⁹⁴⁾

Some of the ions of mass 113 have lost one of the C(6) hydrogen atoms, whilst the remainder retain both. Therefore the m/e 113 ion is formed by two different mechanisms, of which one is given on page 133. There is an alternative mechanism for the loss of benzaldehyde from the m/e 279 ion which gives an m/e 173 ion which can lose methyl formate. The m/e 113 ion, so produced, contains one of the C(6) hydrogen atoms.

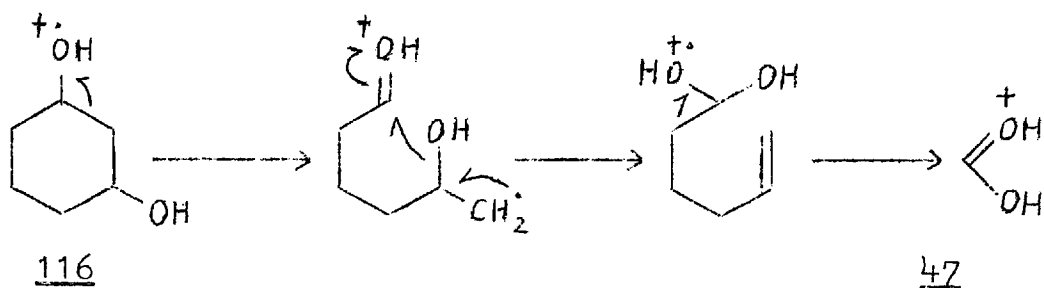


(b) Fission of the C(1)-C(2) bond occurs and is followed by specific migration of the methoxy group from C(3) to C(1), to give an ion which easily fragments to give the abundant ion m/e 75.

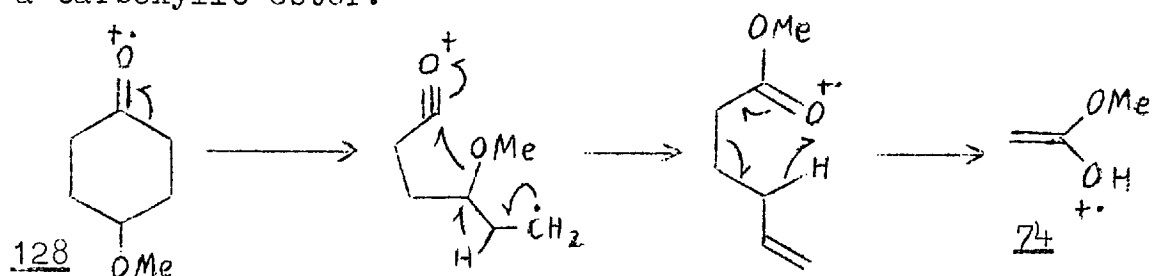


The origin of the methoxy group that migrates to C(1) is shown by the shift of m/e 75 to m/e 78 which occurs in the mass spectrum of the 2,3-di-O- $^{2}\text{H}_3$ -methyl analogue (VII) but not in the spectrum of the 2-O- $^{2}\text{H}_3$ -methyl compound (VI).

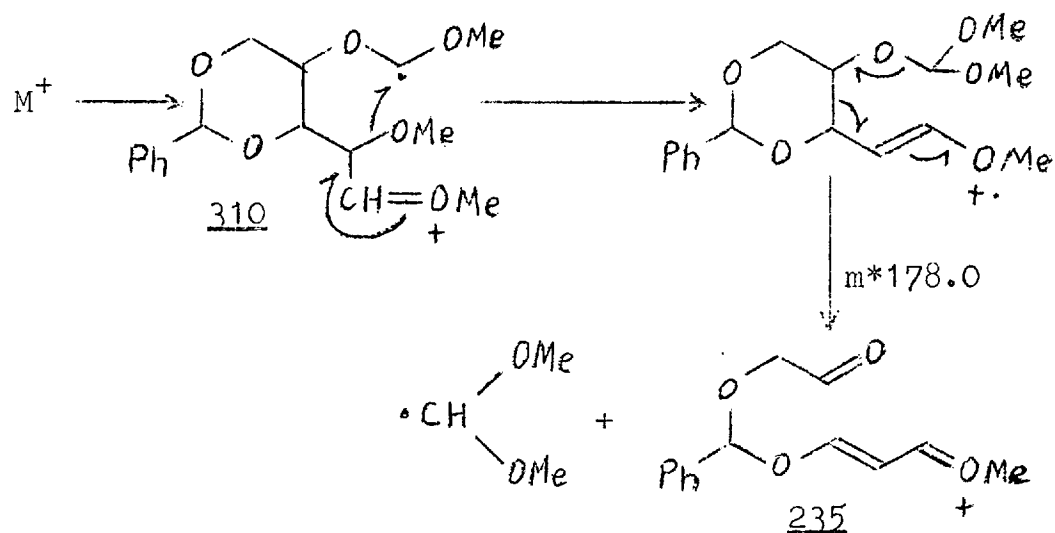
The migration of alkoxy or hydroxy groups appears to be a fairly general process for cyclohexane derivatives which carry oxygen functions at the 1,3 or 1,4 positions. Thus 1,3-dihydroxycyclohexane shows the following rearrangement.⁽⁹⁵⁾



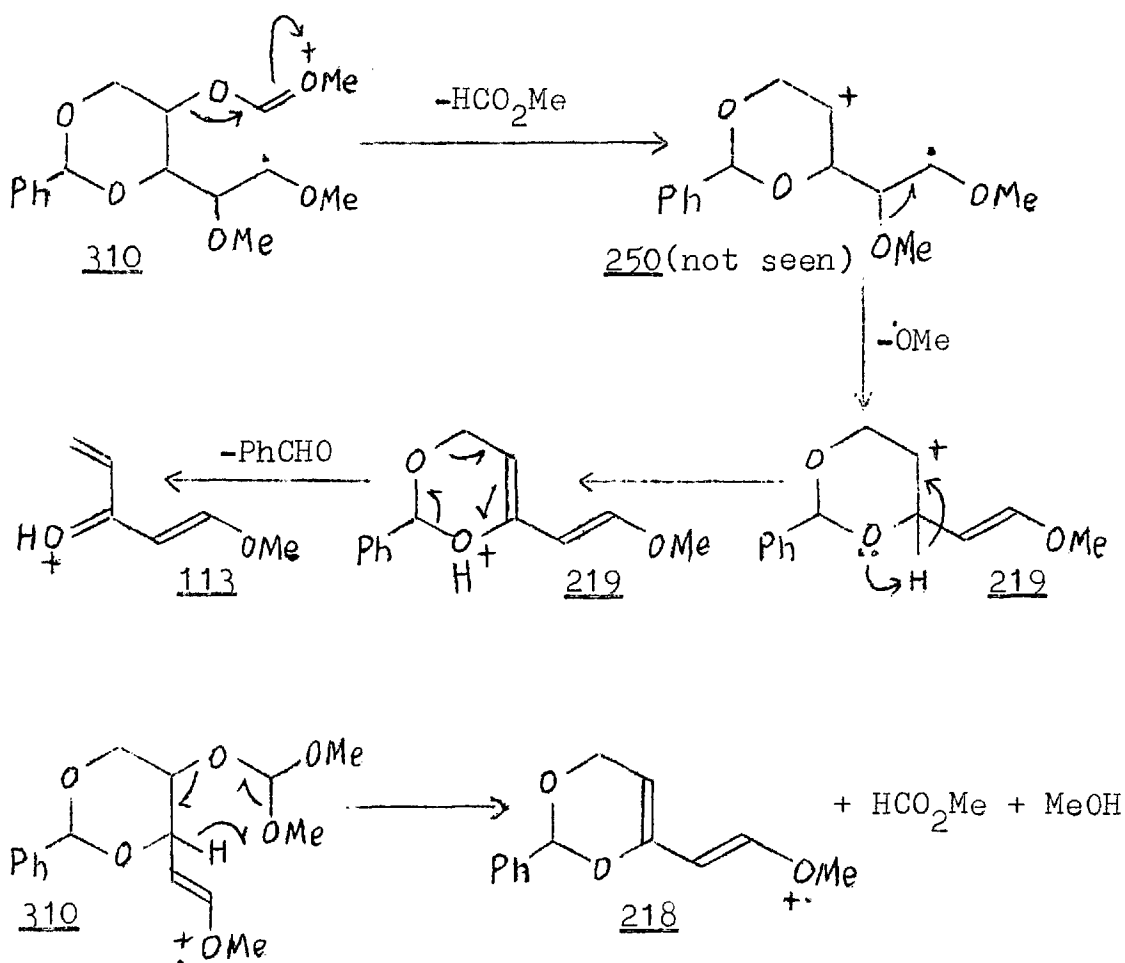
Similarly the main ions in the mass spectrum of 4-methoxycyclohexanone are formed after initial rearrangement to a carboxylic ester.⁽⁹⁶⁾



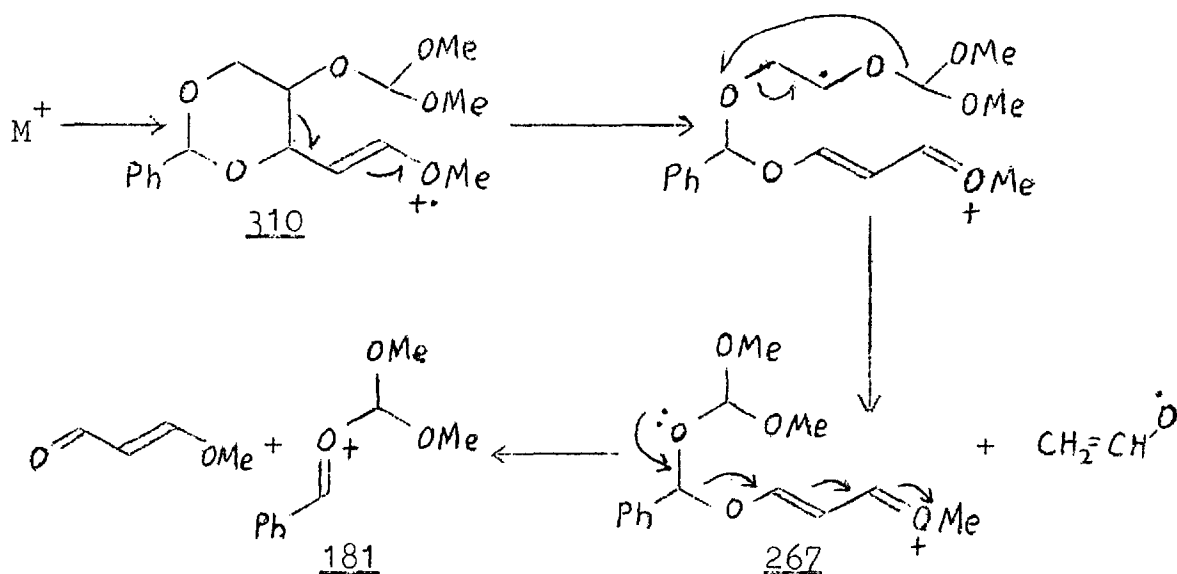
When ionisation of III takes place so that the charge remains on the oxygen atom at C(2) after C(1)-C(2) fission, the decomposition of the rearranged molecular ion can take place along several different pathways, of which the simplest is loss of $\dot{\text{C}}\text{H}(\text{OMe})_2$.



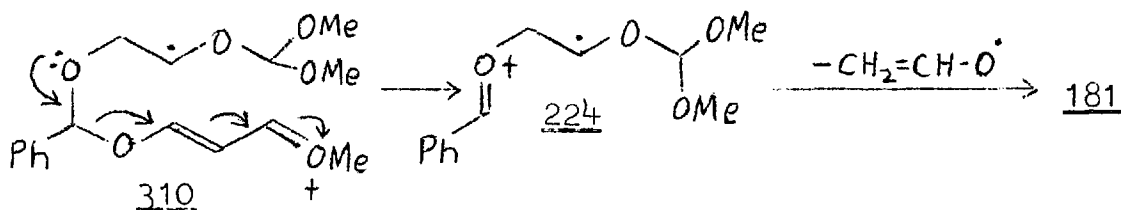
There are ions at m/e 218 and 219 which are analogous to the ions of series B and D in the mass spectra of tetra-acetyl pyranosides. These are also formed by C(1)-C(2) fission, and furthermore the m/e 219 ion can lose benzaldehyde to form the other ion of mass 113.



The ion at m/e 181, $\text{C}_{10}\text{H}_{13}\text{O}_3^+$ is rather surprising because it contains the phenyl ring, the methoxy groups at C(1) and C(3) and only two other carbon atoms. An ion at m/e 267 ($M-43$) has lost C(6) and is probably the precursor of m/e 181 through a rearrangement process.

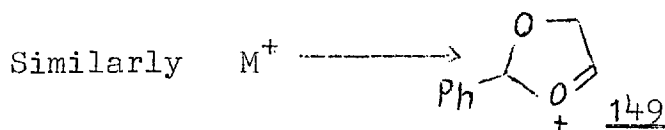
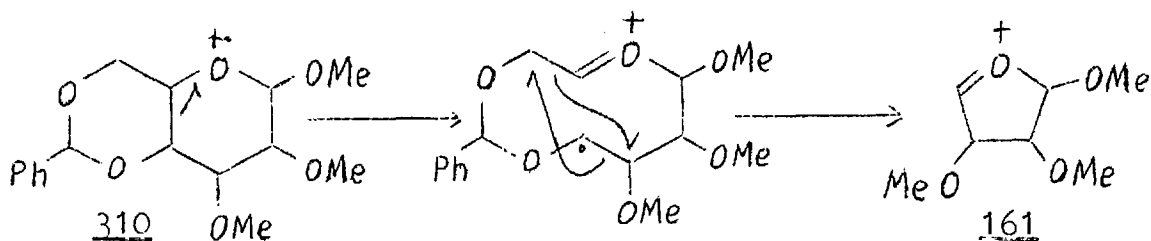


The ion at m/e 224 is formed from the same rearranged molecular ion and may also lose 43 mass units to give m/e 181

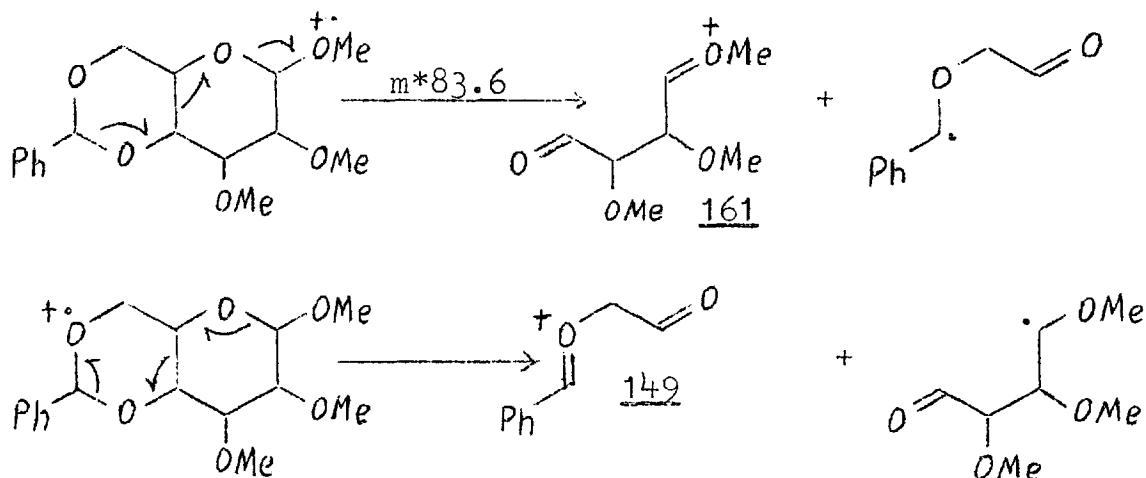


The m/e 181 ion can split into two fragments and give the ion m/e 75 or the benzaldehyde ion of mass 106. However, both of these ions can also be formed directly from the molecular ion.

(c) The molecular ion splits into two parts, giving ions at m/e 161 and 149. The mechanism that was first used to explain this process is shown below.



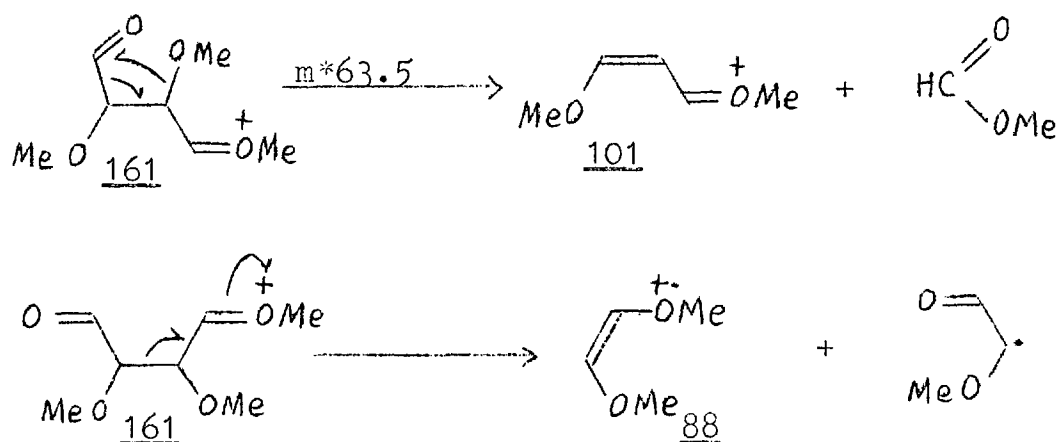
However, Chizhov et al⁽⁹⁷⁾ have recently published a paper on this type of fission which they have designated "h" rupture, and the following mechanism has been proposed for it.



It would be possible to distinguish between these two mechanisms by isotopic labelling of the proton at C(4) or C(5). The mechanism of Chizhov et al is the more attractive of the two because it does not require rupture of a vinylic bond. In addition, it has been shown that "h" rupture is a general fragmentation for compounds containing the group $R-O-C-C-O-C-O-X$ where R can stabilise a radical site and

X is able to donate electrons.

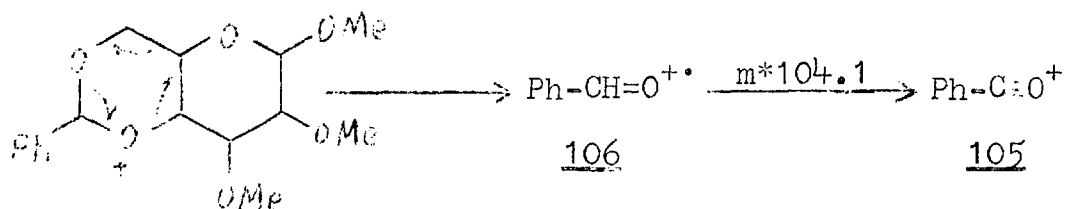
There is a metastable peak at m/e 63.5 due to the formation of the ion m/e 101 from m/e 161. Also, although there is no metastable peak for the process, it is possible to form the ion of mass 88, which contains C(1) and C(2), from m/e 161.



(d) The ion m/e 121 must be formed by a rearrangement process and the data available indicates that it probably has the structure: $\text{Ph} - \text{CH} = \overset{+}{\text{O}} - \text{CH}_3$. The methyl group comes from the methoxy groups at both C(2) and C(3) and the proton on the $-\text{CH} =$ group is the one which was α to the phenyl ring. It is difficult to write a satisfactory mechanism for the formation of this ion, therefore none will be presented.

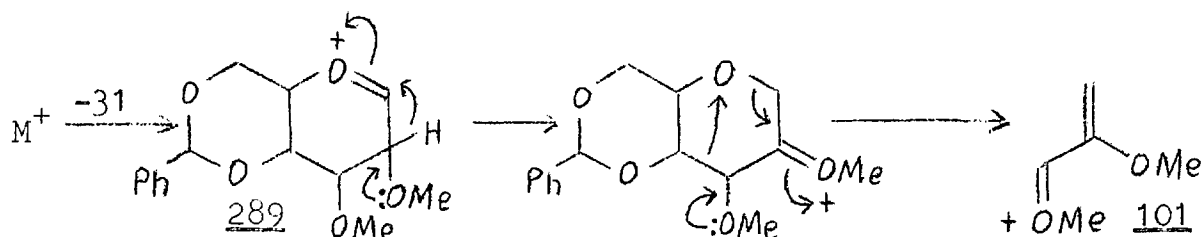
(e) Ions of mass 106 and 105 are formed from the benzylidene part of the molecule. The m/e 106 ion could be formed by fission of certain fragment ions, e.g. m/e 149, 181 and 224,

but can also be readily formed directly from the molecular ion.



Loss of an hydrogen atom from the ion m/e 106 gives the stable benzoyl ion m/e 105.

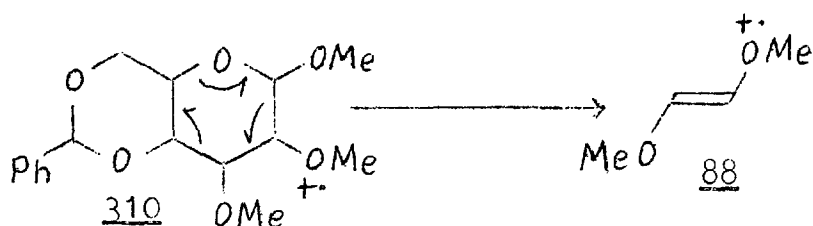
(f) The peak observed at m/e 101 is common in the mass spectra of permethylated compounds, but it has been shown for permethylated pyranosides that this ion mainly carries the methoxy groups from C(2) and C(4).⁽¹⁸⁾ In the case of the benzylidene derivatives this is impossible and most ions of m/e 101 carry the methoxy groups from C(2) and C(3).



Some of the m/e 101 ions have the substituents that were attached to C(1) and C(3) of the parent sugar. These can be formed from m/e 161 as shown on page 136.

Some of the ions may contain the methoxy groups from C(1) and C(2), but since the methoxy group at C(3) has not been separately labelled, it is not possible to prove this.

(g) A mechanism for the formation of the ion m/e 88 from m/e 161 has already been given and this would lead to ions containing the methoxy groups from C(1) and C(2). The labelling results show that this only forms a minor contribution to the peak at m/e 88 and the majority of ions contain the methoxy groups from C(2) and C(3).



(h) The ion m/e 45 is $\text{CH}_2=\text{O}^+-\text{CH}_3$ containing a methoxy group from C(2) or C(3). In the mass spectrum of the $[1-^2\text{H}_1]$ glycoside derivative (XV) m/e 45 is partially shifted to m/e 46, showing one of the sources of the extra proton.

(j) The ion at m/e 145 is a doublet in the mass spectrum of the alkyl glucoside derivative (III). However, in the mass spectrum of the phenyl glucoside derivative (X) it is a singlet with a different elementary composition from the two ions of nominal mass 145 in the spectrum of III. The origins of these ions are not known.

(ii) Phenyl 4,6-O-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside. (Mass spectrum 16)

Mass measurements of the main peaks in the mass spectrum of X are given in table 18 and the shifts in position of the peaks in the spectra of the labelled analogues are in table 19.

Table 18. Mass measurements on phenyl 4,6-O-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside (X).

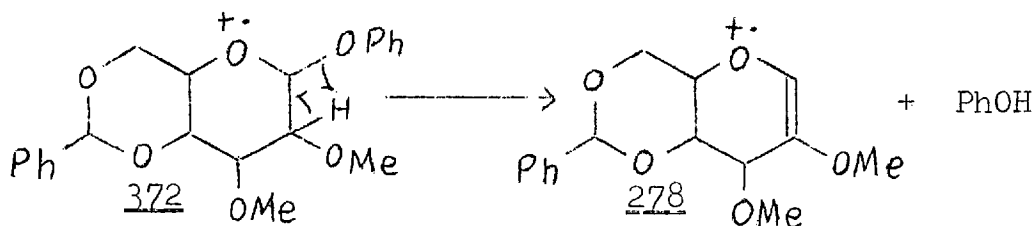
m/e	Observed Mass	Calculated Mass	Formula
279	279.1223	279.1232	C ₁₅ H ₁₉ O ₅
278	278.1157	178.1154	C ₁₅ H ₁₈ O ₅
247	247.0973	247.0970	C ₁₄ H ₁₅ O ₄
173	173.0814	173.0814	C ₈ H ₁₃ O ₄
149	149.0601	149.0603	C ₉ H ₉ O ₂
145	145.0500	145.0501	C ₆ H ₉ O ₄
141	141.0553	141.0552	C ₇ H ₉ O ₃
137	137.0603	137.0603	C ₈ H ₉ O ₂
131	131.0496	131.0497	C ₉ H ₇ O
129	129.0553	129.0552	C ₆ H ₉ O ₃
127	127.0759	127.0759	C ₇ H ₁₁ O ₂
121	121.0652	121.0653	C ₈ H ₉ O
113	113.0601	113.0603	C ₆ H ₉ O ₂
99	99.0446	99.0446	C ₅ H ₇ O ₂

One of the most prominent peaks in the mass spectrum of X is at m/e 278, which has an intensity of 83% of the base peak at m/e 45. It is due to loss of phenol from the molecular ion, and the proton that is abstracted does not come from any of the positions that have been deuterium labelled. Therefore it is probable that a 1,2-elimination

Table 19. Ions in the mass spectra of benzylidene derivatives of aryl glucosides.

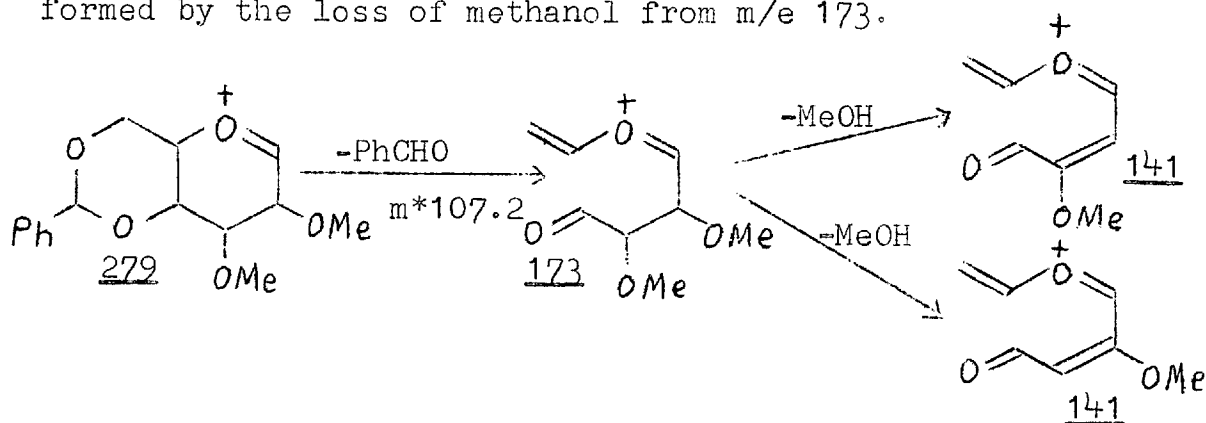
X	XI	XII	XIII	XIV	XV
372	386	378	373	374	373
279	279	285	280	281	280
278	278	284	279	280	279
247	247	250	248	249	248
219	219	222	220	221	219
215	215	215	216	217	215
203	203	203	204	205	203
173	173	179	173	175	174
149	149	149	150	151	149
145	145	148	145	147	146
141	141	144	141	143	142
137	-	140	137	137	138
131	131	131	131	131	132
129	129	135	129	129	130
127	127	133	127	129	128
121	121	124	122	121	121
113	113	116	113	115	114
				114	113
105	105	105	105	105	105
101	101	107	101	101	102
99	99	102	99	101	99
94	108	94	94	94	94
77	91	77	77	77	77
45	45	48	45	45	46
					45

is involved.



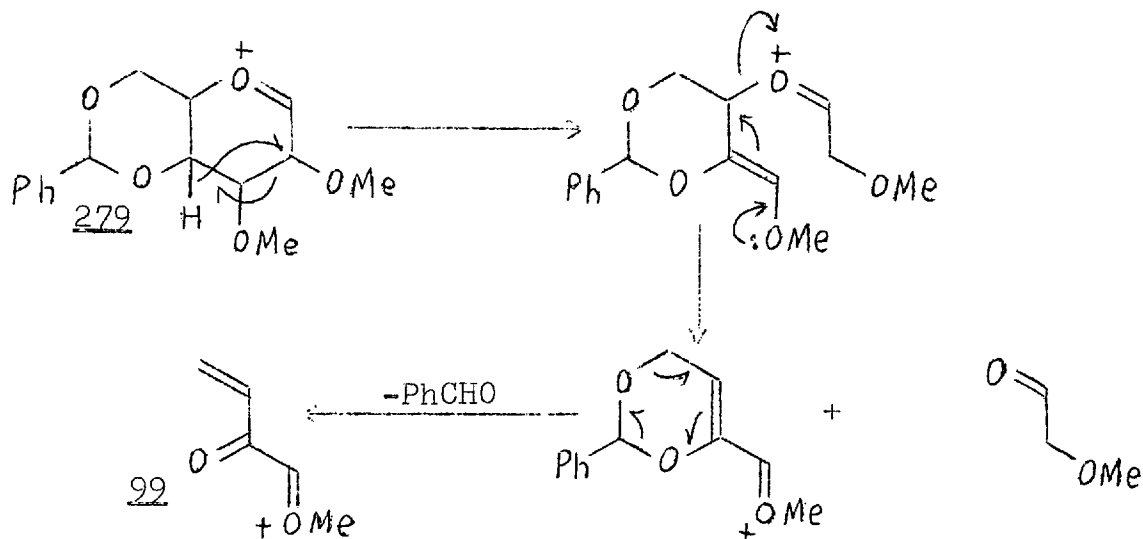
There is a corresponding ion at m/e 94 formed when the charge remains on the phenol.

Fission of the C(1)-Aglycone bond is much more favourable for the phenyl glucoside (X) than the methyl glucoside (III), whereas ions formed by primary fission of the C(1)-C(2) bond are much less abundant for the phenyl glucoside. These are the same observations as were recorded for methyl and phenyl tetra-acetyl glucosides. Thus the ion m/e 279 in the mass spectrum of X has an intensity of 39% of the base peak, and the ions of mass 247 and 173, which are decomposition products of m/e 279, are more abundant. Additionally, there is an ion at m/e 141 which is formed by the loss of methanol from m/e 173.

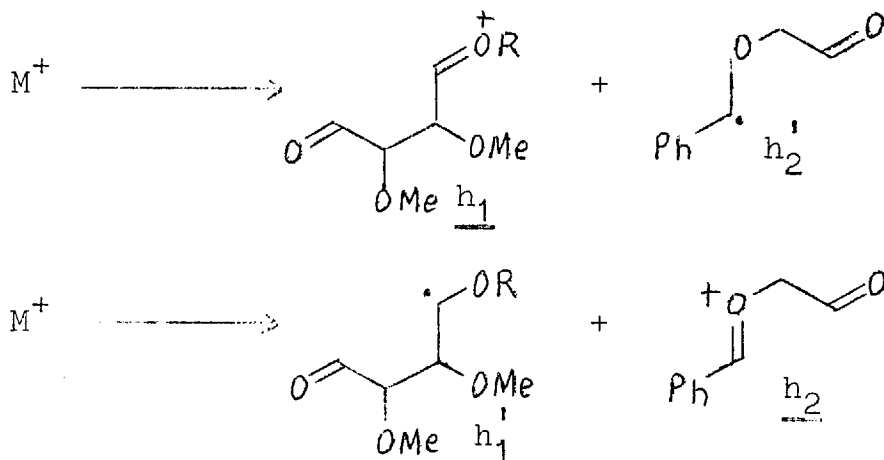


A peak at m/e 99, which is also not found in the mass spectrum of III, is probably associated with the

increased amount of C(1)-O-aglycone fission. Therefore the following pathway from m/e 279 may account for its formation.

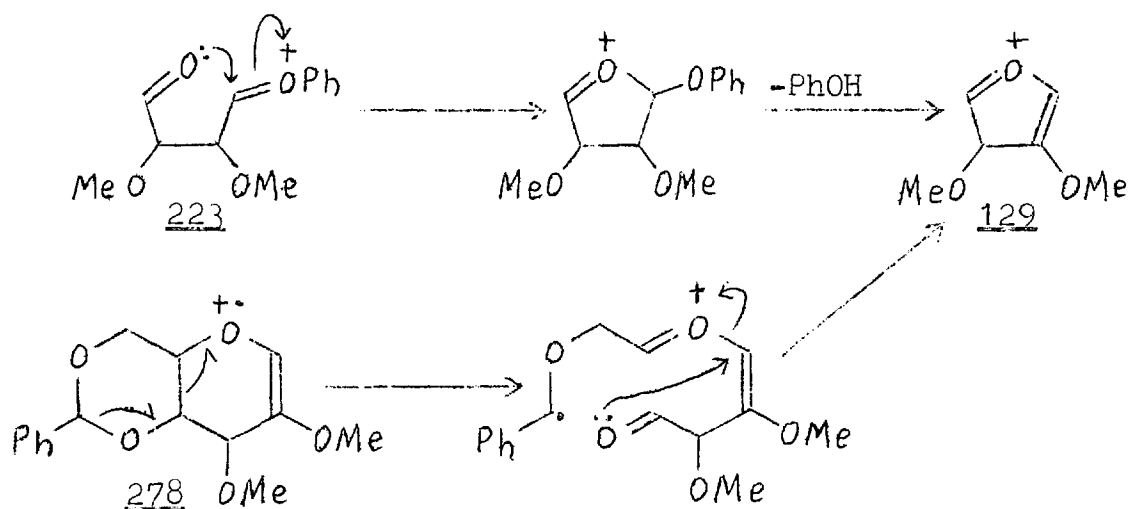


"h"-Rupture is not so important for compound X as for III and gives ions of mass 149 and 223, the latter being of very low intensity. The relative intensities of the two "h" fragments are reversed and Chizhov *et al*⁽⁹⁷⁾ have tried to relate these findings to the stabilities of the ions and radicals formed by "h" rupture.



It was argued that for the methyl glucoside (III) the h₂¹

radical is more stable than the h_1' radical so that the h_1 ion at m/e 161 predominates, whereas for the phenyl glucoside (X), the h_1' and h_2' radicals are of similar stability but the h_2 ion at m/e 149 is more stable and therefore more abundant. It is, in fact, doubtful whether h_1' and h_2' are of similar stability and the reasoning also ignores the fact that for compound III the ion h_2 is more stable than h_1 , so that the total energy requirements for both processes should be considered. There is also a possibility that further fragmentation of the h_1 ion is different for III and X so that the intensity of the peak observed for h_1 is not a true measure of the charge distribution during "h" rupture. Thus an ion of mass 129 present in the mass spectrum of X could be due to loss of phenol from m/e 223. Alternatively the m/e 129 ion could be formed from m/e 278 by a similar process to "h" rupture.



Many of the other peaks in the mass spectrum of X, e.g. those at m/e 219, 121, 113, 105 and 45, are formed by the same mechanisms as were described for the methyl glucoside III. There must be a higher degree of specificity in the formation of the ions at m/e 101 and 88, which are only formed by those mechanisms which lead to ions containing the substituents at C(2) and C(3). The ion of mass 137 is $\text{Ph} - \overset{+}{\text{O}} = \text{CH} - \text{OCH}_3$ formed by migration of the C(3) methoxy group to C(1), but it is much less abundant than the corresponding ion at m/e 75 in the mass spectrum of III because fission of the C(1)-C(2) bond does not occur to a large extent. Finally, m/e 77 is C_6H_5^+ formed by the aglycone and m/e 91 is a tropylium ion C_7H_7^+ formed from the benzyliidene group.

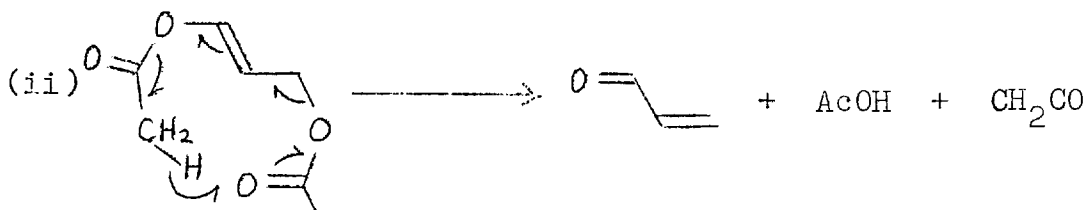
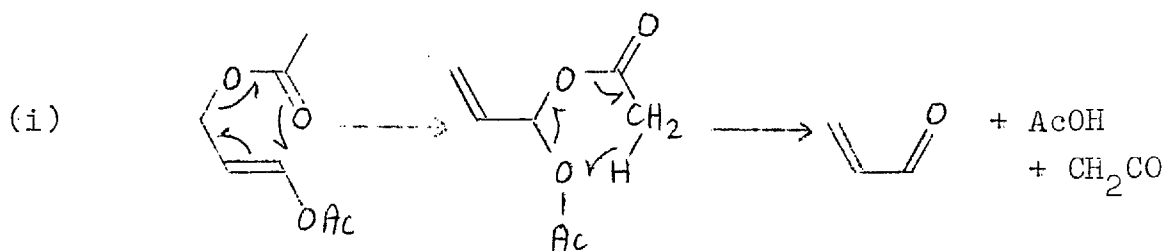
PART 2

Allylic Acetates.

2,1 Preparation of Allylic Acetates

Introduction

In the series A decomposition sequence of acetylated pyranosides it is necessary to postulate a one step loss of 102 mass units from an unsaturated 1,3-diacetate system. Two possible mechanisms have been proposed for this process of which (i) requires an allylic rearrangement⁽⁸⁾ whilst (ii) does not.⁽⁹⁾



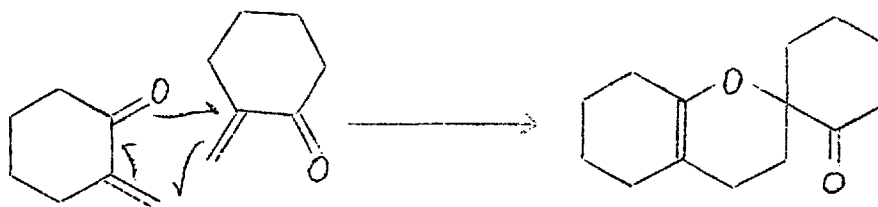
In order to find out whether allylic rearrangements can occur during mass spectrometry, the investigation of a series of allylic acetates has been carried out.

Preparation

Methyl substituted allyl acetates were made by acetylation of the corresponding alcohols. The alcohols were readily available except for 1,1-dimethylallyl alcohol which was made by the reaction of vinyl magnesium chloride

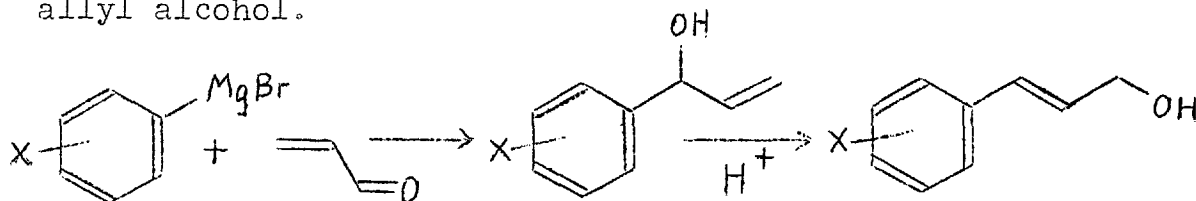
and acetone. Although Grignard reagents have been known for many years, the lack of reactivity of vinylic halides prevented the preparation of vinylic magnesium halides under the normal reaction conditions using diethyl ether as solvent. However, in 1954 Normant demonstrated that vinylic Grignard reagents can be fairly easily prepared using tetrahydrofuran as solvent for the reaction.⁽⁹⁸⁾ Acetylation of the alcohol with acetic anhydride and sodium acetate gave no allylic rearrangement.

The preparation of 2-methylenecyclohexan-1-yl acetate involved the use of 2-methylenecyclohexanone as an intermediate. This is a very unstable compound because on slightly warming it spontaneously dimerises.⁽⁹⁹⁾



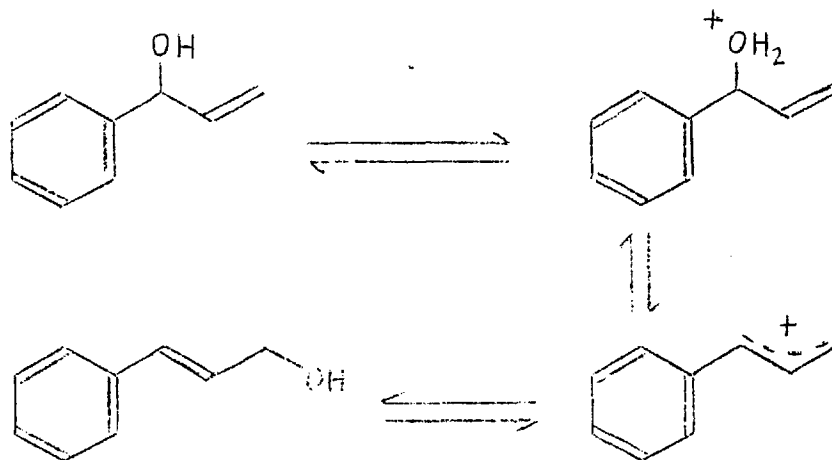
Therefore it has to be kept at a low temperature and reduced to the more stable alcohol as quickly as possible.

Most of the cinnamyl alcohols were made by reacting a substituted phenyl magnesium bromide with acrolein followed by acid catalysed isomerisation of the 1-phenylallyl alcohol.



The substituent has quite a large effect on the rate of isomerisation of the 1-phenylallyl alcohol. The unsubstituted alcohol and alkyl substituted alcohols can be isomerised and acetylated in one step by refluxing with acetic anhydride, but under the same conditions 1- p-bromophenyl -allyl alcohol gives the unrearranged acetate. Hence the conversion of 1- halogenophenyl -allyl alcohols to cinnamyl alcohols was accomplished by prolonged refluxing in aqueous acetone which was 0.2N. with respect to hydrogen chloride. Conversely, 1- o-methoxyphenyl -allyl acetate is very reactive and attempted isomerisation with aqueous acid gave mixtures, so that it is best isomerised by the acetic anhydride method.

The anionotropic rearrangement of allylic alcohols is specifically catalysed by acid,⁽¹⁰⁰⁾ so that the use of acetic anhydride in these preparations relies on the presence of acetic acid as an impurity. Acetylation of substituted 1-phenylallyl alcohols with acetic anhydride and pyridine proceeds without isomerisation. In ionising solvents the isomerisation probably goes through an intermediate carbonium ion which is stabilised by electron donating substituents. The equilibrium mixture consists almost entirely of the cinnamyl alcohol because of the effects of conjugation to the phenyl ring.

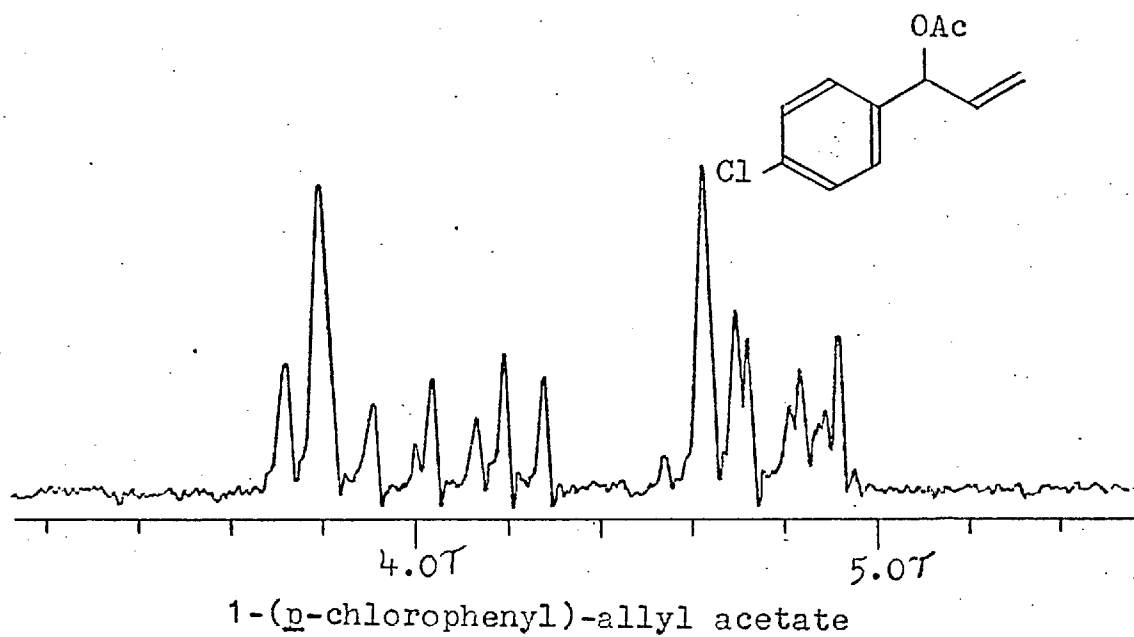
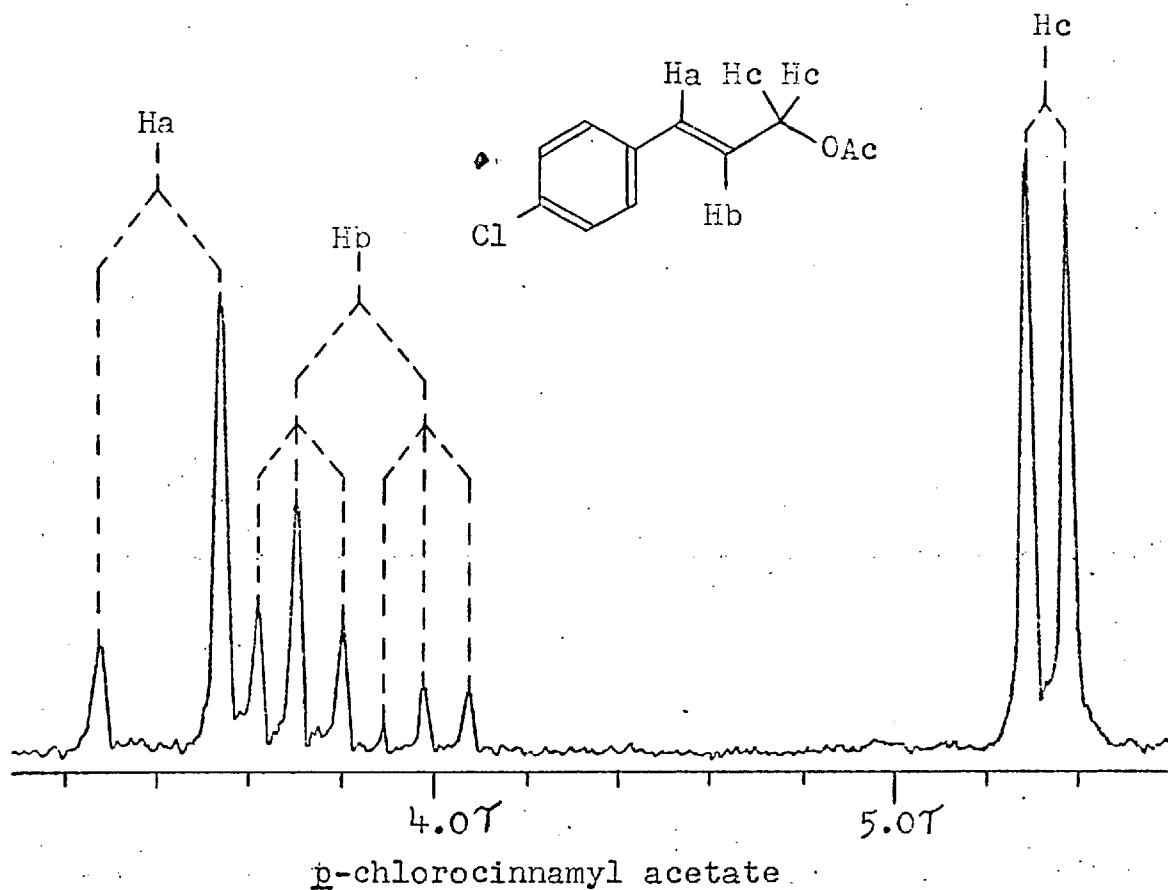


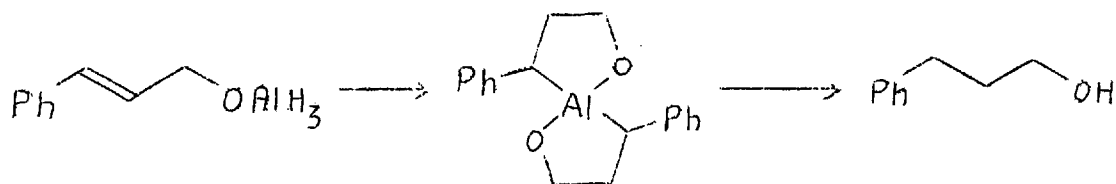
The n.m.r. spectra of the cinnamyl system and the 1-phenylallyl system show characteristic resonances for the vinyl protons which enable them to be distinguished. Thus the allylic rearrangement can be readily followed by n.m.r.spectroscopy and the type of result obtained is illustrated in figure 4, which shows the 60 MHz n.m.r. spectra of p-chlorocinnamyl acetate and 1- p-chlorophenyl -allyl acetate in deuteriochloroform.

Some substituted cinnamyl alcohols were prepared by lithium aluminium hydride reduction of cinnamaldehydes or cinnamic esters. Cinnamaldehyde is reduced to dihydro-cinnamyl alcohol if the compound is added to lithium aluminium hydride, but cinnamyl alcohol is obtained if the addition is the other way around (inverse addition).⁽¹⁰¹⁾ Double bonds are not normally reduced by L.A.H. except in compounds of the general class: $\text{Ph}-\text{CH}=\text{CH}-\text{R}$, where R is a polar group such as CH_2OH , CHO and CH_2NO_2 . In the case of cinnamaldehyde, initial reaction gives cinnamyl alcohol which forms a cyclic aluminium complex on to the double bond.

Figure 4.

60 MHz. n.m.r. spectra





The inverse addition assures that at no time during the addition is there an excess of metal hydride which can react with the double bond.

Normal or inverse addition in the reduction of ethyl cinnamate gave dihydrocinnamyl alcohol, but an electron donating substituent on the phenyl ring enables the reaction to be stopped at the cinnamyl alcohol stage. Thus ethyl p-methoxycinnamate was reduced to p-methoxycinnamyl alcohol by addition of lithium aluminium hydride at 38°C.

Reduction of cinnamaldehyde with lithium aluminium deuteride gave 3-phenyl-[1-²H₁]-prop-2-en-1-ol, but it was nevertheless desired to prepare the [1,1-²H₂] compound to eliminate the complications of deuterium isotope effects in the mass spectral interpretations. Brown et al have reported that ethyl cinnamate can be reduced to cinnamyl alcohol by reaction with lithium borohydride in diglyme at 100°C.⁽¹⁰²⁾ When it was attempted to repeat this reaction using lithium borodeuteride, mixtures were obtained which mainly contained ethyl cinnamate and dihydrocinnamyl alcohol.

A number of deuteriated bromobenzenes were required as starting materials for the preparation of deuteriated

cinnamyl acetates. It has been shown that the protons on a benzene ring can exchange with acids, so that when deuterium oxide is present there is some isotopic exchange. Ingold et al demonstrated that the attack of D_3O^+ on the benzene ring obeyed the rules of aromatic electrophilic substitution.⁽¹⁰³⁾ Thus aniline forms $[2,4,6-^2H_3]$ -aniline on acid catalysed exchange with deuterium oxide. In practice the deuteration was carried out by heating anilinium bromide with several changes of deuterium oxide until the desired incorporation was achieved.⁽¹⁰⁴⁾ A Sandmeyer reaction with cuprous bromide was used to convert $[2,4,6,N,N,N-^2H_6]$ -anilinium bromide to $[2,4,6-^2H_3]$ -bromobenzene.

The complex of bromine with dioxane is a very mild electrophile and can be used to para monobrominate aniline.⁽¹⁰⁵⁾ Therefore this reagent was used to brominate the deuteriated anilinium bromide which was then deaminated by diazotisation and reaction with hypophosphorous acid to give $[3,5-^2H_2]$ -bromobenzene.

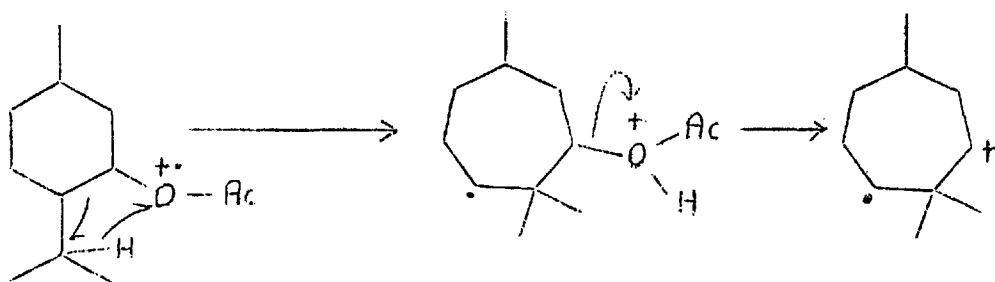
Although a chlorine substituent on a benzene ring is deactivating and ortho/para directing, it was reasoned that the effect of the amino group in p-chloroaniline hydrobromide should outweigh that of the halogen. This proved to be so, because one exchange with an equal amount of deuterium oxide at $100^\circ C$ for two days gave 80% incorporation of deuterium at the positions ortho to the amino group. The n.m.r. spectrum gave no evidence for

exchange ortho to the chloro group. A Sandmeyer reaction converted 4-chloro-[2,6- $^2\text{H}_2$]-anilinium bromide to 4-chloro-[2,6- $^2\text{H}_2$]-bromobenzene.

In order to make o-[$^2\text{H}_3$]-methylcinnamyl acetate it was necessary to prepare o-[$^2\text{H}_3$]-methylbromobenzene by a series of reductions. Ethyl o-bromobenzoate was reduced to o-bromo-[α, α - $^2\text{H}_2$]-benzyl alcohol with lithium aluminium deuteride. The tosylate of this alcohol was formed and reduction of this with lithium aluminium deuteride gave the desired specifically deuteriated o-bromotoluene with a high incorporation.

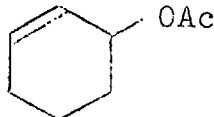
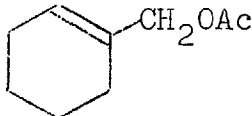
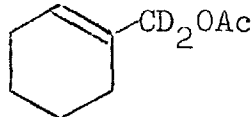
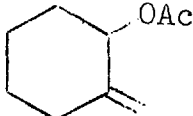
2,2 Mass Spectra of Aliphatic Allylic Acetates

In the examples of the mass spectra of saturated acetates which have been published, the major fragment ion is normally M-60 due to loss of acetic acid. The origin of the proton which is abstracted has been investigated and for n-pentyl acetate there is 55% of 1,2-elimination and 45% of 1,3-elimination.⁽¹⁰⁶⁾ For some cyclic acetates the loss of acetic acid is rather more specific so that 1,2-elimination occurs to an extent of 83% from the molecular ion of cyclohexyl acetate.⁽¹⁰⁷⁾ The mechanism of elimination of acetic acid is altered by the substituents on the cyclohexyl ring, so that menthyl acetate loses 30% of the acetic acid by 1,3-elimination involving a proton from the isopropyl group.⁽¹⁰⁸⁾



By comparison allyl acetates and methyl substituted allyl acetates (compounds I-VI in table 20) exhibit varying degrees of loss of 42, 43, 59 and 60 mass units as shown in table 21. There are several mechanisms available for the loss of acetic acid from these compounds, of which a pyrolytic mechanism must be considered. Thermal 1,2 cis elimination of acetic acid is well known but does not

Table 20. List of aliphatic allylic acetates.

	Compound	Formula
I	Allyl acetate	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{OAc}$
II	1-Methylallyl acetate	$\text{CH}_2=\text{CH}-\text{CHMe}-\text{OAc}$
III	2-Methylallyl acetate	$\text{CH}_2=\text{CMe}-\text{CH}_2-\text{OAc}$
IV	3-Methylallyl acetate	$\text{Me}-\text{CH}=\text{CH}-\text{CH}_2-\text{OAc}$
V	1,1-Dimethylallyl acetate	$\text{CH}_2=\text{CH}-\text{CMe}_2-\text{OAc}$
VI	3,3-Dimethylallyl acetate	$\text{Me}_2\text{C}=\text{CH}-\text{CH}_2-\text{OAc}$
VII	Cyclohex-2-en-1-yl acetate	
VIII	1-Acetoxymethylcyclohex-1-ene	
IX	1-Acetoxy-[$^2\text{H}_2$]-methylcyclohex-1-ene	
X	2-Methylenecyclohexan-1-yl acetate	

normally occur below about 350°C , whilst the mass spectra were obtained at only 100°C . Additionally, there are metastable peaks in the mass spectra of II, III and IV at m/e 25.7 and in the mass spectrum of VI at m/e 36.1, which correspond to the fragmentation $\text{M}^+ \longrightarrow \text{M}-60$, showing that it is, at least partly, a mass spectral elimination.

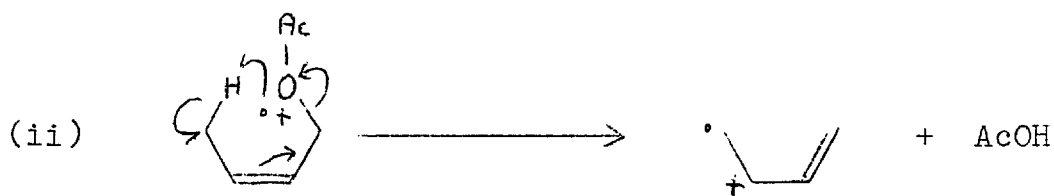
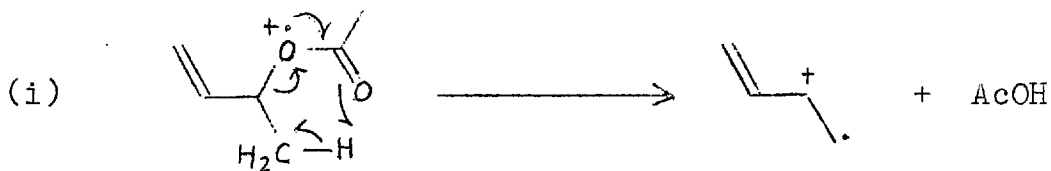
Table 21. Main ions in the mass spectra of allyl acetates.

	Compound					
	I	II	III	IV	V	VI [†]
M ⁺	<u>100</u> 0.6	<u>114</u> 0.1	<u>114</u> 1.5	<u>114</u> 3	<u>128</u> 3	<u>128</u> 0
M-42	<u>58</u> 15	<u>72</u> 26	<u>72</u> 47	<u>72</u> 16	<u>86</u> 10	-
M-43	<u>57</u> 11	<u>71</u> 9	<u>71</u> 6	<u>71</u> 13	<u>85</u> 7	-
M-57	<u>43</u> *100	<u>57</u> 6	<u>57</u> 11	<u>57</u> 7	<u>71</u> 17	-
M-58	<u>42</u> 4	<u>56</u> 1	<u>56</u> 1	<u>56</u> 4	<u>70</u> 12	-
M-59	<u>41</u> 14	<u>55</u> 19	<u>55</u> 9	<u>55</u> 19	<u>69</u> 20	-
M-60	<u>40</u> 4	<u>54</u> 12	<u>54</u> 9	<u>54</u> 13	<u>68</u> 44	<u>68</u> 71
M-61	<u>39</u> 13	<u>53</u> 5	<u>53</u> 5	<u>53</u> 6	<u>67</u> 28	<u>67</u> 100
M-75	-	<u>39</u> 12	<u>39</u> 20	<u>39</u> 20	<u>53</u> 17	<u>53</u> 72

* Mainly CH_3CO^+ which is base peak in five of the spectra.

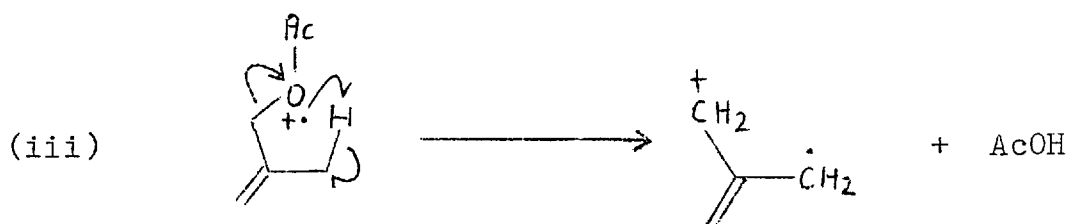
† m/e 43 is 45% and m/e 60 is 32%.

The two main mechanisms which explain the loss of acetic acid, involve either a 1,2-elimination (i) or a 1,4-elimination (ii).



Furthermore, a molecular ion with an α -methyl group as depicted in (i) can undergo allylic rearrangement to form an ion with a γ -methyl group as in (ii), and vice versa.

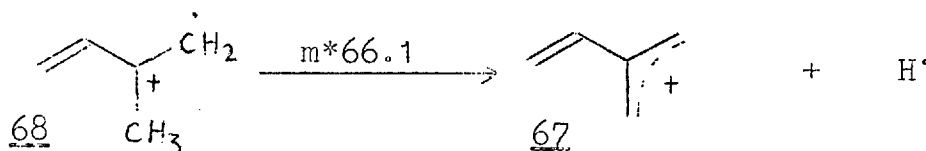
Compounds (I) and (III) which do not have a methyl group attached at either the α or γ positions can not lose acetic acid by mechanism (i) or (ii) and so the M-60 peak is smallest for these two compounds. It is, however, rather larger for III than for I and from this it can be inferred that there is some 1,3-elimination with the proton coming from the β methyl group as shown in (iii).



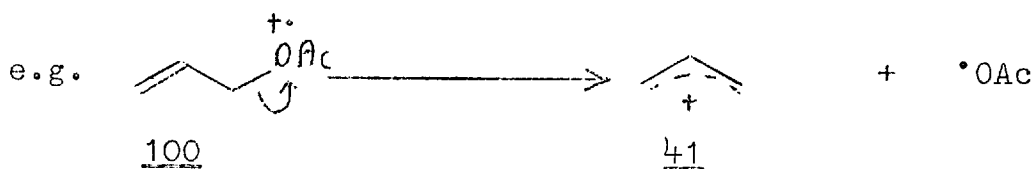
The acetates II and IV together with V and VI provide pairs of isomers which are interconvertible by an allylic rearrangement. The mass spectra of II and IV are very similar and it is possible that the molecular ions of both compounds undergo allylic rearrangements to give the same equilibrium mixture of two isomers. Loss of acetic acid can then proceed by either mechanism (i) or (ii). However, the mass spectra of V and VI are not similar, so that an allylic rearrangement is not being undergone by both compounds. It is possible that VI does partially isomerise in the same manner as II and IV, but V does not. The only

significant ions in the spectrum of V are M-60, M-60-1 and M-60-15 so that acetic acid is lost very rapidly before any allylic rearrangement can occur. This loss of acetic acid is facilitated by the six β protons which can participate in a 1,2-elimination (mechanism i).

All six compounds have an M-61 peak which is due to loss of an hydrogen atom from M-60. It is of greatest intensity in the spectra of V and VI because loss of $H^\bullet$ from the m/e 68 ion gives a carbonium ion with increased resonance stabilisation.

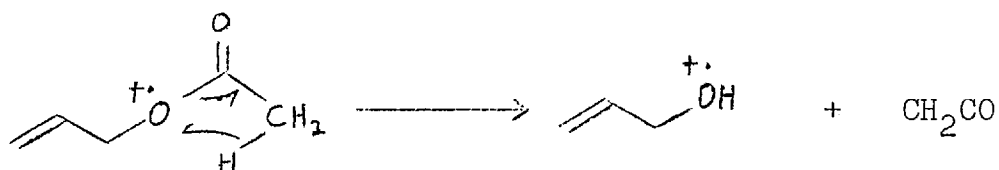


The loss of an acetoxy radical from the molecular ion of an allylic acetate gives a carbonium ion which has allylic stabilisation. This results in the M-59 ion being much more abundant than in the mass spectra of saturated acetates.

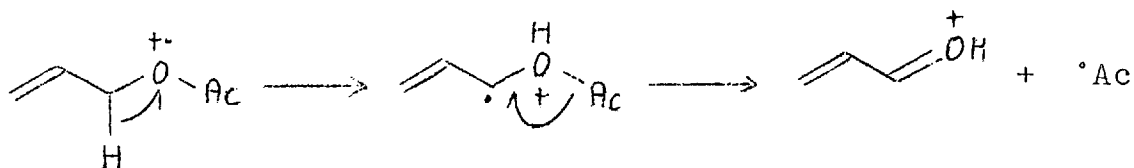


Compared with saturated acetates, the M-42 ion is more prominent in the spectra of some of these allylic acetates, especially compound III. This is probably a consequence of the loss of acetic acid being energetically unfavourable so that

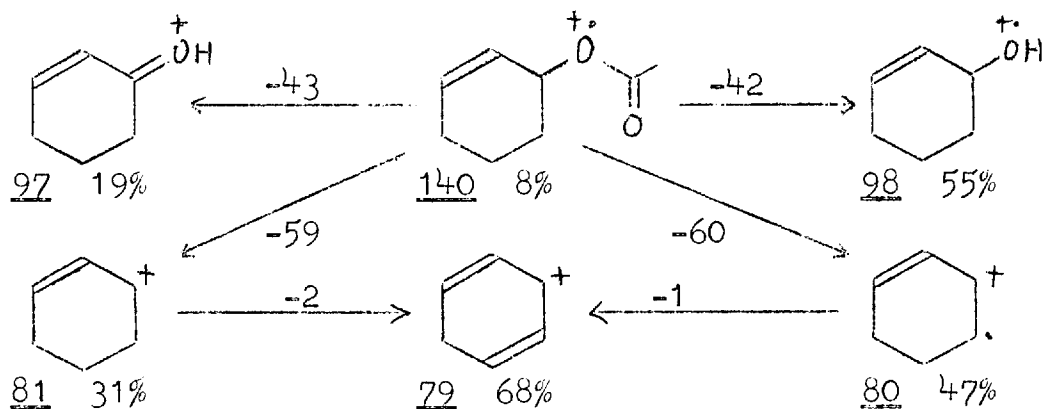
fragmentation takes a different course.



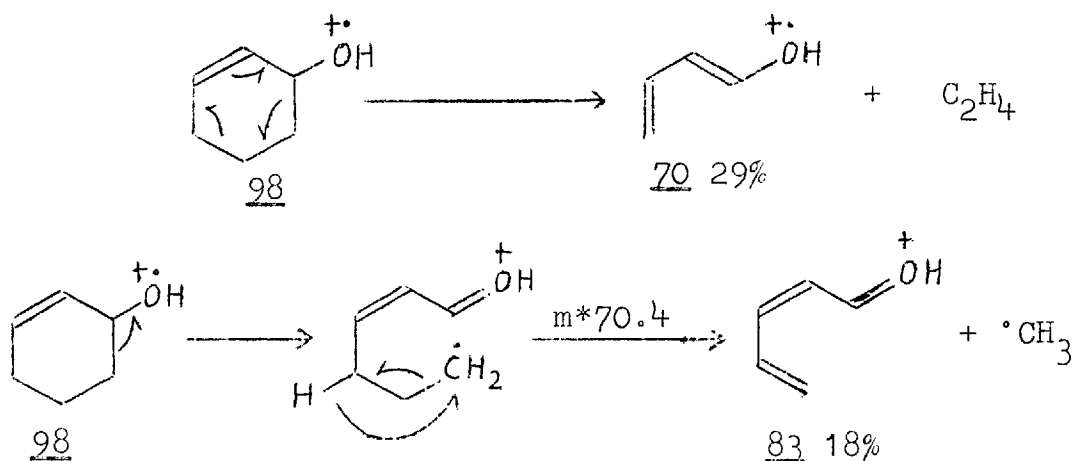
The M-43 peak does not appear in the mass spectra of simple acetates and its occurrence in the spectra of allylic acetates may be explained by the following mechanism. Allylic activation aids the shift of the proton, and the final ion has increased conjugation.



The mass spectrum of cyclohex-2-en-1-yl acetate (VII) shows similar features to those of acyclic allylic acetates. Thus there are M-42, M-43, M-59 (C_6H_9^+) and M-60 (C_6H_8^+) peaks. Metastable peaks at m/e 77.1 and 78.2 are due to the formation of the M-61 ion from M-59 and M-60 respectively.



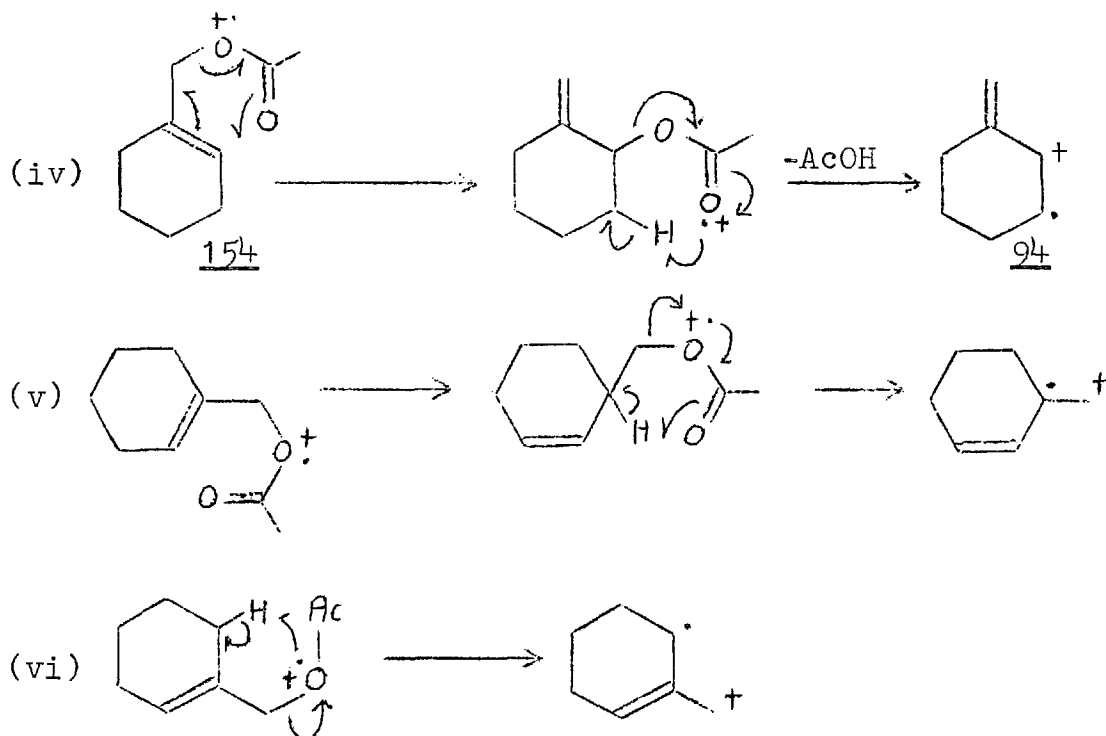
Two other ions appear in the spectrum of VII which are also present in the mass spectrum of cyclohex-2-en-1-ol.⁽¹⁰⁹⁾ This provides evidence that the ion m/e 98 has the same structure as the molecular ion of cyclohexenol and undergoes a retro Diels-Alder reaction to give m/e 70 ($C_4H_6O^+$) or loss of a methyl radical to form m/e 83 ($C_5H_7O^+$).



The intensities of the ions in the mass spectrum of cyclohexenol are m/e 98, 49%; m/e 83, 30% and m/e 70, 100%. Therefore relative to the ion at m/e 98 the intensities of the two fragment ions are reduced in the spectrum of cyclohexenyl acetate. The rate of decomposition of one ion to another is a function of $(1 - E_0/E)$, where E_0 is the activation energy for decomposition and E is the internal energy of the decomposing ion.⁽¹¹⁰⁾ When m/e 98 is formed by loss of ketene from the molecular ion, energy is lost and E is smaller than it is when m/e 98 is formed by direct ionisation. The rate of subsequent decomposition is

therefore slower and fewer fragment ions of mass 83 and 70 appear.

1-Acetoxymethylcyclohex-1-ene (VIII) was chosen as a system which has no proton in the 2 position relative to the acetoxy group and which has the protons in the 4 position sterically fixed so that a six membered transition state of type (ii) is impossible. The line diagram for this compound is shown in mass spectrum 17a and it can be seen that there is a small $M-42$ peak at m/e 112, but the main primary fragmentation is loss of acetic acid. The three possible mechanisms for this loss involve an allylic rearrangement or a double bond migration or a 1,3-elimination.



The mass spectrum of 2-methylenecyclohexan-1-yl acetate (X) (mass spectrum 17c) is very similar to that of VIII,

which implies that the molecular ion of VIII rearranges to give the molecular ion of X before fragmentation. This would be in agreement with mechanism (iv) and provides some proof that allylic rearrangements can occur in the mass spectrometer.

Pyrolysis of compound VIII or X at 500°C gives 1-methylenecyclohex-2-ene in high yield.⁽¹¹¹⁾ If the formulation of the m/e 94 ion is correct, then the behaviour of VIII and X under electron impact and pyrolysis is identical. However, this can not be taken as definite proof of the structure of m/e 94 because it has already been shown that differences occur in the mechanisms of thermal and mass spectral elimination of acetic acid (see page 154)

The only important fragmentation of the M-60 ion is the loss of a methyl radical to form the m/e 79 ion. In the mass spectrum of 1-acetoxy-[²H₂]-methylcyclohex-1-ene (IX), the m/e 94 peak is completely shifted to m/e 96, but the m/e 79 peak is partly shifted to m/e 80 and m/e 81 (see mass spectrum 17b), showing that there are losses of [•]CH₃, [•]CH₂D and [•]CHD₂. To account for this, at least three different mechanisms must be operating in the loss of methyl radical. The mass spectrum of 1-tetralone enol acetate provides another example of a compound which loses a methyl radical when no methyl group is present.⁽¹¹²⁾ The molecular ion loses ketene and then loses [•]CH₃ in which the three protons can come from any of the non-aromatic positions.

2,3 Mass Spectra of Aromatic Allylic Acetates.

This section is concerned with substituted and unsubstituted cinnamyl acetates and the isomeric 1-phenylallyl acetates. They will be discussed according to the substituent that is attached to the system.

(a) Unsubstituted

The low resolution mass spectrum of cinnamyl acetate was reported in the literature in 1960, but no reference was made to the mechanisms of formation of the ions.⁽¹¹³⁾ Mass spectrum 18a was obtained for cinnamyl acetate (XI) at 70eV and the accurate masses of some of the ions are given in table 22. Reducing the electron beam energy to a nominal 16eV (mass spectrum 18b) makes the major peaks more prominent and there is a large decrease in the intensity of the m/e 43 peak.

Table 23 shows the shifts of the main peaks in the mass spectra of $[2,4,6-^2\text{H}_3]$ -cinnamyl acetate (XII), $[3,5-^2\text{H}_2]$ -cinnamyl acetate (XIII), 3-phenyl- $[1-^2\text{H}_1]$ -allyl acetate (XIV) and cinnamyl $[^2\text{H}_3]$ -acetate (XV).

The peaks at m/e 134 and 133 are due to loss of ketene and loss of acetyl radical, respectively, by the same mechanisms as described for aliphatic allylic acetates. The m/e 105 (M-71) peak is a doublet, of which the main peak is due to C_8H_9^+ . There are no metastable peaks for its formation in the mass spectra of the unsubstituted compounds but methoxy cinnamyl acetates which have larger M-43 and

Table 22. Mass measurements on cinnamyl acetates.

Cinnamyl acetate

m/e	Observed Mass	Calculated Mass	Formula
117	117.0709	117.0704	C ₉ H ₉
116	116.0615	116.0626	C ₉ H ₈
115	115.0548	115.0548	C ₉ H ₇
105	105.0333	105.0340	C ₇ H ₅ O
	105.0703	105.0704	C ₈ H ₉
92	92.0626	92.0626	C ₇ H ₈
91	91.0548	91.0548	C ₇ H ₇

p-Methoxycinnamyl acetate

135	135.0449	135.0446	C ₈ H ₇ O ₂
	135.0803	135.0810	C ₉ H ₁₁ O
131	131.0496	131.0497	C ₉ H ₇ O
121	121.0649	121.0653	C ₈ H ₉ O
108	108.0571	108.0575	C ₇ H ₈ O
103	103.0544	103.0548	C ₈ H ₇

3,3-diphenylallyl acetate

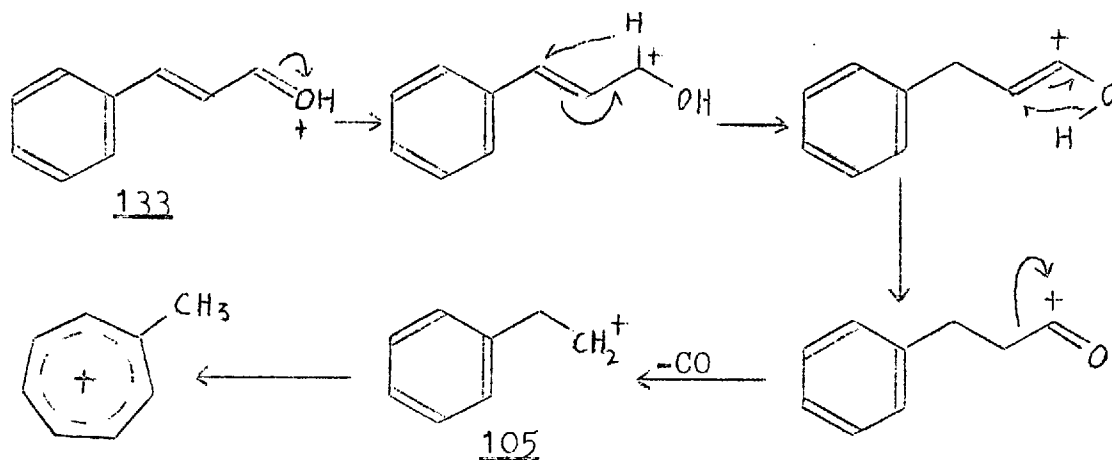
182	182.0729	182.0732	C ₁₃ H ₁₀ O
178	178.0790	178.0782	C ₁₄ H ₁₀
165	165.0701	165.0704	C ₁₃ H ₉
115	115.0546	115.0548	C ₉ H ₇
105	105.0343	105.0340	C ₇ H ₅ O
103	103.0544	103.0548	C ₈ H ₇

Table 23.

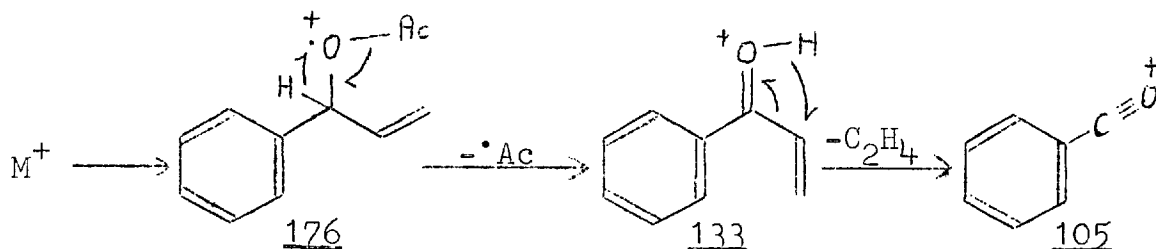
XI	XII	XIII	XIV	XV
176	179	178	177	179
134	137	136	135	135
133	136	135	134	133
117	120	119	118	117
116	119	118	117	116
115	118	117	116	115
	117	116	115	
105	108	107	106	105
92	95	94	93	93
			92	
91	94	93	92	91
			91	

M-71 peaks do have a metastable peak for $M-43 \rightarrow M-71$.

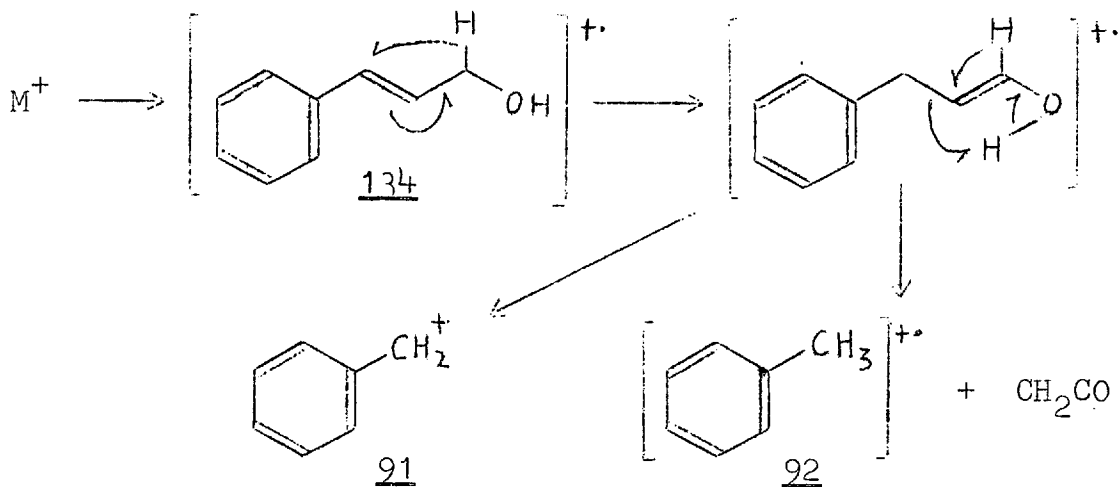
Therefore the following scheme is suggested for the formation of the m/e 105 ion in the mass spectrum of XI.



The less abundant ion at m/e 105, $C_7H_5O^+$ provides evidence for some allylic rearrangement of the molecular ion to that of 1-phenylallyl acetate. This would give an oxygen atom in the correct position for the formation of a benzoyl ion.

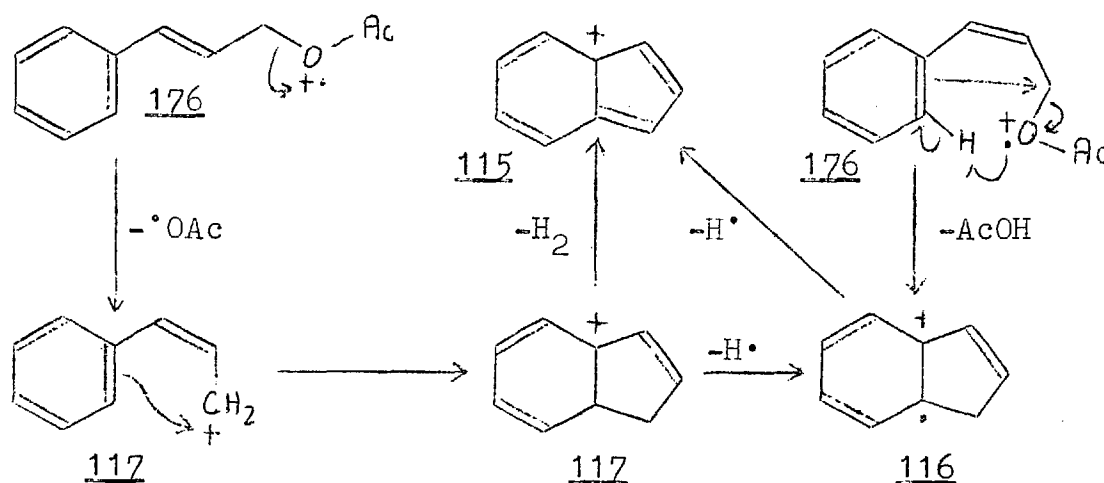


The m/e 92 ion probably has the structure of the toluene molecular ion, which requires migration of two protons to C(3) of the side chain. The results in table 23 show that one of the hydrogen atoms comes from C(1) and the other from the acetate methyl group. A metastable peak at m/e 63.1 is due to the formation of m/e 92 from m/e 134.

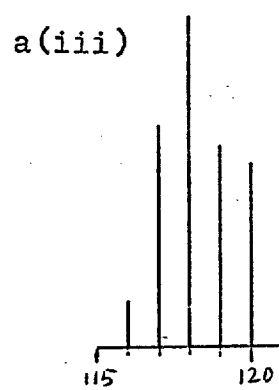
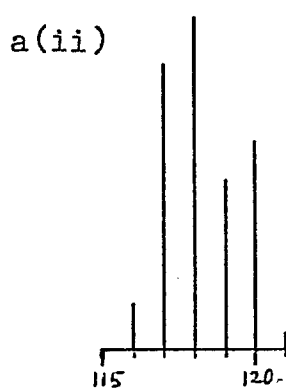
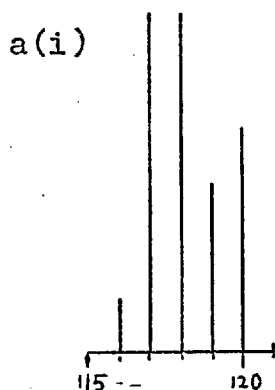


Similarly the ion at m/e 91 contains a proton which was originally at C(1) and is formed from the ion shown above by C(2)-C(3) fission.

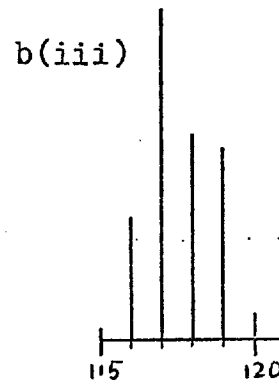
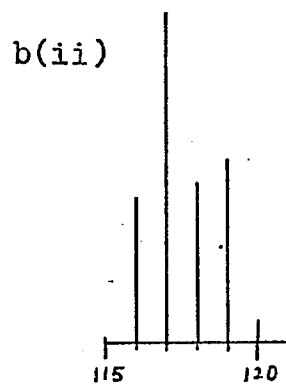
The group of peaks due to loss of 59,60 and 61 mass units appears at m/e 117,116 and 115, and there are metastable peaks due to the fragmentations $117 \rightarrow 116$, $116 \rightarrow 115$, $117 \rightarrow 115$ and $176 \rightarrow 116$. A 1,2-elimination of acetic acid from m/e 176 to form m/e 116 would give an allene, which is unlikely, and so there is the possibility of a mechanism involving loss of an ortho hydrogen from the phenyl ring.



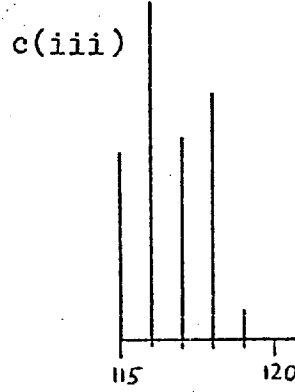
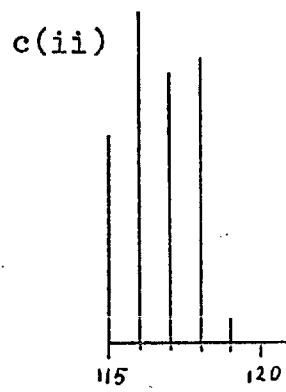
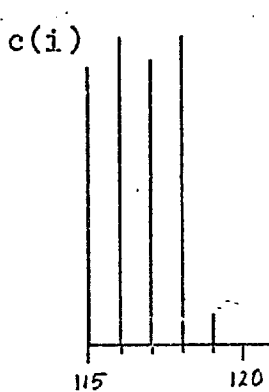
The mass spectra of the undeuteriated compound XI and the three deuteriated analogues XII, XIII and XIV were obtained under identical conditions and comparison of them has provided proof for the proposed mechanism. The mass spectra of the three labelled compounds in the region m/e 115 to 121 are shown in figure 5, diagrams a(iii), b(iii) and c(iii), and the resultant calculation of loss of hydrogen isotope from the ortho and para positions in the fragment ions of XII is as follows.



$[2,4,6-^2\text{H}_3]$ -cinnamyl acetate (XII)



$[3,5-^2\text{H}_2]$ -cinnamyl acetate (XIII)



3-phenyl- $[1-^2\text{H}_1]$ -allyl acetate (XIV)

(1) Heights of the molecular ions of XI and XII are compared and the amounts of mono, di and trideuteriated species in compound XII are calculated.

$$\text{viz. } d_1 \text{ 2.2\%; } d_2 \text{ 22.9\%; } d_3 \text{ 74.9\%.}$$

(2) The peaks between m/e 115 and 121 in the spectra of XI and XII are corrected for ^{13}C isotope peaks.

(3) The value of k is calculated so that:

$$115 + 116 + 117 = k(115' + 116' + 117' + 118' + 119' + 120')$$

Where 115, 116 and 117 are peak heights from (2) for compound XI and 115', 116', etc. are peak heights from (2) for compound XII.

Then let $115 = k115'$, $116 = k116'$, etc.

(4) Let x be the fraction of m/e 116 ions formed by XI which have lost one hydrogen atom from the ortho or para position.

Let y be the fraction of m/e 115 ions formed by XI which have lost one hydrogen atom from the ortho or para position.

Let z be the fraction of m/e 115 ions formed by XI which have lost two hydrogen atoms from ortho or para positions.

Then if deuterium isotope effects are ignored, the following equations relate the peak heights in the undeuteriated compound XI and the deuteriated compound XII.

$$115 = 115d_2z^{1/3} + 115d_1z^{2/3} + 115d_1y^{1/3}$$

$$116 = 115d_3z + 115d_2y^{2/3} + 115d_2z^{2/3} + 115d_1(1-y-z) + \\ 115d_1z^{1/3} + 116d_1x^{1/3} + 115d_1y^{2/3}$$

$$117 = 115d_3y + 115d_2(1-y-z) + 115d_2y^{1/3} + 116d_2x^{2/3} + \\ 116d_1x^{2/3} + 116d_1(1-x)$$

$$\underline{118} = 115d_3(1-y-z) + 116d_3x + 116d_2(1-x) + 116d_2x1/3 + 117d_1$$

$$\underline{119} = 116d_3(1-x) + 117d_2$$

$$\underline{120} = 117d_3$$

(5) The equations are solved for x,y and z. Since there are six equations and three variables there are several possible solutions. The equations selected for solution were those which related to the largest peaks,i.e.the ones involving 116,117 and 118. Solution of these gives:

$$x = 0.43 \quad y = 0.37 \quad z = 0.03$$

Unfortunately these solutions rely on a small difference between two large numbers and must be treated with caution.

The results obtained from all three compounds are summarised in table 24.

Table 24.

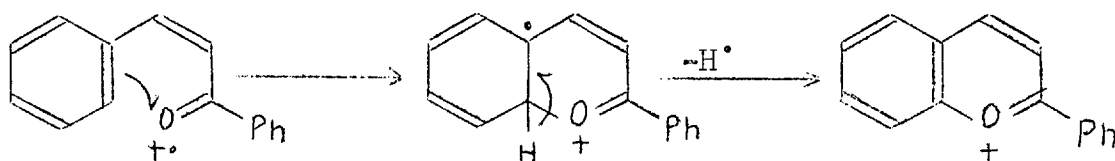
Position from which one proton is lost	Fraction of ions with this loss	
	m/e 116	m/e 115
<u>meta</u>	0.19	0.25
<u>ortho</u> or <u>para</u>	0.43	0.37
C(1) of side chain	0.44	0.87
Positions from which two protons are lost		
<u>meta</u>	-	0.02
<u>ortho</u> or <u>para</u>	-	0.03

The first observation to make on these results is that they are not self consistent. Thus the fraction of m/e 116 ions which have lost a proton from C(1) indicates that a mechanism may be operating whereby a proton is lost from C(1) before it is lost from the phenyl ring. However, the figures for loss of a proton from the ring do not permit this. The proton which is lost from the ring is not lost exclusively from the ortho positions but from all positions. The calculated figures for random loss are meta 0.4 and ortho/para 0.6, so that the experimental results fall well below these values, although they are in the correct ratio for random abstraction, i.e. 0.25/0.37. This probably represents a fairly high isotope effect for discrimination against deuterium, or there may be a small contribution from a second mechanism.

As a further test of the mechanism on page 167, it has been assumed that it accurately represents the fragmentation and the expected spectra for the deuteriated compounds XII, XIII and XIV have been calculated on this basis, using equations such as those on page 169. The results are presented in figure 5, diagrams a(i), b(i) and c(i) and it can be seen that there is some similarity to the observed spectra. However, when an isotope effect of 0.67 is introduced into the calculations, a rather better correlation with the observed spectra is obtained, as shown in diagrams a(ii), b(ii) and c(ii).

Therefore the mechanism for formation of m/e 117, 116 and 115 as shown on page 167 is substantially correct, with the one proviso that there is complete randomisation of the hydrogen atoms on the phenyl ring. Furthermore the mass spectrum of the deuteriated compound XIII has also been obtained at 16eV and this also shows random abstraction of hydrogen atoms from the phenyl ring in the loss of acetic acid.

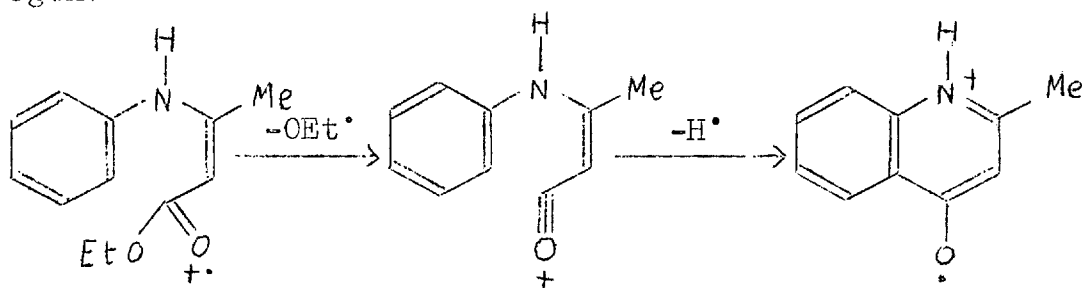
Further examples of the cyclisation of side chains on to a phenyl ring during mass spectrometry are to be found in the literature. Ronayne *et al.*⁽¹¹⁴⁾ have studied some compounds which contain a cinnamoyl group and many of these lose a hydrogen atom from the molecular ion. This can be illustrated for benzalacetophenone.



The spectrum of the $[2,4,6-^2\text{H}_3]$ derivative shows a 50% loss of deuterium in this reaction, indicating that there is a random loss of the hydrogen atom from the phenyl ring. It was suggested that randomisation was caused by migration of a hydrogen atom to the radical site followed by further migrations to new radical sites as they are formed. This is a plausible mechanism, and where randomisation of the substituents attached to a phenyl ring is mentioned in this

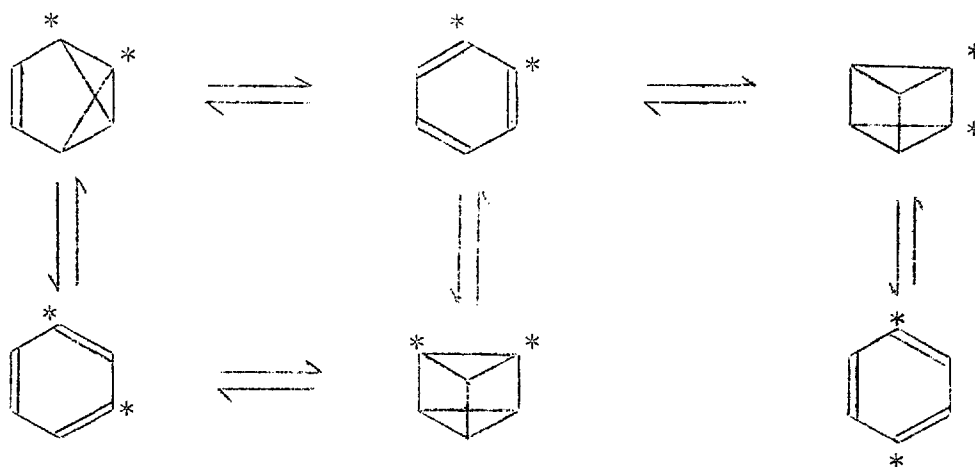
section, radical migration could explain the findings as well as the theory of prismane and benzvalene intermediates, which is described below.

Some aromatic compounds exhibit randomisation of the hydrogen atoms attached to the aromatic ring, but others do not. Thus benzene⁽¹¹⁵⁾ and pyridine⁽¹¹⁶⁾ show complete randomisation of the hydrogen atoms in the molecular ion, even at 15eV, whereas the enamine shown below exhibits no randomisation at 70eV and loses only the ortho hydrogen.⁽¹¹⁷⁾



The behaviour of benzonitrile is intermediate between the two extremes.⁽¹¹⁸⁾ The main decomposition of the molecular ion is loss of hydrogen cyanide and with the electron beam energy in the range 20-70eV the hydrogen atom is abstracted equally from all positions. However, at lower electron volts, the specificity of hydrogen abstraction increases so that at 13eV it is exclusively ortho.

Williams has suggested that randomisation does not take place by C-H bond cleavage but by reversible rearrangement of the benzene nucleus to prismanes and benzvalenes.^(116,118)



Such rearrangements would be analogous to those of benzene on photoexcitation. Two electronically excited species have been postulated to account for observed photochemical reactions,⁽¹¹⁹⁾ and these biradicals are shown below.



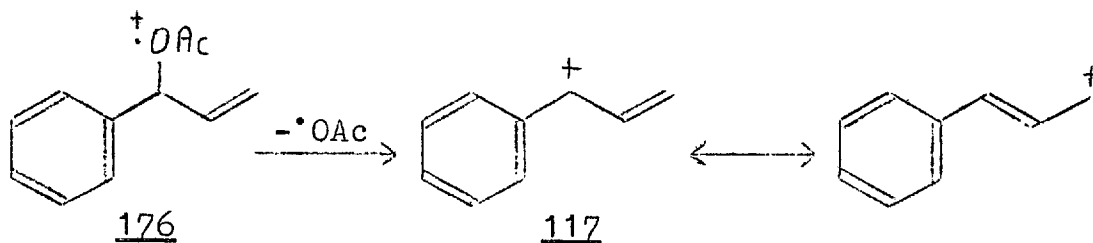
It will be noticed that these two species can readily form benzvalene and prismane.

If the behaviour of benzene and the benzene radical ion can be compared, it is concluded that the molecular ion must be in an electronically excited state if randomisation of the benzene ring substituents is to occur. If this theory is applied to the case of benzonitrile, it shows that the energy of the ionising electrons must be greater than 20eV before they all supply a sufficiently large quantum of energy to the benzonitrile molecule to excite

it out of the ground state.

Johnstone and Payling have recently used a different criterion for distinguishing between ground and excited state reactions of benzene derivatives in mass spectrometry. In an electronically excited state there is a higher resonance contribution from meta substituents than there is in the ground state. Thus the lack of correlation of meta substituents in an Hammett plot indicates an excited state.⁽⁷⁶⁾

Mass spectrum 18c is that of 1-phenylallyl acetate, and it can be seen that it is very similar to the spectrum of cinnamyl acetate except for a difference in height of some of the peaks. It has already been pointed out that an allylic rearrangement of cinnamyl acetate gives one of the peaks at m/e 105, so that the similarity of the two spectra is probably due to partial interconversion of the molecular ions. The m/e 116 and 115 ions have the same structure as those formed by cinnamyl acetates, but this does not imply that an allylic rearrangement of the molecular ion of 1-phenylallyl acetate is necessary. The allylic carbonium ion m/e 117 is essentially the same, whichever compound it is formed from.



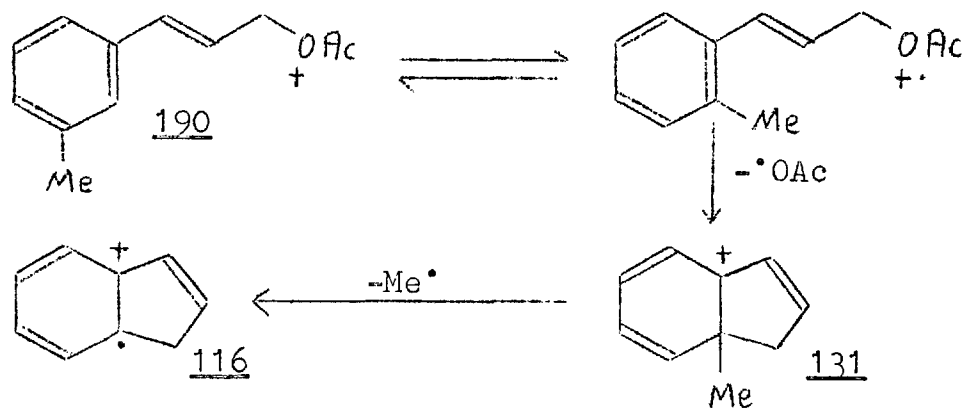
(b) Alkyl Substituted

The mass spectra of meta and para-methylcinnamyl acetate are almost identical and the main peaks in the spectrum of the meta compound are listed in table 25.

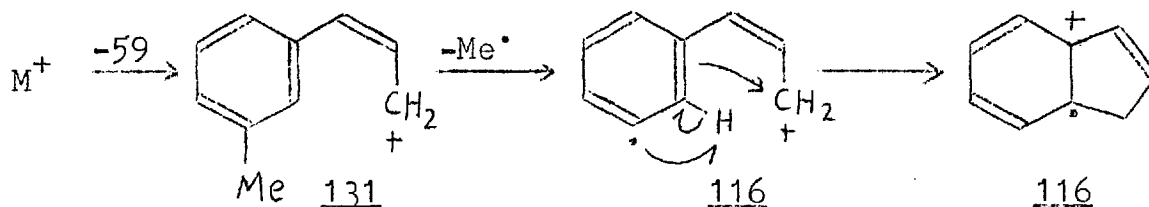
Table 25.

	M ⁺	M-42	M-43	M-59	M-60	M-61	M-71	M-74	M-75
m/e	190	148	147	131	130	129	119	116	115
%	34	34	22	28	18	27	29	17	50

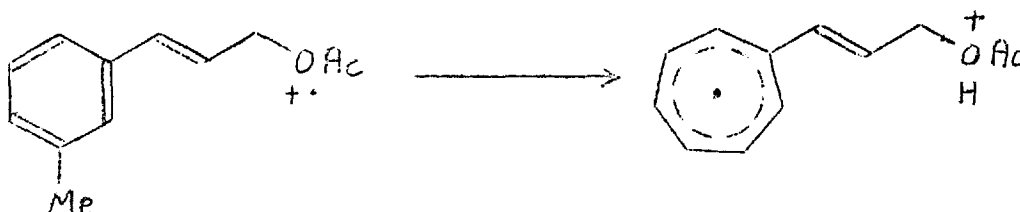
The mass spectra of these two compounds are very similar to that of cinnamyl acetate, with most of the peaks moved 14 units to higher mass. The exceptions to this are ions at m/e 116 and 115 which have lost the methyl group in the formation of an indene ion. If the methyl group is lost and there is cyclisation, then two mechanisms are possible. There can either be a randomisation of the substituents through prismane intermediates, or loss of a methyl radical followed by hydrogen atom migration to the radical site.



or

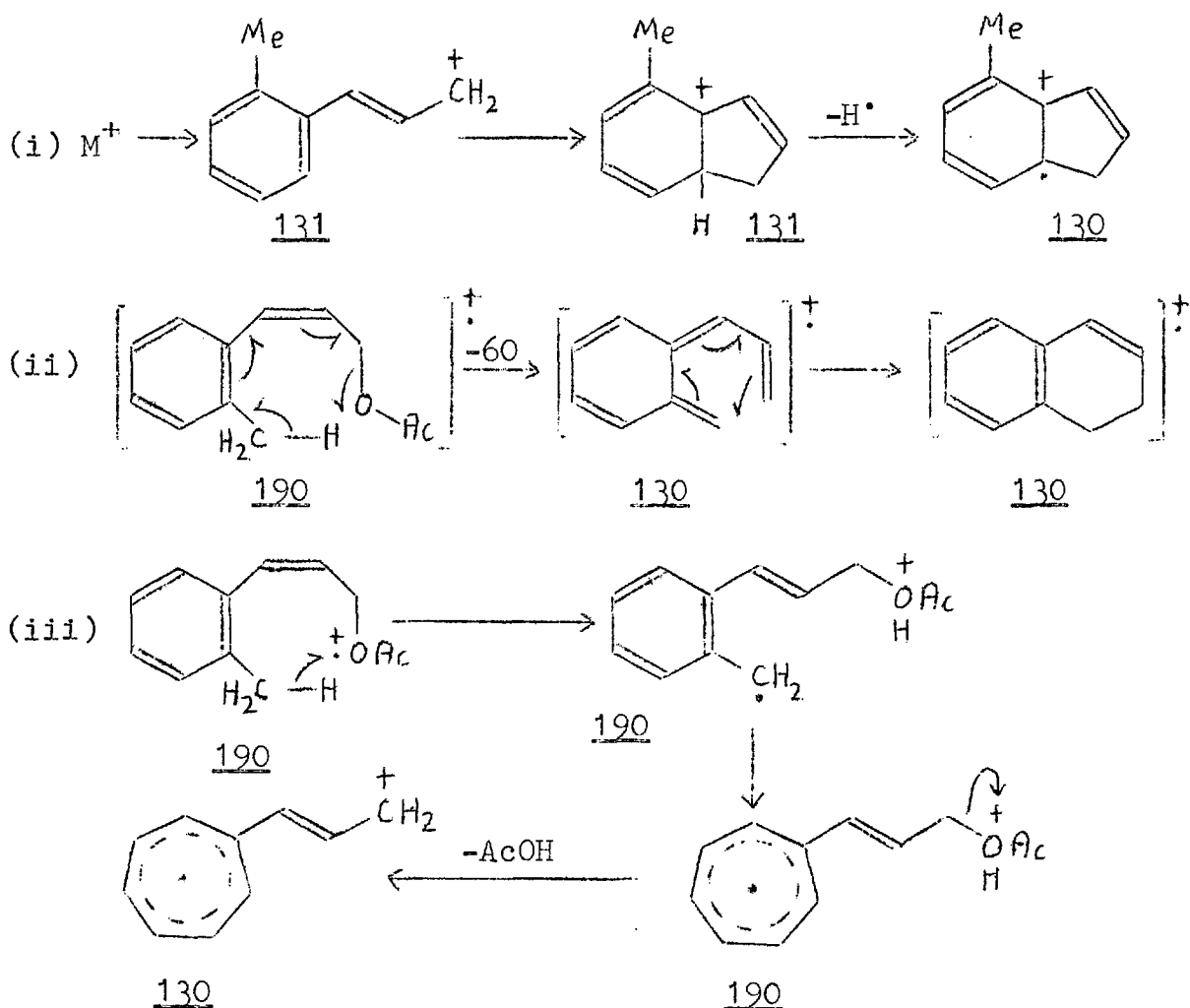


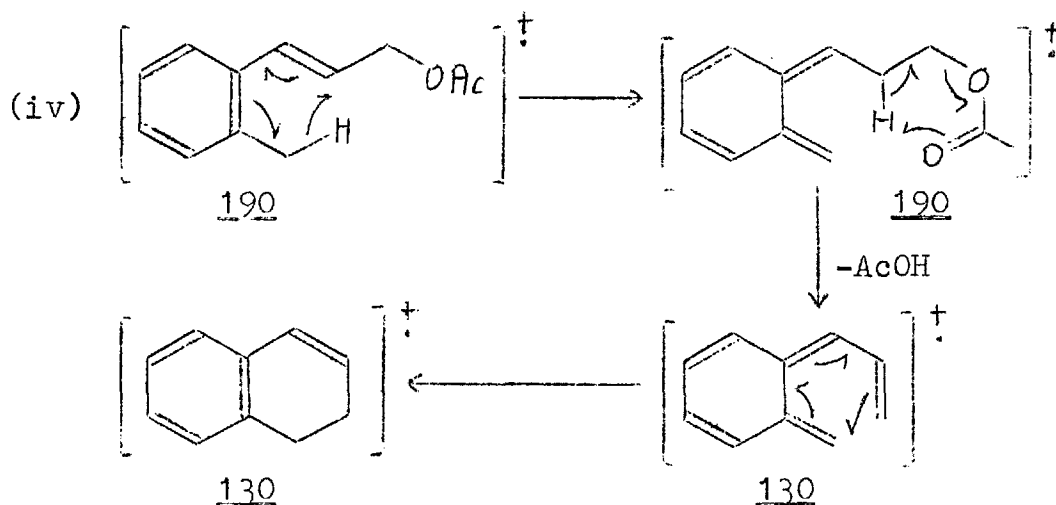
If ring expansion occurs to give a rearranged molecular ion of the type shown below, it is unlikely that a methyl radical would be lost, and it is expected that the mass spectra of cinnamyl acetate and methylcinnamyl acetates would show some significant differences. Hence ring expansion does not occur to any extent for the meta and para compounds.



The mass spectrum of o-methylcinnamyl acetate (XVI) (mass spectrum 19a) is somewhat different, because although it has peaks at the same m/e values, the intensity of the $M-60$ ion at m/e 130 is much increased, and it is the base peak, whilst m/e 43 which is normally the base peak, only has a relative intensity of 54%. The peak remains at m/e 130 in mass spectrum 19b which is that of o-methylcinnamyl $[\text{}^2\text{H}_3]$ -acetate (XVII), but is shifted mainly to m/e 132 in mass spectrum 19c which is that of o- $[\text{}^2\text{H}_3]$ -methylcinnamyl acetate (XVIII).

Reduction of the electron beam energy to 16eV gives the fragmentation patterns shown in mass spectrum 20. The m/e 130 ion formed by XVI becomes the only important fragment ion in the spectrum and m/e 129 and m/e 131 are much reduced in intensity, enabling the mechanism of loss of acetic acid to be studied more clearly. Compound XVIII shows a 65% shift of m/e 130 to 132 and 35% shift to 133, showing that 65% of the acetic acid is lost as $C_2H_3DO_2$ and the remainder as $C_2H_4O_2$. There are four possible mechanisms for the loss of acetic acid.



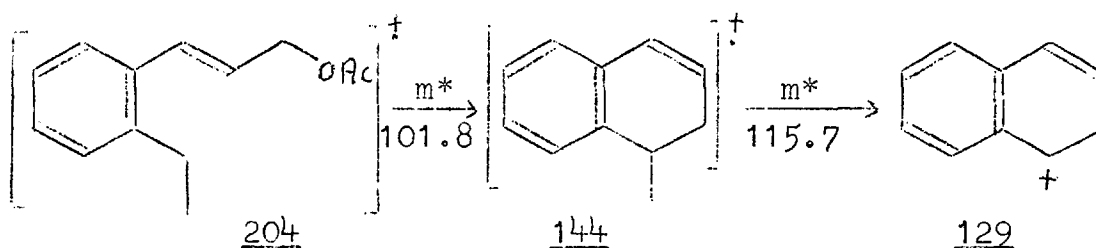


Mechanism (i) would give a loss of $C_2H_4O_2$, in the spectrum of XVIII, and both (ii) and (iii) would give a loss of $C_2H_3DO_2$, whereas (iv) would give an equal loss of both species (excluding isotope effects). If mechanism (i) is operating but (iv) is not, then there must be 65% of acetic acid lost by mechanism (ii) or (iii). On the other hand, if (iv) is operating but (i) is not, then mechanisms (ii) and (iii) are only responsible for 30% of the loss of acetic acid. Therefore it can be stated conclusively that the eight membered transition state represented by (ii) or (iii) is occurring to an extent of between 30 and 65%. A metastable peak at m/e 89.0 is due to a one step loss of acetic acid from the molecular ion.

The fact that the ortho compound gives a different mass spectrum from the meta and para isomers shows that complete randomisation of the substituents in the molecular ion does not occur. This is probably a kinetic factor, so that loss of acetic acid involving a proton from the ortho

methyl group occurs at a faster rate than any randomisation. Alternatively, if a radical migration mechanism as shown on page 177 is responsible for the apparent randomisation, then loss of acetic acid from the ortho compound is faster than loss of a methyl radical.

The main peaks in the mass spectrum of o-ethylcinnamyl acetate are at m/e 144 and 129 and these can be rationalised on the basis of a mechanism of type (ii).



(c) Halogen Substituted

Mass spectrum 21a is that of p-chlorocinnamyl acetate (XIX); the spectra of the ortho and meta isomers are almost identical to this, so that no ortho effect is exhibited. This is in direct contrast to the diester shown below which loses chlorine only from the ortho isomer.⁽¹²⁰⁾

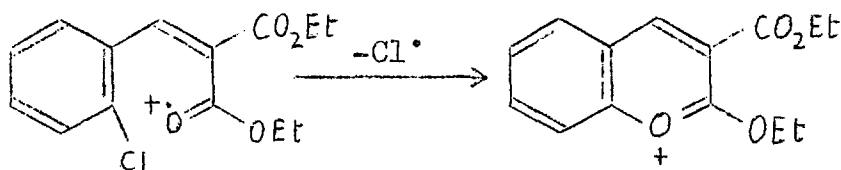


Table 26 compares the heights of the significant peaks in the spectra of XIX, p-bromocinnamyl acetate (XX) and

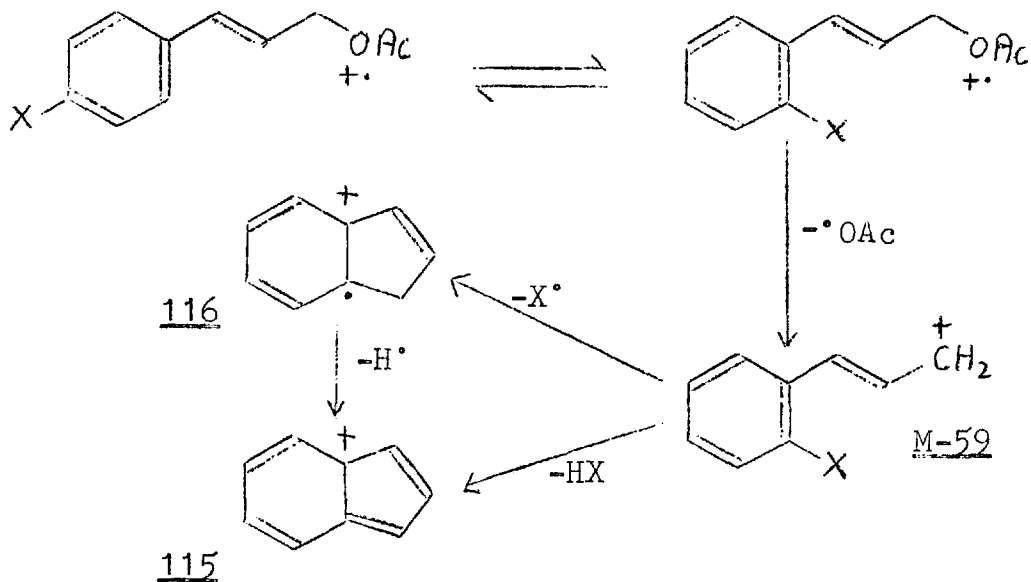
p-fluorocinnamyl acetate (XXI).

Table 26

Comp		M ⁺	M-42	M-43	M-59	M-60	M-61	M-71			
XIX	m/e	210	168	167	151	150	149	139	133	116	115
	%*	21	29	20	14	7	4	12	10	15	48
XX	m/e	254	212	211	195	194	-	183	133	116	115
	%*	22	35	17	5	5	-	7	8	29	43
XXI	m/e	194	152	151	135	134	133	123	-	-	115
	%	21	37	25	21	22	47	12	-	-	5

* Where there is an halogen isotope peak at higher mass, it has been added to the lower mass one, which is recorded in table.

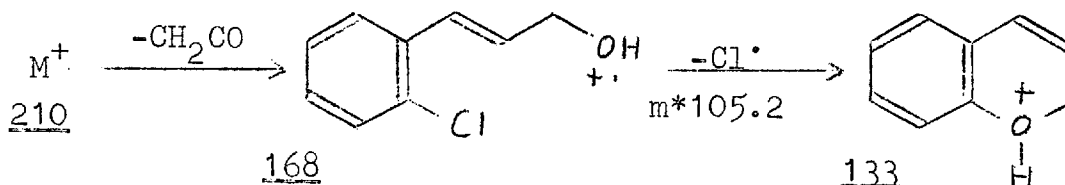
It can be seen from this that there are two competing fragmentations of the M-59 ion which can lose either an hydrogen atom to give M-60 or an halogen atom to give m/e 116.



The extent to which hydrogen and halogen are lost can be related to the strength of the C-X bond. The C-F bond energy is 93 K.cal/mole and C-H is 87 K.cal/mole, so that the m/e 133 peak is larger than the m/e 115 peak in the spectrum of XXI. However, the C-Cl bond (70 K.cal/mole) and the C-Br bond (58 K.cal/mole) are much weaker than the C-H bond, so that the halogen is preferentially lost to give large peaks at m/e 115 and 116 in the mass spectra of XIX and XX.

The mechanism for the formation of m/e 115 requires randomisation of the substituents on the phenyl ring in the molecular ion, and that there should be no loss of hydrogen from the phenyl ring during the formation of m/e 115. This was tested by running the mass spectrum of 4-chloro-[2,6-²H₂]-cinnamyl acetate, which lost only 16% of the deuterium (corrected for incomplete deuteration) in the formation of the ion with the structure of m/e 115 shown on the previous page.

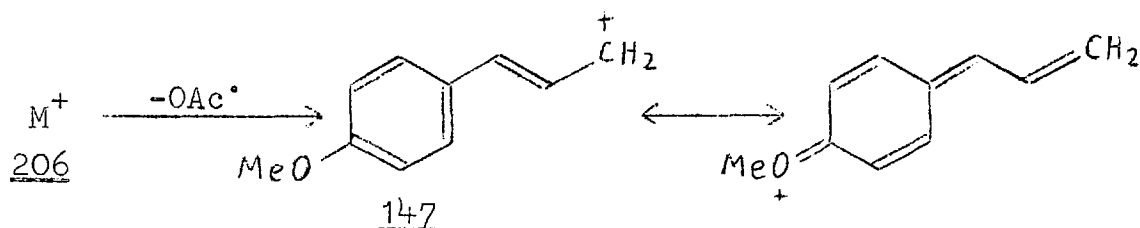
The weakness of the C-Cl and C-Br bonds is further demonstrated by the presence of an ion m/e 133 in the spectra of XIX and XX which is due to loss of ketene, followed by loss of a halogen atom.



The mass spectra of 1- p-chlorophenyl -allyl acetate and its bromo and fluoro analogues have been obtained and they give peaks at the same m/e values as the cinnamyl isomers. There are differences in intensities, such that the molecular ion is very weak (2-4%) and the M-43 peak is much smaller, but M-60 is rather larger.

(d) Methoxy Substituted

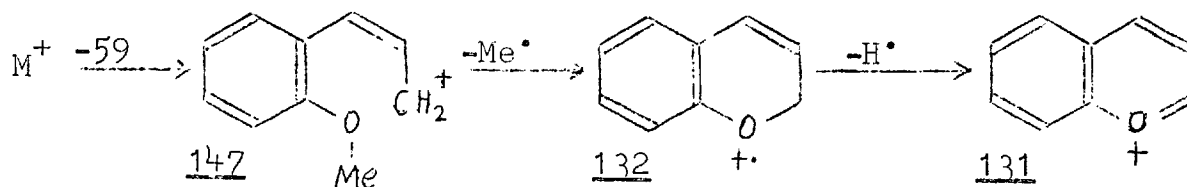
The elementary compositions of some ions in the mass spectrum of p-methoxycinnamyl acetate are given in table 22 and the line diagram is shown in mass spectrum 21b. There are peaks at m/e 164 and 163 due to loss of ketene and acetyl radical respectively, but the main fragment peak is at M-59. The M-59 ion is stabilised by the electron donating properties of the methoxy group so that there is little tendency for it to fragment further and the m/e 115 and M-60 ions are not very abundant.



Two different ions are responsible for the M-71 peak and these are formed by similar mechanisms to those given on page 165 for cinnamyl acetate.

Certain other prominent ions are not formed by the compounds so far discussed, namely the ions at m/e 131, 108 and 103. The m/e 132 ion which is probably formed by loss of

a methyl group from a rearranged M-59 ion, loses an hydrogen atom to form a fully aromatic system which is the m/e 131 ion.



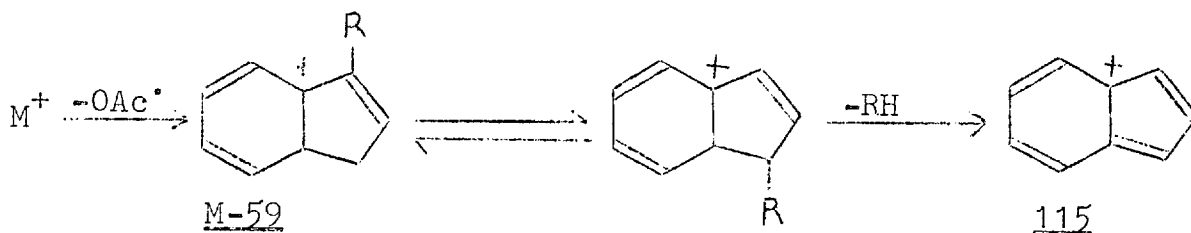
In the absence of any labelling experiments, there will be no speculation on the origin of m/e 108 or 103 ions. The remaining ion which has been mass measured is m/e 121 which proves to be a methoxytropylium ion corresponding to m/e 91 in the mass spectrum of cinnamyl acetate.

The mass spectrum of o-methoxycinnamyl acetate is very similar, except that the m/e 147 ion is not quite so abundant.

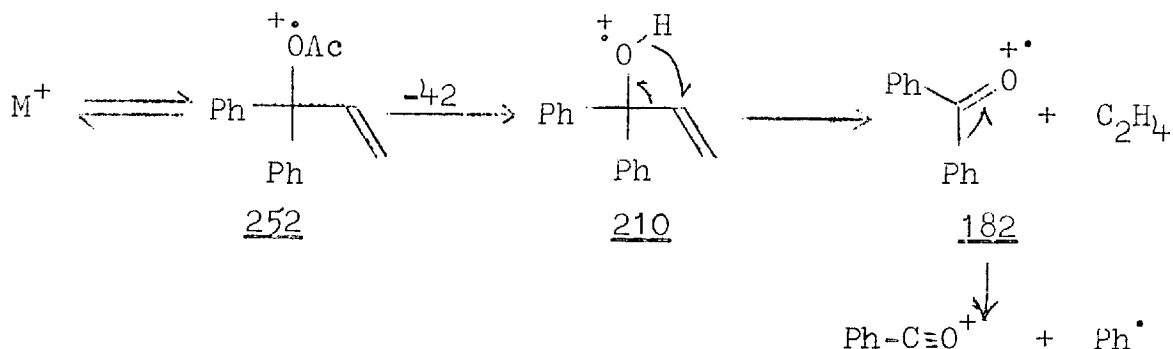
(e) Side Chain Substituted

The mass spectrum of 3,3-diphenylallyl acetate (XXII) and 3-methyl-3-phenylallyl acetate (XXIII) are shown in mass spectrum 22 and mass measurements on XXII are given in table 22. Both compounds form M-42, M-43, M-59, M-60, M-61 and M-71 ions by the usual mechanisms, but in addition these two compounds form an m/e 115 ion which has lost the substituent from the side chain of the cinnamyl acetate. There is a metastable peak in the spectrum of XXII at m/e 68.5 which is due to the formation of m/e 115 from m/e 193 (M-59). This represents a one step loss of $H^\bullet$ and $Ph^\bullet$ and as such it is difficult to write a satisfactory mechanism, unless it is postulated that there is scrambling of the substituents on

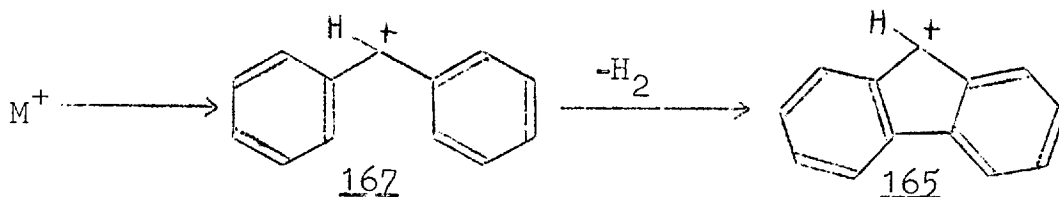
the five membered ring.



The other major peaks in the mass spectrum of XXII are not given by ordinary cinnamyl acetates, and the following mechanisms are suggested for some of them. The m/e 182 ion is formed after an allylic rearrangement of the molecular ion.

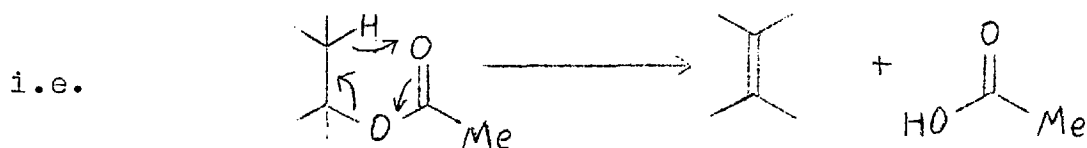


The ion of mass 167 is equivalent to the m/e 91 ion formed by cinnamyl acetate, but in this case it can lose a hydrogen molecule to form m/e 165.



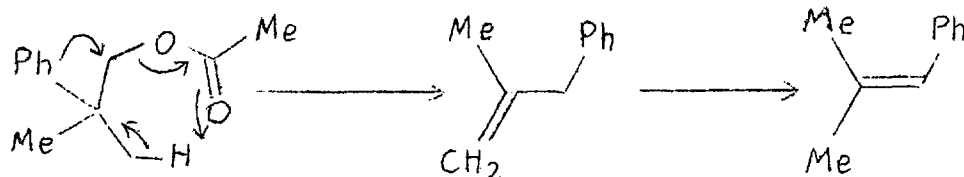
2,⁴ Pyrolysis of Cinnamyl Acetate

Pyrolysis of acetates at temperatures between 350° and 600°C results in the cracking out of acetic acid and the formation of an olefin. This reaction has been reviewed by De Puy and King⁽¹²¹⁾ who have correlated the evidence on the mechanism, which in most cases is a concerted cis 1,2-elimination.



Substituent effects indicate that there is a small amount of charge separation in the breaking of the C-O bond. The cis elimination is often accompanied by a small amount of trans elimination and the latter may be due to formation of a carbonium ion or a radical intermediate.

Kwart and Hoster have reported the pyrolysis of neophyl acetate at 550-600°C, which is described as the first example of a gas phase skeletal rearrangement.⁽¹²²⁾



Formation of a carbonium ion followed by a Wagner-Meerwein type migration of the phenyl group would be an attractive

mechanism, but both ionic and radical mechanisms were disproved and the 7-membered concerted transition state proposed.

It is postulated in this thesis that the electron impact induced elimination of acetic acid from cinnamyl acetate gives an indene ion. Therefore cinnamyl acetate has been pyrolysed to find out whether there is any correlation between mass spectral and thermal behaviour.

At a temperature of 500°C and a pressure of 1mm., cinnamyl acetate was distilled unchanged through the apparatus, but at 600° in a stream of nitrogen or 750° under a pressure of 1mm., the ester was 95% destroyed. The gas chromatograms of the two pyrolysates were nearly identical but the run at atmospheric pressure was accompanied by extensive charring, so that for the preparative work the pyrolysis was carried out under reduced pressure.

The gas chromatogram of the neutral volatile fraction is shown in figure 6 and the compounds which have been identified are listed below. The yields of the various compounds in this fraction have been obtained from the areas under the peaks in the chromatogram.

A Styrene	25%
B Phenylacetylene	6%
C Indene	39%
D (1,2-Dihydronaphthalene)	
(1,4-Dihydronaphthalene)	9%

Figure 6.

188.

Gas Chromatogram of crude Pyrolysate from
Cinnamyl Acetate at 750° and 1mm pressure.

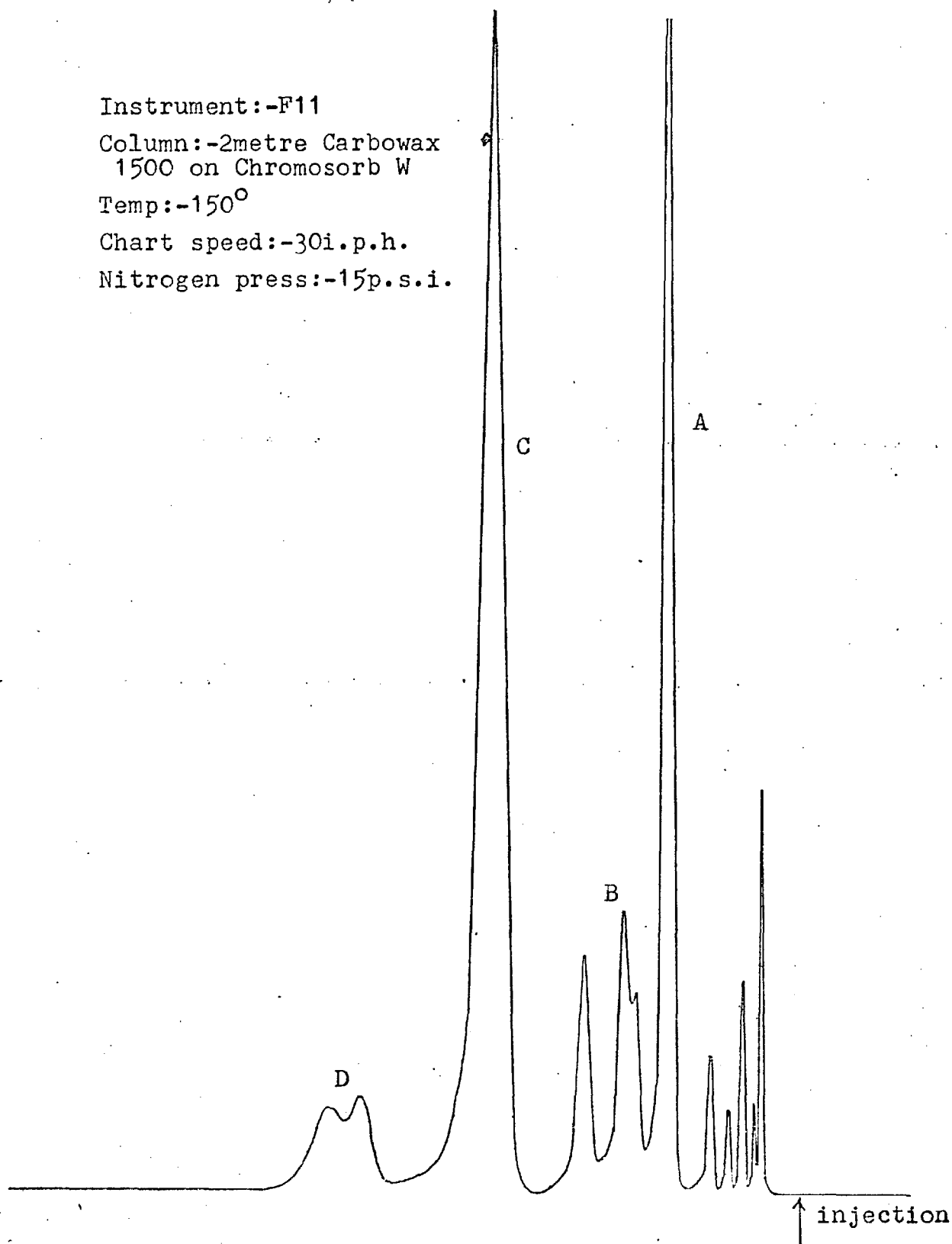
Instrument:-F11

Column:-2metre Carbowax
1500 on Chromosorb W

Temp:-150°

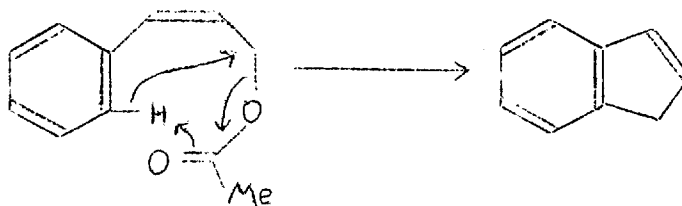
Chart speed:-30i.p.h.

Nitrogen press:-15p.s.i.

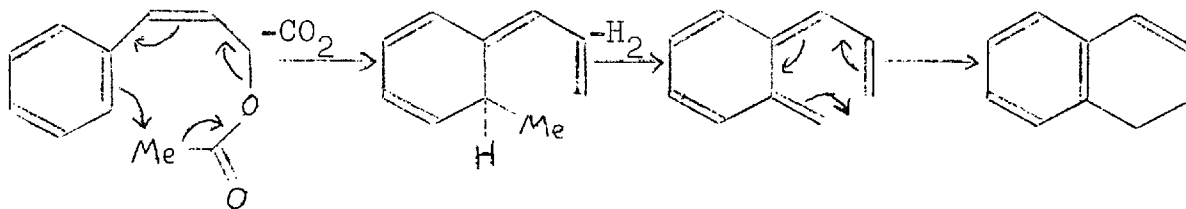


When this fraction was allowed to stand for a few days without any precautions being taken to exclude light or air, another compound could be detected by gas chromatography which had a longer retention time than D and was shown to be naphthalene. This is probably formed by oxidation of the dihydronaphthalenes and there is a precedent for this, because exposure of 1,2 or 1,4-dihydronaphthalene to ultra-violet light rapidly converts them to naphthalene.⁽¹²³⁾

The elimination of acetic acid from cinnamyl acetate is shown below as concerted, by analogy to the pyrolytic behaviour of neophyl acetate, but radical or ionic mechanisms could equally apply.



The formation of C_{10} hydrocarbons on pyrolysis is very interesting, because assuming it is a unimolecular reaction, a carbon atom from the acetate group must enter the carbocyclic system.



PART 3

Experimental

General.

Infra-red spectra were obtained using a Unicam SP200 spectrometer, with the sample in the form of a nujol mull, or a liquid film (L.F.) between sodium chloride plates, or in solution in a sodium chloride cell. Ultra-violet spectra were recorded on a Unicam SP800 instrument. Optical rotations were obtained in 10cm cell in a Hilger-Watts Microptic polarimeter.

Nuclear magnetic resonance spectra were obtained on either a Varian Associates 100 MHz HA-100, or a 60 MHz A-60 spectrometer. In all cases, tetra-methyl silane was used as an internal standard.

The purity of liquids was checked using a Perkin-Elmer F11 gas chromatograph fitted with a 2 metre Carbowax 1500 on Chromosorb W column.

The purity of solids and high boiling liquids was checked by thin layer chromatography (t.l.c.) on silica gel; using iodine vapour for detection of components.

Melting points were determined on a Kofler Micro Heating Stage.

Unless otherwise stated, solutions were dried over anhydrous sodium sulphate.

3,1 Glycosides

Tri-[²H₃]-acetyl fusicoccin-A

[²H₆]-Acetic anhydride (30mg.) was added to an ice-cold solution of fusicoccin-A (10mg.) in pyridine (0.2ml.). The solution was kept at room temperature for 24hrs., poured into ice-water (10ml.) and extracted with chloroform. The chloroform solution was washed with water, dried and evaporated under reduced pressure to yield a syrup. It was crystallised from a small volume of ethanol. Yield 7.5mg.(66%); m.p.mainly 97-103° but only completely at 130°.

Hexa-acetyl fusicoccin-F

Fusicoccin-F (12mg.), (supplied by Dr.R.Thomas), pyridine (0.2ml.) and acetic anhydride (0.1ml.) were allowed to react as above. The product was a syrup which could not be crystallised. It was purified by preparative t.l.c.on silica gel, developing with 2vols.benzene/1vol.acetone. Yield 7mg.(40%).

Methyl 6-O-trityl- α -D-glucopyranoside

A solution of methyl α -D-glucopyranoside (14.0g.) and trityl chloride (20.0g.) in pyridine (100ml.) was warmed on the steam bath for 2hrs. The solution was cooled, poured into water(600ml) and the product extracted with chloroform. The chloroform solution was washed well with water, dried and the solvent was evaporated under reduced pressure to give a syrup which was crystallised from ethanol. Yield 28g.(89%); m.p.partially at 80° and finally at 130-132°. (lit.⁽²³⁾ m.p.80°, 128-130° or 151-152°, depending on degree of solvation)

Methyl 6-O-trityl-2,3,4-tribenzoyl- α -D-glucopyranoside⁽²³⁾

Methyl 6-O-trityl- α -D-glucopyranoside (15g.) in pyridine (100ml.) was treated with benzoyl chloride (18ml.) with ice cooling. The mixture was kept at room temperature overnight and the product isolated as above to give a yellow uncrystallisable syrup. Chromatography on silica gel using benzene/10% acetone gave the main component (fastest running) as a syrup. Yield 15g.(78%); $\nu_{\max}(\text{CCl}_4)$ 1715 & 1595 cm^{-1}

Methyl 2,3,4-tribenzoyl- α -D-glucopyranoside⁽²³⁾

Methyl 6-O-trityl-2,3,4-tribenzoyl- α -D-glucopyranoside (5.5g.) in glacial acetic acid (10ml.) was cooled in ice and treated with 45% hydrogen bromide in acetic acid (1.5ml.). After 5 min.the precipitated trityl bromide was filtered off and the solution poured into ice-water. The syrup was extracted with chloroform,washed with saturated aqueous sodium bicarbonate,water,and dried. Removal of the solvent under reduced pressure gave a syrup,containing three compounds. The mixture was chromatographed on silica gel using benzene/15% acetone and the second component eluted,was collected and isolated as a colourless glass. Yield 2.45g.(66%);

$\nu_{\max}(\text{CHCl}_3)$ 3520,1715 & 1595 cm^{-1}

Methyl 6-O-methyl-2,3,4-tribenzoyl- α -D-glucopyranoside⁽²³⁾

Methyl 2,3,4-tribenzoyl- α -D-glucopyranoside (2.45g.), methyl iodide (20ml.) and silver oxide (5.5g.) were stirred and refluxed for 5hrs. The product was filtered and the filtrate concentrated to a glass. The least polar compound

was isolated by chromatography on silica gel using benzene/10% acetone. Yield 1.94g. (77%); m.p. 119-120° (ethanol); $\nu_{\max}$ (nujol) 1720 cm^{-1} , (lit⁽²³⁾ m.p. 116-117°).

Methyl 6-O-methyl-2,3,4-triacetyl- α -D-glucopyranoside

Methyl 6-O-methyl-2,3,4-tribenzoyl- α -D-glucopyranoside (1.9g.) in chloroform (10ml.) was treated with a solution of sodium (0.49g.) in ethanol (10ml.) at 0°C. After $\frac{1}{2}$ hr. at 0°, water and solid carbon dioxide were added, and the mixture evaporated to dryness. The residue was extracted with hot acetone, and the acetone solution evaporated to a syrup, which was treated with pyridine (6ml.) and acetic anhydride (3ml.). After 2 days the solution was poured into ice-water and the product isolated in the usual way. The syrup was purified by chromatography on silica gel using benzene/10% acetone, and then distilled. Yield 0.92g. (75%); b.p. 122-124°/0.001mm.; $[\alpha]_D^{21} +141^\circ$ (c1.7 in CHCl_3); $\nu_{\max}$ (CCl_4) 1750 cm^{-1} ; τ 7.96, 7.93 and 7.90 (OAc, singlets, 3H), 6.63 and 6.57 (OMe, singlets, 3H). (Found: C, 50.13; H, 6.86. $\text{C}_{14}\text{H}_{22}\text{O}_9$ requires C, 50.30; H, 6.63%).

Methyl 6-O-trityl-2,3,4-triacetyl- α -D-glucopyranoside

Methyl 6-O-trityl- α -D-glucopyranoside (2.1g) was treated with acetic anhydride (5ml.) and pyridine (10ml.). After 1 day the solution was poured into water and the product extracted with chloroform. Yield 1.7g. (63%); m.p. 134-135° (ethanol). (lit⁽¹²⁴⁾ m.p. 136°).

Methyl 2,3,4-triacetyl- α -D-glucopyranoside

Methyl 6-O-trityl-2,3,4-triacetyl- α -D-glucopyranoside

(1.3g.) in glacial acetic acid (2.6ml.) was cooled in ice and 45% hydrogen bromide in glacial acetic acid (0.4ml.) added. After 5 min. the mixture was filtered, the filtrate poured into ice-water and extracted with chloroform. The solution was washed with 2% aqueous sodium hydroxide, water, and then dried. The product was obtained as a colourless syrup. Yield 0.57g. (77%); $\nu_{\max}$ (CHCl₃) 1740 and 3500 cm⁻¹

Methyl 2-O-methyl-3,4,6-triacetyl- α -D-glucopyranoside ⁽²⁷⁾

Methyl 2,3,4-triacetyl- α -D-glucopyranoside (380mg.) and silver oxide (2g.) were stirred in refluxing methyl iodide (5ml.) for 8 hrs. The product was filtered and the filtrate evaporated to dryness. Yield 93mg. (24%); m.p. 120-122° (ethanol). (lit ⁽²⁷⁾ m.p. 120°).

Methyl 4,6-O-benzylidene- α -D-glucopyranoside ⁽¹²⁵⁾

Methyl α -D-glucopyranoside (28g.), powdered anhydrous zinc chloride (21g.) and benzaldehyde (70ml.) were stirred together for 3 hrs. and then kept at 0°C overnight. Water (200ml.) was added and the solid filtered off, washed with water, petroleum ether, and dried. It was recrystallised from ethanol.

Yield 16.5g. (40%); m.p. 167°; $\nu_{\max}$ (nujol) 3460, 3200, 1470, 1460, 760 and 700 cm⁻¹ (lit ⁽¹²⁵⁾ m.p. 164-165°).

Methyl 4,6-O-benzylidene-2-O-tosyl- α -D-glucopyranoside ⁽³¹⁾

Tosyl chloride (4.8g.) in pyridine (15ml.) was added to a solution of methyl 4,6-O-benzylidene- α -D-glucopyranoside (5.6g.) in pyridine (15ml.) with ice cooling. After 2 days at room temperature the product was poured into water and extracted

with chloroform. Yield 6.45g. (75%); m.p. 122-136° (ethanol); $\nu_{\max}$ (nujol) 3500, 1590, 1470 and 1460 cm^{-1} (lit⁽³¹⁾ m.p. 153-154°)

Methyl 4,6-O-benzylidene-3-O-methyl-2-O-tosyl- α -D-glucopyranoside

Methyl 4,6-O-benzylidene-2-O-tosyl- α -D-glucopyranoside (1.0g.) was methylated with methyl iodide (10ml.) and silver oxide (3g.) at reflux temperature for 4 hrs. Yield 0.59g. (57%); m.p. 145-153° (methanol); $\nu_{\max}$ 1590 and 1465 cm^{-1} (lit⁽³¹⁾ m.p. 151-152°).

Methyl 4,6-O-benzylidene-3-O-methyl- α -D-glucopyranoside⁽¹²⁶⁾

A stirred solution of methyl 4,6-O-benzylidene-3-O-methyl-2-O-tosyl- α -D-glucopyranoside (470mg.) in methanol (30ml.) was treated with 2½% sodium amalgam (20g.) over 2 hrs. After a further 2 hrs. the solution was decanted from the mercury; water (5ml.) and solid carbon dioxide added, and the solution evaporated to dryness. The residue was extracted with chloroform to give a product which was crystallised from ether. Yield 210mg. (68%); m.p. 136-142°; $\nu_{\max}$ (nujol) 3450, 1470 and 1460 cm^{-1} (lit⁽¹²⁶⁾ m.p. 150-151°).

Methyl 3-O-methyl-2,4,6-triacetyl- α -D-glucopyranoside⁽¹²⁷⁾

Methyl 4,6-O-benzylidene-3-O-methyl- α -D-glucopyranoside (200mg.), methanol (0.65ml.) and 0.01N. sulphuric acid (0.65ml.) were heated on the steam bath for 2 hrs. The product was neutralised with barium carbonate, filtered, and evaporated down to a colourless syrup (100mg.). The syrup was dissolved in pyridine (2ml.) and treated with acetic anhydride (1ml.).

After 24 hrs.the solution was poured into water and extracted with chloroform. The resulting syrup was purified by chromatography on silica gel using benzene/10% acetone,and then distilled at 10^{-4} mm.pressure in a bent tube. The product would not crystallise. Yield 150mg.(66%); $\nu_{\max}(\text{CHCl}_3)$ 1740 cm^{-1}

Methyl 2,3-di-O-methyl- α -D-glucopyranoside

Methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside (500mg.) was hydrolysed with methanol (2ml.) and 0.01N.sulphuric acid (2ml.) according to the method of Irvine and Scott.⁽¹²⁸⁾ Yield 290mg.(81%);m.p.81-83°(benzene); (lit⁽¹²⁸⁾ m.p.80-82°).

Methyl 2,3-di-O-methyl-4,6-diacetyl- α -D-glucopyranoside

Methyl 2,3-di-O-methyl- α -D-glucopyranoside (100mg.) was acetylated with acetic anhydride (0.4ml.) in pyridine (1ml.) for 2 days. The product would not crystallise but was distilled in a bent tube at 3×10^{-4} mm. Yield 110mg.(80%); $\nu_{\max}(\text{CCl}_4)$ 1745 and 1245 cm^{-1}

o-Cresyl β -D-glucopyranoside tetra-acetate

o-Cresyl glucoside (Calbiochem) (200mg.0.0007mole) in pyridine (3ml.) was treated with acetic anhydride (0.64ml. 0.007mole). After 24 hrs.at room temperature,the solution was poured into water and extracted with chloroform,washed well with water,dried,and evaporated to dryness under reduced pressure. The product was crystallised from ethanol. Yield 230mg.(71%);m.p.145-146°.

The compounds in table 27 were made by the same method.

Table 27, (i) Tetra-acetyl glucosides

Aglycone	Source of free glycoside	Yield	m.p.
<u>p</u> -acetoxyphenol	Koch-Light	63%	145-146°
<u>o</u> -hydroxybenzyl acetate	Unknown	84%	132°
<u>o</u> -nitrophenol	B.D.H.	65%	160°

Table 27, (ii) Tetra-acetyl- β -galactosides

<u>o</u> -nitrophenol	B.D.H.	65%	173°
<u>p</u> -nitrophenol	B.D.H.	71%	145-146°
4-methyl-7-hydroxy-coumarin	B.D.H.	53%	100-102°
6-bromo-2-naphthol	B.D.H.	63%	70-72°

p-Methoxyphenyl β -D-glucopyranoside tetra-acetate

p-Hydroxyphenyl β -D-glucopyranoside (Koch-Light) (500mg.) in acetone (3ml.) was heated with potassium carbonate (330mg.) and methyl iodide (0.45ml.) for 12 hrs. The solvents were evaporated off and the residue extracted with ethanol to give p-methoxyphenyl β -D-glucopyranoside (300mg.) as a syrup. This was acetylated with acetic anhydride (2ml.) and pyridine (4ml.). Yield 100mg.(12%);m.p.93-94°(ethanol);(lit⁽¹²⁹⁾m.p. 95.5-96.5°).

6-(Tetra-acetyl- β -D-glucosyloxy)-7-methoxycoumarin

This was prepared from 6-(β -D-glucosyloxy)-7-hydroxy-coumarin (Koch-Light) as above. Yield 48%;m.p.191-192° (ethanol).

Tri-O-methylphloridzin tetra-acetate

The method of Muller and Robertson⁽¹³⁰⁾ was used to make this compound from phloridzin (B.D.H.). Yield 37%; m.p. 76-80° (ethanol). (lit⁽¹³⁰⁾ m.p. 87-88°).

Penta-acetyl-β-D-glucose

D-Glucose (25g.), acetic anhydride (135ml.) and powdered anhydrous sodium acetate (11g.) were stirred together and heated to 100° for 2 hrs. The product was poured into water (400ml.) and the solid filtered off after 1 hr. It was recrystallised from ethanol. Yield 34g. (63%); m.p. 125-129°.

Phenyl β-D-glucopyranoside tetra-acetate⁽³⁸⁾

Penta-acetyl-β-D-glucose (35g.), phenol (34g.) and p-toluenesulphonic acid (450mg.) were fused together at 100° for 1 hr. under reduced pressure. The cooled product was dissolved in benzene (200ml.), washed with water, 3% aqueous sodium hydroxide, water, and dried. The benzene was distilled off under reduced pressure and the residue crystallised from ethanol. Yield 19g. (50%); m.p. 126°. (lit⁽³⁸⁾ m.p. 125-126°).

p-Bromophenyl β-D-glucopyranoside tetra-acetate

Penta-acetyl-glucose (2.0g.), p-bromophenol (2.5g.) and p-toluenesulphonic acid (20mg.) were fused together as described above. Yield 500mg. (20%); m.p. 130-131° (ethanol). (lit⁽¹³¹⁾ m.p. 131.5-132°).

p-Cresyl β-D-glucopyranoside tetra-acetate

Penta-acetyl-glucose (2.0g.), p-cresol (2.0g.) and p-toluenesulphonic acid (20mg.) were fused together as above.

Yield 600mg.(27%);m.p.120°(ethanol). (lit⁽¹³²⁾m.p.120°).

5-Indanyl β -D-glucopyranoside tetra-acetate

Penta-acetyl glucose (2.0g.),5-hydroxyindane (2.0g.) and p-toluenesulphonic acid (20mg.) were fused together as described above. Yield 780mg.(33%); m.p.163°(ethanol) ; $[\alpha]_D^{21.5}$ -14.4°(c 2.7 in CHCl₃); (Found:C,59.6;H,6.00. C₂₃H₂₈O₁₀ requires C,59.5;H,6.03%).

p-Formylphenyl β -D-glucopyranoside tetra-acetate⁽¹³³⁾

p-Hydroxybenzaldehyde (500mg. 0.0041mole) and aceto-bromoglucose (1.9g. 0.0046mole) were dissolved in acetone (10ml.) and treated with 7% aqueous sodium hydroxide (2.5ml. 0.0044mole). After 24 hrs.the acetone was distilled off under reduced pressure,and water (20ml.) added. The product was extracted with chloroform,washed with water,and dried. The solution was evaporated to dryness and the residue crystallised from ethanol. Yield 300mg.(16%); m.p.142-144° (lit⁽¹³³⁾m.p.144-145°).

m-Acetylphenyl β -D-glucopyranoside tetra-acetate

This was prepared from m-hydroxyacetophenone(300mg.), acetobromoglucose(1.05g.) and 7% aqueous sodium hydroxide (1.4ml.) in acetone (5ml.). Yield 200mg.(19%); m.p.124-126° (ethanol). (lit⁽¹³⁴⁾m.p.123-124°).

m-Nitrophenyl β -D-glucopyranoside tetra-acetate

This was made from m-nitrophenol (1.0g.),acetobromo-glucose (2.1g.) and 4% aqueous sodium hydroxide (10ml.) in

acetone (15ml.). Yield 600mg.(18%); m.p.138-139°(ethanol).
(Found:N,3.01%. $C_{20}H_{23}NO_{12}$ requires N,2.99%)

9-Anthrlyl β -D-glucopyranoside tetra-acetate (135)

This compound was prepared from anthrone (0.4g.), aceto-bromoglucose (0.85g.) and 1% aqueous potassium hydroxide (12.5ml.) in acetone (25ml.). Yield 350mg.(32%); m.p.205-207°(ethanol); τ 7.78, 7.92, 8.01 and 8.25 (OAc, singlets, 3H). (lit⁽¹³⁵⁾ m.p.205°)

m-Benzoyloxyphenyl β -D-glucopyranoside tetra-acetate

Silver oxide (1.5g.) was added to a stirred solution of resorcinol monobenzoate (1.25g.) and acetobromoglucose (2.5g.) in quinoline (5ml.). After 2hrs.the product was extracted with acetic acid (40ml.), filtered, and the filtrate poured into water (200ml.). The glucoside was extracted with chloroform, washed with aqueous sodium bicarbonate, water, and dried. The chloroform was distilled off and the residue crystallised from ethanol. Yield 0.95g.(29%); m.p.124°.

m-Acetoxyphenyl β -D-glucopyranoside tetra-acetate

A solution of m-benzoyloxyphenyl β -D-glucopyranoside tetra-acetate (0.9g.) in methanol (20ml.) was saturated with dry ammonia at 0°C. After 18 hrs. at 0° the solvent was evaporated off and the residue treated with pyridine (2ml.) and acetic anhydride (1ml.). After 24 hrs.the product was isolated in the usual way. Yield 330mg.(41%); m.p.116-117°(ethanol); $[\alpha]_D^{21.5}$ -23.4°(c 2.4 in $CHCl_3$). (Found:C,54.56; H,5.31. $C_{22}H_{26}O_{12}$ requires C,54.78;H,5.43%).

Ethyl β -D-glucopyranoside tetra-acetate

Powdered anhydrous calcium sulphate (5g.) was added to a stirred solution of ethanol (1.1g.) and acetobromoglucose (2.8g.) in dry ether (15ml.), which was contained in a darkened flask. After 15 mins., freshly prepared, dry silver oxide (2.0g.) was added and the mixture stirred at room temperature for 24 hrs. The mixture was filtered, and the filtrate evaporated to dryness. The residue was crystallised from ethanol. Yield 1.59g. (62%); m.p. 106-107°. (lit⁽¹³⁶⁾ m.p. 107°)

The following glucosides were prepared by the Koenigs-Knorr procedure, as described above.

Cyclohexyl β -D-glucopyranoside tetra-acetate⁽¹³⁷⁾

This was made from acetobromoglucose (1.2g.), cyclohexanol (4.0g.), silver oxide (0.6g.) and ether (16ml.). Yield 400mg. (32%); m.p. 119-120° (ethanol). (lit⁽¹³⁷⁾ m.p. 120-121°)

tert-Butyl β -D-glucopyranoside tetra-acetate

tert-Butanol (0.8g.) was allowed to react with acetobromoglucose (2.2g.), calcium sulphate (5g.) and silver carbonate (1.45g.) in benzene (16ml.). Yield 500mg. (23%); m.p. 146-147° (ethanol). (lit⁽¹³⁸⁾ m.p. 148°)

5,5-Dimethyl-3-oxocyclohex-1-en-1-yl β -D-glucopyranoside tetra-acetate⁽¹³⁹⁾

This was made by reaction of dimedone (1.05g.), acetobromoglucose (3.08g.), calcium sulphate (3g.), silver oxide

(1.2g.) and quinoline (1 drop) in ether (10ml.). The product crystallised immediately. Yield 1.6g. (45%); m.p. 143-145° (ethanol); τ 7.71 and 7.78 (CH₂, singlets, 2H), 7.90-7.98 (OAc, 12H), 8.92 (CH₃, singlet, 6H). (lit⁽¹³⁹⁾ m.p. 142-144°)

Allyl β -D-glucopyranoside tetra-acetate⁽¹⁴⁰⁾

This was prepared from allyl alcohol (2.9g.), acetobromoglucose (2.0g.), silver oxide (1.5g.) and calcium sulphate (5g.) in chloroform (10ml.). Yield 920mg. (49%); m.p. 86-87° (ethanol). (lit⁽¹⁴⁰⁾ m.p. 88°)

Cyclohex-2-en-1-yl β -D-glucopyranoside tetra-acetate

dl-Cyclohex-2-en-1-ol (500mg.) was reacted with silver oxide (1.6g.), calcium sulphate (4.0g.) and acetobromoglucose (1.65g.) in benzene (15ml.). The product was a mixture of 6 compounds, which was chromatographed on silica gel using benzene/20% acetone. The second component eluted was collected and crystallised from ethanol. Yield 262mg. (15%); m.p. 148-153°; $[\alpha]_D^{21}$ 5.5-25.4° (c 3.0 in CHCl₃); $\nu_{\max}$ 1745, 1260, 1240 and 740 cm⁻¹ (Found: C, 56.05; H, 6.66. C₂₀H₂₈O₁₀ requires C, 56.07; H, 6.59%).

Benzyl β -D-glucopyranoside tetra-acetate⁽¹³⁷⁾

This was made from benzyl alcohol (5.0g.), silver oxide (0.7g.) and acetobromoglucose (1.0g.) in ether (15ml.). Yield 450mg. (42%); m.p. 97-101° (ethanol). (lit⁽¹³⁷⁾ m.p. 96-101°)

Cholesteryl β -D-glucopyranoside tetra-acetate

This was made by the method of Meystre and Miescher.⁽¹⁴¹⁾

Yield 4%; m.p. 148-150° (ethanol).

dl-Cyclohex-3-en-1-ol

A cis/trans mixture of cyclohexan-1,4-diol (20g.) and 60% sulphuric acid (0.1ml.) were heated in a distillation apparatus at 180-200° for 1 hr. A distillate b.p. 100-145° was collected, washed with saturated aqueous sodium bicarbonate, water, and dried. The product was distilled; the fraction b.p. 150-165° collected, and redistilled under reduced pressure. Yield 4.74g. (28%); b.p. 68°/15mm.; n_D^{20} 1.4880; $\nu_{\max}$ 3370 and 1645 cm^{-1}

Cyclohex-3-en-1-yl β -D-glucopyranoside tetra-acetate

This was made by the Koenigs-Knorr reaction of dl-cyclohex-3-en-1-ol (1.0g.), silver oxide (1.1g.), calcium sulphate (3g.) and acetobromoglucose (1.5g.) in dry benzene (15ml.) The syrupy product was chromatographed on silica gel using benzene/10% acetone and the least polar of the two main components was isolated. Yield 536mg. (34%); m.p. 106-107° (ethanol); $[\alpha]_D^{21.5}$ -19.8° (c 2.1 in CHCl_3); $\nu_{\max}$ 1745 and 1240 cm^{-1} (Found: C, 55.90; H, 6.61. $\text{C}_{20}\text{H}_{28}\text{O}_{10}$ requires C, 56.07; H, 6.59%)

2-Hydroxyethyl β -D-glucopyranoside tetra-acetate

This was made by the method of Karjala and Link.⁽¹⁴²⁾ Yield 63%; m.p. 101-102° (water). (lit.⁽¹⁴²⁾ m.p. 105-106°)

cis-2-Hydroxycyclohexyl β -D-glucopyranoside tetra-acetate

cis-Cyclohexane-1,2-diol (1.0g.) was condensed with

acetobromoglucose (2.0g.) and silver oxide (2.0g.) and calcium sulphate (5g.) in benzene (15ml.). The glucoside crystallised from an ethanolic solution of the reaction product. Yield 650mg.(30%); m.p.125-126°; $[\alpha]_D^{22}$ -10.4° (c 2.1 in CHCl_3); ν_{max} 3500, 1740 and 1230 cm^{-1} (Found: C, 53.97; H, 6.98. $\text{C}_{20}\text{H}_{30}\text{O}_{11}$ requires C, 53.80; H, 6.77%)

cis-2-Acetoxycyclohexyl β -D-glucopyranoside tetra-acetate

cis-2-Hydroxycyclohexyl β -D-glucopyranoside (50mg.) was acetylated with acetic anhydride (0.1ml.) in pyridine (0.2ml.) for 2 days at room temperature. The syrupy product crystallised from ethanol after standing at -10° for 24 hrs. Yield 30mg.(55%); m.p.121-124°; $[\alpha]_D^{22.5}$ -25.8° (c 2.1 in CHCl_3); ν_{max} 1735 and 1240 cm^{-1}

trans-2-Hydroxycyclohexyl β -D-glucopyranoside tetra-acetate

dl-trans-Cyclohexane-1,2-diol (1.0g.) was reacted with silver oxide (2.0g.), calcium sulphate (5g.) and acetobromoglucose (2.0g.) in benzene (15ml.). The required product crystallised from an ethanolic solution of the reaction product. Yield 550mg.(25%); m.p.169°; $[\alpha]_D^{22}$ -13.0° (c 2.5 in CHCl_3); ν_{max} 3480, 1735 and 1240 cm^{-1} (Found: C, 53.58; H, 6.96. $\text{C}_{20}\text{H}_{30}\text{O}_{11}$ requires C, 53.80; H, 6.77%).

trans-2-Acetoxycyclohexyl β -D-glucopyranoside tetra-acetate

The previous compound was acetylated with acetic anhydride and pyridine. Yield 73%; m.p.143° (ethanol); ν_{max} 1745, 1710, 1250 and 1225 cm^{-1}

[²H₃]-Acetyl chloride

[²H₃]-Acetic acid (Yeda) (2.0g.) and benzoyl chloride (13.0g.) were heated in a distillation apparatus for about 45 min., at such a rate that the temperature at the still head did not exceed 52°. The acetyl chloride was collected in a receiver cooled by ice-salt mixture. Yield 2.0g. (78%).

Phenyl β-D-glucopyranoside

Phenyl β-D-glucopyranoside tetra-acetate (19g.) was dissolved in methanol (540ml.) and the solution saturated with dry ammonia at 0°C. After 2 days at 0° the solvent was evaporated off and the residue crystallised from water. Yield 9.9g. (86%); m.p. 174-175°

Phenyl β-D-glucopyranoside tetra-[²H₃]-acetate

(a) [²H₃]-Acetyl chloride (0.05ml.) was added to an ice-cold solution of phenyl β-D-glucopyranoside (25mg.) in pyridine (0.2ml.). The mixture was stirred and then allowed to stand for 1 hr. The solution was poured into ice-water and extracted with chloroform. Yield 20mg. (48%); m.p. 124-125°

(b) [²H₆]-Acetic anhydride (Merck, Sharp & Dohme) (50mg.) was added to a solution of phenyl β-D-glucopyranoside (20mg.) in pyridine (0.2ml.). After 24 hrs. the product was isolated as above. Yield 28mg. (82%); m.p. 125-126° (ethanol).

3,4,6-Triacetyl-2-trichloroacetyl-β-D-glucopyranosyl chloride

Carbon tetrachloride (20ml.) was added to a mixture of penta-acetyl-β-D-glucopyranose (39g.) and powdered phosphorus pentachloride (89g.) and the mixture was heated on a steam

bath for 5 hrs. with exclusion of water. The solution was concentrated under reduced pressure and dissolved in ether (80ml.). The ethereal solution was washed with saturated aqueous sodium bicarbonate, water, dried, and set aside at 0° for 12 hrs. to crystallise. The solid was filtered off, washed with methanol (10ml.) and ether (10ml.) and dried. Yield 13g. (28%); m.p. $139-140^{\circ}$ (ether); $\nu_{\max}$ (nujol) 1760, 1740, and 1230 cm^{-1} (lit⁽⁶³⁾ m.p. 140°)

3,4,6-Triacetyl- β -D-glucopyranosyl chloride

Dry ether (200ml.) was saturated with ammonia at 20°C . The solution was cooled to 0° and 3,4,6-triacetyl-2-trichloroacetyl- β -D-glucopyranosyl chloride (10g.) added. After 5 min. the product was filtered off and dried in vacuum. Yield 5.9g. (86%); m.p. $95-120^{\circ}$ (not recrystallised); $\nu_{\max}$ 3380, 1740, 1700 and 1240 cm^{-1} (lit⁽⁶⁰⁾ m.p. $156-158^{\circ}$ after 10 recrystallisations from ethyl acetate)

p-Nitrophenyl α -D-glucopyranoside 3,4,6-triacetate

3,4,6-Triacetyl- β -D-glucosyl chloride (3.0g.), p-nitrophenol (1.5g.), silver oxide (2.5g.) and calcium sulphate (5g.) were stirred in chloroform (30ml.) for 24 hrs. The product was filtered, washed with water, 2% aqueous sodium hydroxide, water, dried, and evaporated to dryness. The residue was dissolved in hot ethanol and allowed to crystallise. Yield 550mg. (14%); m.p. $149-150^{\circ}$; $\nu_{\max}$ 3500, 1740, 1715 and 1245 cm^{-1} (lit⁽⁶⁴⁾ m.p. $148-149^{\circ}$)

p-Nitrophenyl β -D-glucopyranoside 3,4,6-triacetate-2- $[\text{}^2\text{H}_3]$
acetate

p-Nitrophenyl α -D-glucopyranoside 3,4,6-triacetate (20mg.) was acetylated with $[\text{}^2\text{H}_6]$ -acetic anhydride (10mg.) and pyridine (0.2ml.). A crystalline product was obtained, but it was a mixture of α and β forms. The mixture was separated by preparative t.l.c. on silica gel developing twice with benzene. Slow component: m.p. 177-178°; (lit⁽³⁸⁾ β form has m.p. 174-175°). Faster component: m.p. 113-114°; (lit⁽³⁸⁾ α form has m.p. 113°). The ratio of products was 2 parts β / 1 part α .

1,2-O-Isopropylidene- α -D-glucuronolactone⁽⁶⁷⁾

D-Glucuronolactone (12.5g.), dry acetone (250ml.) and concentrated sulphuric acid (0.5ml.) were stirred together for 2 days. The solution was passed through a column of Amberlite IRA-410 (OH form) resin (25ml.); and then evaporated to 40ml., filtered, and ether added. The precipitated compound was crystallised from ether/petroleum ether. Yield 9g. (59%); m.p. 120-121°; ν_{max} 3430, 1770 and 1380 cm^{-1} (lit⁽⁶⁷⁾ m.p. 120°)

$[\text{}^6,6\text{}^2\text{H}_2]$ - D-glucose

Sodium borodeuteride (200mg.) (Merck Sharp & Dohme) was added to a stirred solution of 1,2-O-isopropylidene- α -D-glucuronolactone (1.0g.) in water (25ml.) at 5°C. After $\frac{1}{2}$ hr. the reaction was allowed to warm to room temperature, and after 2 hrs., further sodium borodeuteride (150mg.) was added. The reaction was allowed to go to completion overnight and

was then heated with 6N.sulphuric acid (4ml.) to 100° for 1 hr. The cooled solution was passed through a column packed with Amberlite IRA-410 (OH form) (25ml.), Amberlite IR-120 (H form) (25ml.) and Amberlite IRA-410 (OH form) (25ml). The resulting solution was evaporated down to a glass under a pressure of 1mm. Yield 520mg.(62%).

Penta-acetyl-[6,6-²H₂]-β-D-glucopyranose

Syrupy [6,6-²H₂]-D-glucose (315mg.) was acetylated with acetic anhydride (3ml.) and sodium acetate (0.3g.) at 100° for 2hrs. Yield 110mg.(16%); m.p.123-128°(ethanol).

Phenyl [6,6-²H₂]-β-D-glucopyranoside tetra-acetate

Penta-acetyl-[6,6-²H₂]-β-D-glucose (107mg.) was fused with phenol (200mg.) and p-toluenesulphonic acid (2mg.) at 100° for 1 hr., under reduced pressure. The product was isolated as in the preparation of the undeuteriated compound. Yield 28mg.(24%); m.p.124-125°(ethanol); $\nu_{\max}$ 1735, 1595, 1585, 1230, 765 and 695 cm⁻¹

Methyl [6,6-²H₂]-α-D-glucopyranoside

Syrupy [6,6-²H₂]-D-glucose (200mg.), methanol (2ml.) and saturated methanolic hydrogen chloride (0.2ml.) were refluxed together for 4 hrs. The solution was concentrated to small bulk and seeded with a crystal of the undeuteriated compound. It was recrystallised from methanol. Yield 19mg.(9%); m.p.159-164°.

Methyl $[6,6-^2\text{H}_2]$ - α -D-glucopyranoside tetra-acetate

The glucoside (4mg.) was acetylated with acetic anhydride (0.05ml.) and pyridine (0.1ml.). Yield 6.7mg. (91%); m.p. 99-100° (ethanol).

$[1-^2\text{H}_1]$ -D-Glucose

A suspension of D-glucono- δ -lactone (2.5g.), anhydrous sodium hydrogen oxalate (8.25g.), oxalic acid (5.5g.) and deuterium oxide (10ml.) in ether (100ml.) was reduced with 5% sodium amalgam (32.5g.), by the method of Bentley and Bhate.⁽⁶⁸⁾ The product was obtained as a glass. Yield 1.3g. (51%).

Penta-acetyl- $[1-^2\text{H}_1]$ - β -D-glucopyranose

Syrupy $[1-^2\text{H}_1]$ -D-glucose (1.3g.) was acetylated with acetic anhydride (12ml.) and anhydrous sodium acetate (1.2g.) at 100° for 1 hr. Yield 700mg. (25%); m.p. 126-129° (ethanol); ν_{max} 1740 and 1240 cm^{-1}

Phenyl $[1-^2\text{H}_1]$ - β -D-glucopyranoside tetra-acetate

Penta-acetyl $[1-^2\text{H}_1]$ - β -D-glucose (245mg.), phenol (240mg.) and p-toluenesulphonic acid (4mg.) were fused at 100° for an hour under reduced pressure. Yield 75mg. (28%); m.p. 125-126° (ethanol); ν_{max} 1740, 1600, 1585, 1230, 765 and 695 cm^{-1}
Mass spectrometry shows 79% incorporation of deuterium.

Octa-acetyl barbaloin

Barbaloin (65mg.) (provided by Professor L.N.Owen) was treated with acetic anhydride (0.2ml.) in pyridine (0.5ml.). After 24 hrs. the solution was poured into ice-water and the

solid filtered off (80mg.). The powder contained 6 compounds and was chromatographed twice on silics gel using 50:50 benzene/acetone. The second fastest, highly fluorescent, component was isolated as a pale yellow glass. Yield 40%.

Hepta-acetyl barbaloin

Barbaloin (75mg.), acetic anhydride (0.5ml.) and anhydrous zinc chloride were allowed to react exothermally. The product was heated at 100° for 10 mins. and then kept at room temperature for 3 hrs. The solution was poured into water, extracted with ether, washed with saturated aqueous sodium bicarbonate, water and dried. The main component was separated firstly by t.l.c. on silica gel using benzene/acetone (2:1), and then by t.l.c. developing with chloroform/ethyl acetate (2:1). It was a greenish-yellow glass. Yield 30%.

Methyl 4,6-O-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside

Methyl 4,6-O-benzylidene- α -D-glucopyranoside (2.0g.), methyl iodide (20ml.) and silver oxide (5g.) were stirred together and refluxed for 4 hrs. The product was filtered and the filtrate evaporated to dryness. The solid was recrystallised from ethanol. Yield 1.8g. (82%); m.p. 122°; $\nu_{\max}$ (nujol) 1460, 750 and 705 cm^{-1} τ 4.61 (benzylidene H, singlet), 5.37 (C(1)-H, doublet $J=3.5\text{Hz}$), 6.49, 6.61 and 6.67 (OMe, singlets, 3H). (lit⁽⁹⁰⁾ m.p. 122-123°)

Methyl 4,6-O-p-tolylidene- α -D-glucopyranoside

Methyl α -D-glucopyranoside (550mg.), anhydrous powdered zinc chloride (450mg.) and p-tolualdehyde (1.5g.) were

stirred together for 3 hrs. and kept at 0° overnight. The viscous mixture was poured into water and the solid filtered off, washed with petroleum ether (40-60°) and dried. It was recrystallised from ethanol. Yield 300mg.(36%): m.p.185-186°; $\nu_{\max}$ 3400, 1470 and 820 cm^{-1}

Methyl 4,6-O-p-tolylidene-2,3-di-O-methyl- α -D-glucopyranoside

Methyl 4,6-O-p-tolylidene- α -D-glucopyranoside (80mg.) was methylated with methyl iodide (3ml.) and silver oxide (300mg.) at reflux temperature for 4 hrs. Yield 60mg.(69%); m.p.150-156°(ether); $[\alpha]_D^{21} +94^\circ$ (c 2.1 in CHCl_3); $\nu_{\max}$ 1465 and 825 cm^{-1} (Found: C, 62.98; H, 7.33. $\text{C}_{17}\text{H}_{24}\text{O}_6$ requires C, 63.00; H, 7.40%)

Methyl 4,6-O-benzylidene-2,3-di-O- $[\text{}^2\text{H}_3]$ -methyl- α -D-glucopyranoside

Methyl 4,6-O-benzylidene- α -D-glucopyranoside (10mg.) in ether (2ml.) was treated with $[\text{}^2\text{H}_3]$ -methyl iodide (Ciba) (0.05ml.) and silver oxide (50mg.). The suspension was stirred and refluxed for 4 hrs., and the product isolated as usual. Yield 7.8mg.(70%); m.p.122-123°(ethanol).

Methyl 4,6-O-benzylidene-2- $[\text{}^2\text{H}_3]$ -methyl-3-O- $[\text{}^1\text{H}_3]$ -methyl- α -D-glucopyranoside.

Methyl 4,6-O-benzylidene-3-O-methyl- α -D-glucopyranoside (15mg.) (see page 196.) in ether (2ml.) was methylated with $[\text{}^2\text{H}_3]$ -methyl iodide (0.05ml.) and silver oxide (50mg.). Yield 8.2mg.(52%); m.p.122-123°(ethanol).

Ethyl 4,6-O-benzylidene- β -D-glucopyranoside

An ice cold solution of ethyl β -D-glucopyranoside tetraacetate (1.4g.) in chloroform (20ml.) was treated with a solution of sodium (135mg.) in methanol (9ml.). After 1 hr. at 0°, excess water was added and the aqueous extract was neutralised with solid carbon dioxide. The aqueous solution was evaporated to dryness, extracted with acetone, and the acetone solution evaporated down to a gum (703mg.). This was stirred with benzaldehyde (1.8ml.) and zinc chloride (0.6g.) for 4hrs. and then kept at 0° overnight. The usual isolation procedure gave the title compound. Yield 350mg. (32%); m.p. 182-187° (ethanol).

Ethyl 4,6-O-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside

Ethyl 4,6-O-benzylidene- β -D-glucopyranoside (160mg.) was methylated with methyl iodide (5ml.) and silver oxide (0.6g.). Yield 80mg. (46%); m.p. 123-124° (ethanol); $\nu_{\max}$ 1470, 1460, 750 and 700 cm^{-1}

Methyl 4,6-O-benzylidene-2,3-di-O-methyl-[6,6-²H₂]- α -D-glucopyranoside

Methyl [6,6-²H₂]- α -D-glucopyranoside (15mg.), benzaldehyde (0.2ml.) and anhydrous zinc chloride (20mg.) were stirred together overnight. Water and petroleum ether were added and the solid filtered off. This was methylated with methyl iodide (2ml.) and silver oxide (100mg.) to give a syrup from which the product was obtained by preparative t.l.c., developing with benzene/10% acetone. Yield 1.5mg. (6%);

m.p. 121-123° (40-60° petroleum ether).

2-Phenyl-2-morpholino-acetonitrile

A solution of benzaldehyde (2.12g.) and morpholine perchlorate (4.12g.) in morpholine (20ml.) was stirred at 60° for 45 min. It was cooled and sodium cyanide (1.08g.) in a minimum of water added. The solution was heated at 80° for $\frac{1}{2}$ hr., cooled and poured into ice-water. The precipitate was recrystallised from 60-80° petroleum ether. Yield 3.46g. (86%); m.p. 68-69°; τ 5.2 (tertiary H, singlet). (lit⁽¹⁴³⁾ m.p. 68-70°)

2-Phenyl-2-morpholino-[2-²H₁]-acetonitrile

53.3% Sodium hydride dispersion (2.7g.) was added to a stirred solution of 2-phenyl-2-morpholino-acetonitrile (6.0g.) in dry dimethylformamide (60ml.), under dry, oxygen-free nitrogen. After 1 hr., deuterium oxide (6ml.) was added, and after 5 min. the solution was neutralised with solid carbon dioxide. Excess water was added, and the solid filtered off. Yield 4.0g. (60%); m.p. 68-69° (60-80° petroleum ether); no n.m.r. signal at τ 5.2.

[α -²H₁]-Benzaldehyde

2-Phenyl-2-morpholino-[2-²H₁]-acetonitrile (4.0g.) was refluxed with 6N. hydrochloric acid (400ml.) for 1 hr. The solution was cooled and extracted with chloroform (3x100ml.). Chloroform extracts were dried and evaporated under reduced pressure. Yield 2.04g. (97%); n_D^{24} 1.532; $\nu_{\max}$ (L.F.) 2120, 2060 and 1680 cm.⁻¹ The n.m.r. signal at τ 0.00 indicated 10% of

undeuteriated benzaldehyde.

Methyl 4,6-O-[α -²H₁]-benzylidene-2,3-di-O-methyl- α -D-glucopyranoside

Methyl α -D-glucopyranoside (80mg.) was reacted with [α -²H₁]-benzaldehyde (0.2ml.) and zinc chloride (80mg.) in the usual way. The crude benzylidene derivative (30mg.) was methylated with methyl iodide (2ml.) and silver oxide (150mg.). Yield 13mg. (10% over 2 stages); m.p. 121-123° (ethanol).

Phenyl 4,6-O-[α -²H₁]-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside

Phenyl β -D-glucopyranoside (80mg.) was reacted with [α -²H₁]-benzaldehyde (0.2ml.) and zinc chloride (80mg.) in the usual way. The crude benzylidene derivative (61mg.) was methylated with methyl iodide (3ml.) and silver oxide (300mg.). Yield 44mg. (38% over 2 stages); m.p. 178-179° (ethanol); n.m.r. spectrum shows no signal at τ 4.5 due to benzylidene H.

Phenyl 4,6-O-benzylidene- β -D-glucopyranoside

Phenyl β -D-glucopyranoside (1.0g.), anhydrous zinc chloride (0.6g.) and benzaldehyde (1.9ml.) were stirred together for 2 hrs. and then kept at 0° overnight. The product was added to water, the solid filtered off, washed with petroleum ether (40-60°) and recrystallised from ethanol. Yield 940mg. (70%); m.p. 194-196°. (lit⁽¹⁴⁴⁾ m.p. 195°)

Phenyl 4,6-O-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside

Phenyl 4,6-O-benzylidene- β -D-glucopyranoside (150mg.) was methylated with methyl iodide (5ml.) and silver oxide (600mg.).

Yield 110mg.(66%); m.p.177-178°(ethanol); $\nu_{\max}$ 1600,1590, 1500,1470,1460,765,755 and 700 cm^{-1}

Phenyl 4,6-O-benzylidene-2,3-di-O-[$^2\text{H}_3$]-methyl- β -D-glucopyranoside

Phenyl 4,6-O-benzylidene- β -D-glucopyranoside (10mg.) in ether (2ml.) was refluxed with [$^2\text{H}_3$]-methyl iodide (0.05ml.) and silver oxide (50mg.) for 4 hrs. Yield 4mg.(37%); m.p.178-179°(ethanol).

o-Cresyl 4,6-O-benzylidene- β -D-glucopyranoside

o-Cresyl β -D-glucopyranoside (200mg.), benzaldehyde (0.4ml.) and zinc chloride (120mg.) were stirred for 5 min.until the mixture solidified. After standing for 20 hrs.the product was isolated as usual. Yield 180mg.(68%); m.p.191-193°(ethanol)

o-Cresyl 4,6-O-benzylidene-2,3-di-O-methyl- β -D-glucopyranoside

o-Cresyl 4,6-O-benzylidene- β -D-glucopyranoside (50mg.) was methylated with methyl iodide (2ml.) and silver oxide (200mg.). Yield 26mg.(48%); m.p.172-174°(ethanol); $\nu_{\max}$ (nujol) 1605,1595,1500,1470,1460,765 and 700 cm^{-1}

Phenyl [6,6- $^2\text{H}_2$]- β -D-glucopyranoside

Phenyl [6,6- $^2\text{H}_2$]- β -D-glucopyranoside tetra-acetate (22mg.) in methanol (2ml.) was treated with 1.5% methanolic sodium methoxide (0.35ml.) at 0°C. After 3 hrs.at 0°,water (4ml.) was added and the solution neutralised with carbon dioxide, evaporated to dryness and extracted with acetone. The acetone solution was evaporated to dryness and the residue

crystallised from water. Yield 7mg.(53%); m.p.173-174°.

Phenyl 4,6-O-benzylidene-2,3-di-O-methyl-[6,6-²H₂]-β-D-glucopyranoside

Phenyl [6,6-²H₂]-β-D-glucopyranoside (7mg.), benzaldehyde (0.1ml.) and zinc chloride (10mg.) were stirred together overnight and the derivative isolated as usual. The crude product (8mg.) was methylated with methyl iodide (2ml.) and silver oxide (50mg.). Yield 3.7mg.(37% over 2 stages); m.p.178-179°; $\nu_{\max}$ 1600, 1585, 1500, 1460, 765, 745 and 700 cm.⁻¹

Phenyl [1-²H₁]-β-D-glucopyranoside

Phenyl [1-²H₁]-β-D-glucopyranoside tetra-acetate (50mg.) in methanol (1.5ml.) was saturated with dry ammonia at 0°. After 4 hrs.at 0° the solvent was distilled off and the residue crystallised from water. Yield 16mg.(53%); m.p.173-175°.

Phenyl 4,6-O-benzylidene-2,3-di-O-methyl-[1-²H₁]-β-D-glucopyranoside

Phenyl [1-²H₁]-β-D-glucopyranoside (16mg.) was converted into the title compound in the usual manner. Yield 4mg.(17%); m.p.180-181°.

3,2 Allylic AcetatesAllyl acetate

Allyl alcohol (6g.) and acetic anhydride (12g.) were refluxed together for 2 hrs. The cooled product was poured into ice-water and the organic phase separated, washed with aqueous sodium bicarbonate, water and dried. The liquid was distilled at atmospheric pressure. Yield 3.2g.(31%); b.p. 103-105°; n_D^{21} 1.405.

1-Methylallyl alcohol

The Grignard reagent was prepared from methyl iodide (20g.) and magnesium (3.3g.) in ether (100ml.). A solution of acrolein (6.2g.) in dry ether (15ml.) was added over 1 hour at 0°, and after a further hour at room temperature, the complex was decomposed with saturated aqueous ammonium chloride (24ml.). The ethereal solution was separated, dried and distilled. Yield 4.8g.(60%); b.p. 95-98°; n_D^{20} 1.4142.

1-Methylallyl acetate

1-Methylallyl alcohol (2.3g.) was heated at reflux with acetic anhydride (5.5ml.) for 2 hrs. The product was isolated as for allyl acetate. Yield 1.8g.(50%); b.p. 111-113°; n_D^{20} 1.4033; τ 8.68 (1-Me, doublet $J=6.5$ Hz, 3H), 7.94 (OAc, singlet, 3H), 3.8-5.0 (multiplet, 4H).

2-Methylallyl acetate

2-Methylallyl alcohol (5.1g.) was acetylated with acetic anhydride (12ml.). Yield 5.6g.(70%); b.p. 122-123°; n_D^{19} 1.4131; τ 8.23 (2-Me, broad singlet, 3H), 7.90 (OAc, singlet 3H), 5.48

(CH₂, broad singlet, 2H), 5.03 (=CH₂, broad singlet, 2H).

(lit⁽¹⁴⁵⁾ b.p. 124°; n_D^{20} 1.4129)

3-Methylallyl alcohol

Crotonaldehyde (7.0g.) was added to a well stirred solution of sodium borohydride (1.0g.) in water (50ml.) over 1 hr., with ice cooling. After a further 1½ hrs. the solution was acidified with 2N. sulphuric acid (12ml.) and the product extracted with ether, washed with aqueous sodium bicarbonate, water, dried, and distilled. Yield 2.5g. (35%); b.p. 118-121°; n_D^{25} 1.4253; $\nu_{\max}$ (L.F.) 3350 and 1665 cm⁻¹

3-Methylallyl acetate

3-Methylallyl alcohol (2.1g.) was acetylated with acetic anhydride (5ml.). Yield 1.95g. (59%); b.p. 129-130°; n_D^{21} 1.4166; $\nu_{\max}$ 1730, 1665 and 1240 cm⁻¹; τ 8.28 (3-Me, doublet, J=4.5Hz, 3H), 7.95 (OAc, singlet, 3H), 5.49 (CH₂, doublet J=5Hz, 2H), 4.1-4.5 (vinyl H, multiplet, 2H).

1,1-Dimethylallyl alcohol⁽¹⁴⁶⁾

All reagents and solvents were dried thoroughly. Vinyl chloride was passed into tetrahydrofuran (108g.) until the gain in weight was 31g. This solution (10ml.) and ethyl bromide (1ml.) were added to magnesium (12.0g.) under dry nitrogen, in a flask equipped with a solid carbon dioxide/acetone condenser. The suspension was stirred and warmed until reaction began, and then the vinyl chloride solution was added over 2 hrs. with the temperature at 40-50°. After

a further 1 hr. at this temperature, the solution was cooled and acetone added (29.0g.), so that the temperature was $<30^{\circ}$. After stirring for 1 hr. at room temperature, water (126ml.) and concentrated hydrochloric acid (45ml.) were added and the organic phase separated. The aqueous phase was extracted with xylene (2x25ml.) and combined extracts were washed with water and dried over potassium carbonate. The liquid was distilled using a Vigreux column. Yield 14g. (33%); b.p. $95-96^{\circ}$; $\nu_{\max}$ 3400, 1640 and 1375 cm^{-1} ; τ 8.78 (1-Me, singlet, 6H), 7.80 (OH, singlet, 1H), 5.14 (double doublet, $J=10$ and 1.5Hz, 1H), 4.93 (double doublet, $J=17$ and 1.5Hz), 4.10 (double doublet, $J=17$ and 10Hz, 1H). (lit⁽¹⁴⁶⁾ b.p. $95-96^{\circ}$)

1,1-Dimethylallyl acetate

1,1-Dimethylallyl alcohol (4.0g.), anhydrous sodium acetate (4g.) and acetic anhydride (6ml.) were heated at 100° for 20 hrs. The cooled product was poured into water (30ml) and neutralised with sodium bicarbonate. The ester was extracted with ether (10ml.), washed with water, dried, and distilled. Yield 3.5g. (59%); b.p. $121-122^{\circ}$; n_D^{20} 1.4160; $\nu_{\max}$ 1730, 1640, 1375, 1255 and 935 cm^{-1} ; τ 8.50 (1-Me, singlet, 6H), 8.02 (OAc, singlet, 3H), 4.94 (double doublet $J=10.5$ and 1.5Hz, 1H), 4.85 (double doublet $J=17.5$ and 1.5Hz, 1H), 3.90 (double doublet $J=17.5$ and 10.5Hz, 1H).

3,3-Dimethylallyl alcohol

3,3-Dimethylacrylic acid (30g.) was reduced with lithium aluminium hydride (12g.) by the method of Bolleter et al.⁽¹⁴⁷⁾

Yield 20g.(78%); b.p.52-54°/16mm.; n_D^{18} 1.4412.
(lit⁽¹⁴⁷⁾ b.p.48-50°/12mm.)

3,3-Dimethylallyl acetate

3,3-Dimethylallyl alcohol (1.0g.) was acetylated with acetic anhydride (1.8g.). Yield 450mg.(30%); b.p.116°/30cm.; n_D^{22} 1.4282; τ 8.30 (3-Me, singlet, 3H), 8.27 (3-Me, doublet $J=1.5\text{Hz}$, 3H), 7.98 (OAc, singlet, 3H), 5.45 (CH_2 , doublet $J=7\text{Hz}$, 2H), 4.66 (vinyl H, triple quadruplet $J=7$ and 1.5Hz).

Cyclohex-2-en-1-yl acetate

Cyclohex-2-en-1-ol (2.0g.) (provided by D.G.B.Boocock) was acetylated with acetic anhydride (3ml.). Yield 2.0g.(70%); b.p.74-76°/18mm.; n_D^{20} 1.4600; ν_{max} (L.F.) 1725, 1645 and 1245cm^{-1}

Cyclohexanone cyanohydrin

Sodium cyanide (41.6g.), water (100ml.) and cyclohexanone (100g.) were stirred with ice-cooling, until reaction ceased. The precipitate was decomposed with 40% aqueous sulphuric acid (175ml.) which was added over 2 hrs., and the upper organic phase was then decanted. The lower aqueous phase was extracted with ether (3x25ml.) and the ethereal extracts were combined with the organic phase, dried and distilled. Yield 95g.(75%); b.p.95-100°/0.5mm.; m.p.ca 30°; n_D^{30} 1.4615; ν_{max} 3600, 3450, 2250 and 1690cm^{-1} (lit⁽¹⁴⁸⁾ b.p.109-113°/9mm.; m.p.29°; n_D^{25} 1.4613)

1-Cyanocyclohex-1-ene

A solution of cyclohexanone cyanohydrin (20g.) in pyridine

(26ml.) was cooled in ice-salt mixture, and thionyl chloride (12.5ml.) was added over 1 hr. The cooling bath was removed and the temperature allowed to rise to ca 60° by an exothermic reaction. The cooled product was poured into ice-water (200ml.) and the organic phase separated, washed with 2N. aqueous hydrochloric acid, water, dried and distilled. Yield 13.5g. (79%); b.p. $88^{\circ}/10\text{mm.}$; n_D^{21} 1.4818; ν_{max} (L.F.) 2210 and 1635 cm^{-1}

1-Carbethoxycyclohex-1-ene

This was prepared from 1-cyanocyclohex-1-ene (15g.) by the method of Lythgoe et al.⁽¹⁴⁹⁾ Yield 17g. (79%); b.p. $95-102^{\circ}/12\text{mm.}$; n_D^{24} 1.4710; ν_{max} 1705 and 1645 cm^{-1} (lit⁽¹⁴⁹⁾ b.p. $104-111^{\circ}/25\text{mm.}$)

1-Hydroxymethylcyclohex-1-ene

1-Carbethoxycyclohex-1-ene (10g.) was reduced with lithium aluminium hydride (1.9g.) by the method of Lythgoe et al.⁽¹⁴⁹⁾ Yield 5.5g. (76%); b.p. $90-91^{\circ}/10\text{mm.}$; n_D^{20} 1.4903; ν_{max} (L.F.) 3350 and 1665 cm^{-1} (lit⁽¹⁴⁹⁾ b.p. $96^{\circ}/18\text{mm.}$)

1-Acetoxymethylcyclohex-1-ene

1-Hydroxymethylcyclohex-1-ene (2.0g.) was acetylated with acetic anhydride (2.8ml.). Yield 2.2g. (80%); b.p. $100^{\circ}/13\text{mm.}$; n_D^{21} 1.4661; ν_{max} 1730, 1665 and 1235 cm^{-1} ; τ 7.95 (OAc, singlet, 3H), 5.54 (CH_2 , singlet, 2H), 4.25 (vinyl H, multiplet, 1H). (Found: C, 70.18; H, 8.95. $\text{C}_9\text{H}_{14}\text{O}_2$ requires C, 70.10; H, 9.15%)

1-Acetoxy-[²H₂]-methylcyclohex-1-ene

1-Carbethoxycyclohex-1-ene (800mg.) was reduced with lithium aluminium deuteride (150mg.) and the product (520mg.) was acetylated with acetic anhydride (0.8ml.). Yield 500mg. (62% over 2 stages); n_D^{23} 1.4620; $\nu_{\max}$ 2240, 2180, 2121, 1730 and 1665 cm^{-1} . No n.m.r. resonance at τ 5.54.

2-Hydroxymethylcyclohexanone

Cyclohexanone (50g.) was condensed with 40% aqueous formaldehyde (34ml.), water (8ml) and calcium hydroxide (200mg.) by the method of Mannich and Brose.⁽¹⁵⁰⁾ Yield 14g. (21%); b.p. 117-120°/20mm.; n_D^{21} 1.4803; $\nu_{\max}$ 3450 and 1700 cm^{-1} (lit⁽¹⁵⁰⁾ b.p. 114-115°/16mm.)

2-Methylenecyclohexan-1-ol

This was made from 2-hydroxymethylcyclohexanone (10g.) according to the method of Dreiding and Hartman.⁽¹⁵¹⁾ Yield 1.05g. (12%); b.p. 88-91°/30mm.; n_D^{20} 1.4855; $\nu_{\max}$ 3370 and 1645 cm^{-1} (lit⁽¹⁵¹⁾ b.p. 83-85°/26mm.; n_D^{25} 1.4893)

2-Methylenecyclohexan-1-yl acetate

2-Methylenecyclohexan-1-ol (1.0g.) was acetylated with acetic anhydride (1.5ml.). Yield 880mg. (64%); b.p. 83-84°/15mm.; n_D^{20} 1.4595; $\nu_{\max}$ 1735 and 1650 cm^{-1} ; τ 7.91 (OAc, singlet, 3H), 5.20 (=CH₂, triplet J=1Hz, 2H), 4.76 (broad, 1H). (lit⁽¹¹¹⁾ b.p. 83-84°/20mm.; n_D^{25} 1.4552)

1-o-Methylphenyl-allyl alcohol

o-Bromotoluene (20.0g., 0.12mole) was added to a stirred suspension of magnesium (2.9g., 0.12mole) and ethyl bromide (0.2 ml.) in anhydrous ether (50ml.) at such a rate that the reaction mixture refluxed gently. When reaction was complete, the solution was cooled in ice-salt mixture and freshly distilled acrolein (8g., 0.14mole) in ether (30ml.) was added over 1 hr. Stirring was continued at room temperature for 2 hrs. and saturated aqueous ammonium chloride (150ml.) added. The ethereal solution was separated, washed with water, dried over potassium carbonate and distilled. Yield 9.5g. (55%); b.p. 72-79°/0.5mm.; n_D^{21} 1.5436; $\nu_{\max}$ 3380, 1640 and 1605 cm^{-1} ; τ 7.69 (Ar-CH₃, singlet, 3H) and vinyl protons are similar to figure 4.

The substituted 1-phenylallyl alcohols shown in table 28 were made by the same method.

Table 28.

Substituent	% Yield	n_D^*	b.p.
<u>m</u> -methyl	44%	1.5388(20°)	76-79°/0.7mm.
<u>o</u> -ethyl	49%	1.5367(22°)	80-81°/0.9mm.
<u>o</u> -chloro	26%	1.5630(18°)	81-82°/0.8mm.
<u>m</u> -chloro	80%	1.5560(20°)	81-84°/0.5mm.
<u>p</u> -chloro	58%	1.5565(19°)	91-94°/0.9mm.
<u>p</u> -bromo	32%	1.5780(23°)	103-105°/1mm.
<u>p</u> -fluoro	52%	1.5185(22°)	64-68°/0.8mm.
<u>o</u> -methoxy	63%	1.5485(20°)	86-90°/0.5mm.

*Temperature in parenthesis.

p-Chlorocinnamyl alcohol⁽¹⁵²⁾

1-p-Chlorophenyl-allyl alcohol (2.0g., 0.012mole) in acetone (100ml.), water (60ml.) and concentrated hydrochloric acid (2.8ml.) was refluxed for 2 days. The product was evaporated down to 40ml. bulk under reduced pressure and extracted with ether. The ethereal solution was evaporated to dryness and recrystallised from 40-60° petroleum ether. Yield 1.4g. (70%); m.p. 54-55°; $\nu_{\max}$ 3300, 1650, 1590, 1500 and 860 cm^{-1} (lit⁽¹⁵²⁾ m.p. 57°)

m-Chlorocinnamyl alcohol

This compound was prepared as above, but it would not crystallise. It was purified by chromatography on silica gel; eluting with benzene/10% acetone. Yield 55%.

o-Chlorocinnamyl alcohol

1-o-Chlorophenyl-allyl alcohol (1.08g.) was isomerised as above to give a product which was a liquid. This was purified by chromatography on silica gel using benzene/10% acetone. Yield 865mg. (80%).

p-Bromocinnamyl alcohol

1-p-Bromophenyl-allyl alcohol (2.8g.) was isomerised as above. Yield 1.8g. (64%); m.p. 68° (40-60° petroleum ether). (lit⁽¹⁵²⁾ m.p. 67°)

p-Fluorocinnamyl alcohol

1-p-Fluorophenyl-allyl alcohol (2.0g.) was isomerised as above. Yield 950mg. (48%); m.p. 54-57° (40-60° petroleum ether);

$\nu_{\max}$ (nujol) 3300, 1655, 1600, 1520 and 860 cm^{-1} (lit⁽¹⁵²⁾ m.p. 57°)

o-Chlorocinnamyl acetate

o-Chlorocinnamyl alcohol (865mg., 0.0052mole) was refluxed with acetic anhydride (1.5ml.) for 2 hrs. The solution was cooled, poured into ice-water, neutralised with sodium bicarbonate, extracted with ether, dried and distilled. Yield 610mg. (56%); b.p. 102°/0.2mm.; n_D^{19} 1.5550; $\nu_{\max}$ 1735, 1590, 1240 and 760 cm^{-1} (Found: C, 62.58; H, 5.18; Cl, 16.86. $\text{C}_{11}\text{H}_{11}\text{ClO}_2$ requires C, 62.73; H, 5.26; Cl, 16.84%). N.m.r. spectra of the cinnamyl acetates were all similar to figure 4 and were in agreement with assigned structures.

m-Chlorocinnamyl acetate

m-Chlorocinnamyl alcohol (1.1g.) was acetylated as above. Yield 1.0g. (73%); b.p. 104°/0.3mm.; n_D^{22} 1.5546; $\nu_{\max}$ 1730, 1650, 1595, 1565, 890, 785 and 690 cm^{-1} (Found: C, 62.44; H, 5.33; Cl, 16.97. $\text{C}_{11}\text{H}_{11}\text{ClO}_2$ requires C, 62.73; H, 5.26; Cl, 16.84.)

p-Chlorocinnamyl acetate

This was made from p-chlorocinnamyl alcohol (1.0g.). Yield 1.05g. (84%); b.p. 109°/0.3mm.; n_D^{22} 1.5585; $\nu_{\max}$ 1735, 1655, 1590, 1500, 1235 and 860 cm^{-1} (Found: C, 62.70; H, 5.08; Cl, 16.76. $\text{C}_{11}\text{H}_{11}\text{ClO}_2$ requires C, 62.73; H, 5.26; Cl, 16.84%.)

p-Bromocinnamyl acetate

This was prepared by acetylation of p-bromocinnamyl alcohol (1.1g.). Yield 0.9g. (68%); b.p. 128°/0.8mm.; n_D^{19} 1.5797; $\nu_{\max}$ 1735, 1660, 1585 and 1245 cm^{-1} (Found: C, 51.82;

H, 4.46; Br, 31.37. $C_{11}H_{11}BrO_2$ requires C, 51.80; H, 4.35; Br, 31.32)

p-Fluorocinnamyl acetate

This was prepared by acetylation of p-fluorocinnamyl alcohol (900mg.). Yield 850mg. (74%); b.p. $83^{\circ}/0.3\text{mm.}$; n_D^{20} 1.5250; ν_{max} 1730, 1655, 1600, 1515, 1230 and 860 cm^{-1} (Found: C, 68.11; H, 5.93; F, 9.59. $C_{11}H_{11}FO_2$ requires C, 68.05; H, 5.71; F, 9.78%)

o-Methylcinnamyl acetate

1-o-Methylphenyl-allyl alcohol (5.0g.) and acetic anhydride (6ml.) were refluxed together for 6 hrs. and the product isolated as usual. Yield 5.2g. (81%); b.p. $102-104^{\circ}/0.9\text{mm.}$; n_D^{20} 1.5370; ν_{max} 1735, 1650, 1600 and 755 cm^{-1}

m-Methylcinnamyl acetate

1-m-Methylphenyl-allyl alcohol (2.0g.) was acetylated as above. Yield 1.5g. (58%); b.p. $106-107^{\circ}/1\text{mm.}$; n_D^{20} 1.5420; ν_{max} 1735, 1650, 1600, 1580, 780 and 695 cm^{-1}

o-Ethylcinnamyl acetate

1-o-Ethylphenyl-allyl alcohol (2.0g.) was acetylated as above. Yield 1.1g. (44%); b.p. $100^{\circ}/0.6\text{mm.}$; n_D^{23} 1.5335; ν_{max} 1735, 1650, 1600, 1240 and 760 cm^{-1} ; τ 8.80 (ethyl CH_3 , triplet $J=7.5\text{Hz}$, 3H), 7.93 (OAc, singlet, 3H), 7.30 (ethyl CH_2 , quartet $J=7.5\text{Hz}$, 2H), 5.27 ($\text{CH}_2\text{-O}$, doublet $J=6\text{Hz}$, 2H), 3.88 (double triplet $J=16$ and 6Hz , 1H), 3.08 (doublet $J=16\text{Hz}$, 1H), 2.6-2.9 (aromatic, multiplet, 4H). (Found: C, 76.21; H, 7.80. $C_{13}H_{16}O_2$ requires C, 76.44; H, 7.90%)

o-Methoxycinnamyl acetate

1-o-Methoxyphenyl-allyl alcohol (2.0g.) was acetylated as above. Yield 1.3g.(52%); b.p.108°/0.2mm.; n_D^{20} 1.5518; $\nu_{\max}$ 1730,1650,1595,1580,1250 and 760 cm^{-1} (Found:C,69.68; H,6.75. $\text{C}_{12}\text{H}_{14}\text{O}_3$ requires C,69.88;H,6.84%)

1-p-Chlorophenyl-allyl acetate

1-p-Chlorophenyl-allyl alcohol (1.7g.),pyridine (2ml.) and acetic anhydride (2.1ml.) were warmed to 65° for 1 hr. The solution was cooled,poured into ice-water,extracted with ether,washed with water,dried over potassium carbonate and distilled. Yield 1.5g.(71%); b.p.80-81°/0.3mm.; n_D^{21} 1.5240; $\nu_{\max}$ 1730,1635,1595,1240 and 830 cm^{-1} . The n.m.r.spectrum was as shown in figure 4,and the following two acetates gave similar n.m.r.spectra.

1-p-Bromophenyl-allyl acetate

This was prepared as above. Yield 67%; b.p.101-104°/1mm.; n_D^{20} 1.5435; $\nu_{\max}$ 1740,1640 and 1595 cm^{-1}

1-p-Fluorophenyl-allyl acetate

This was prepared as above. Yield 58%; b.p.56°/0.3mm.; n_D^{22} 1.4930; $\nu_{\max}$ 1730,1640,1600,1515,1230 and 845 cm^{-1}

p-Methoxycinnamyl alcohol

A solution of ethyl p-methoxycinnamate (2.0g.) in ether (25ml.) was gently refluxed and a solution of lithium aluminium hydride (230mg.) in ether (50ml.) added over 2 hrs. The reaction mixture was stirred at room temperature

overnight, and then water (5ml.) and 10% aqueous sulphuric acid (12ml.) were added. The ethereal layer was washed with saturated aqueous sodium bicarbonate, water, dried and evaporated to dryness. Yield 0.8g. (50%); m.p. 76-78° (60-80° petroleum ether); $\nu_{\max}$ 3350, 1655, 1605 and 1575 cm^{-1} (lit⁽¹⁵²⁾ m.p. 80°)

p-Methoxycinnamyl acetate

p-Methoxycinnamyl alcohol (600mg.) was acetylated with acetic anhydride (2ml.). Yield 410mg. (55%); b.p. 112-114°/0.2mm.; n_D^{19} 1.5568; $\nu_{\max}$ 1730, 1655, 1610, 1580 and 1520 cm^{-1}

3-Methyl-3-phenylallyl acetate

3-Methyl-3-phenylallyl alcohol (1.0g.) (provided by D.R. Buckle) was acetylated with acetic anhydride (1.3ml.). Yield 1.1g. (86%); b.p. 98-99°/0.8mm.; n_D^{23} 1.5322; $\nu_{\max}$ 1735, 1655, 1600, 1500, 1240, 770 and 705 cm^{-1} ; τ 7.97 (OAc, singlet, 3H), 7.92 (CH_3 , singlet, 3H), 5.25 (CH_2 , doublet $J=7\text{Hz}$, 2H), 4.13 (vinyl H, triplet $J=7\text{Hz}$, 1H), 2.6-2.9 (aromatic, multiplet, 5H).

3,3-Diphenylprop-1-yn-3-ol

Ferric nitrate (100mg.) and sodium (6.7g.) were added to liquid ammonia (300ml.) and acetylene was passed through for 2 hrs. during which time the colour changed from blue to grey. Powdered benzophenone (40g.) was then added over 15 mins., and after a further 6 hrs. the flow of acetylene was cut off and ether (100ml.) added. When the ammonia had evaporated off, water (50ml.) was cautiously added, and the solution acidified with 4N. sulphuric acid. Ether (200ml.) was added,

and the ethereal layer separated, washed with water, dried over potassium carbonate and distilled. The product was recrystallised from 40-60° petroleum ether. Yield 37.5g. (82%); b.p. 116-118°/0.35mm.; m.p. 47-48°; $\nu_{\max}$ (nujol) 3550, 3280, 2120 and 1595 cm^{-1} ; τ 7.21 ($=\text{CH}$, singlet, 1H), 7.15 (OH, singlet, 1H), 2.3-2.8 (aromatic, multiplet, 10H). (lit⁽¹⁵³⁾ m.p. 48.5-49.5°)

3,3-Diphenylprop-2-en-1-al

3,3-Diphenylprop-1-yn-3-ol (10.0g.) was isomerised by the method of Hennion and Fleck.⁽¹⁵³⁾ Yield 8.1g. (81%); m.p. 37-38° (40-60° petroleum ether); $\nu_{\max}$ 1665, 1595 and 1575 cm^{-1} (lit⁽¹⁵³⁾ m.p. 44.5-45.5°)

3,3-Diphenylprop-2-en-1-ol

A solution of lithium aluminium hydride (210mg.) in ether (50ml.) was added to a solution of 3,3-diphenylprop-2-en-1-al (4.0g.) in ether (25ml.) at 0° over 1 hr. After stirring at room temperature for 2 hrs. water (5ml.) and 10% aqueous sulphuric acid (10ml.) were added. The ethereal solution was separated, washed with aqueous sodium bicarbonate, water, dried and distilled. The distillate solidified and was recrystallised from 60-80° petroleum ether. Yield 3.5g. (87%); m.p. 62-63°; b.p. 142-143°/0.5mm.; $\nu_{\max}$ 3350, 1625, 1595 and 1570 cm^{-1}

3,3-Diphenylprop-2-en-1-yl acetate

3,3-Diphenylprop-2-en-1-ol (2.0g.) was acetylated with acetic anhydride (3ml.). Yield 2.0g. (83%); b.p. 140-143°/0.5mm.; n_D^{22} 1.5843; $\nu_{\max}$ 1735, 1660, 1600 and 1575 cm^{-1} ; τ 7.98 (acetate,

singlet, 3H), 5.38 (CH₂, doublet J=7Hz, 2H), 3.82 (=CH-, triplet J=7Hz, 1H), 2.6-2.9 (aromatic, multiplet, 10H).

(lit⁽¹⁵⁴⁾ b.p. 205°/15mm.; $n_D^{10.7}$ 1.5859)

Cinnamyl [²H₃]-acetate

Cinnamyl alcohol (200mg.) was acetylated with [²H₆]-acetic anhydride (250mg.). Yield 234mg. (88%); n_D^{26} 1.5368; $\nu_{\max}$ 1735, 1600, 1580, 755 and 700 cm⁻¹ No acetate resonance in the n.m.r. spectrum.

o-Methylcinnamyl [²H₃]-acetate

1-o-Methylphenyl-allyl alcohol (200mg.) was heated with [²H₆]-acetic anhydride (250mg.) for 6 hrs. Yield 190mg. (73%); n_D^{25} 1.5278; $\nu_{\max}$ 1735, 1600 and 1255 cm⁻¹ No n.m.r. signal at τ 7.91.

3-Phenyl-[1-²H₁]-prop-2-en-1-ol

A suspension of lithium aluminium deuteride (78mg.) in ether (5ml.) was added to an ice-cold solution of cinnamaldehyde (1.0g.) in ether (5ml.) over $\frac{1}{2}$ hr. After a further $\frac{1}{2}$ hr. the complex was decomposed with 10% aqueous sulphuric acid (3ml.). The ethereal extract was washed with aqueous sodium bicarbonate, water, dried, and distilled. Unchanged cinnamaldehyde was removed by chromatography on silica gel, eluting with benzene. Yield 210mg. (21%).

3-Phenyl-[1-²H₁]-prop-2-en-1-yl acetate

3-Phenyl-[1-²H₁]-prop-2-en-1-ol (160mg.) was acetylated with acetic anhydride (0.4ml.). Yield 150mg. (72%); $\nu_{\max}$ 2200, 1740, 1650 and 1600 cm⁻¹; τ 7.91 (acetate, singlet, 3H),

5.31 (CHD, double triplet, $J=5.5$ and 1.5Hz , 1H), 3.78 (vinyl H, double doublet $J=5.5$ and 16Hz , 1H), 3.32 (vinyl H, doublet $J=16\text{Hz}$, 1H).

[2,4,6-N,N,N- $^2\text{H}_6$]-Anilinium bromide

Anilinium bromide (6g.) and deuterium oxide (3ml.) were heated together at 100° for 24 hrs. The water was distilled off under reduced pressure and the residue subjected to two more such exchanges. The crude product was used for the next stage.

[2,4,6- $^2\text{H}_3$]-Bromobenzene

[2,4,6-N,N,N- $^2\text{H}_6$]-Anilinium bromide (6.0g.) was converted to the title compound by the method of Akawie *et al.*⁽¹⁰⁵⁾
Yield 2.2g. (40%); b.p. $90^\circ/90\text{mm.}$; n_D^{25} 1.5590.

1-[2,4,6- $^2\text{H}_3$]-Phenyl-allyl alcohol

The Grignard reagent prepared from [2,4,6- $^2\text{H}_3$]-bromobenzene (2.1g.) and magnesium (320mg.) was caused to react with acrolein (1ml.). Yield 1.05g. (58%); b.p. $62-66^\circ/0.5\text{mm.}$; n_D^{24} 1.5440; ν_{max} (L.F.) 3400, 2280, 1640 and 1580 cm^{-1}

[2,4,6- $^2\text{H}_3$]-Cinnamyl acetate

1-[2,4,6- $^2\text{H}_3$]-Phenyl-allyl alcohol (1.0g.) was refluxed with acetic anhydride (2ml.) for 6 hrs. Yield 900mg. (69%); b.p. $82^\circ/0.3\text{mm.}$; n_D^{23} 1.5422; ν_{max} 2280, 1730, 1655 and 1240 cm^{-1}
 τ 2.70 (aromatic, broadened singlet, 2.2H); mass spectrum shows D_3 75%, D_2 23%, D_1 2%.

[3,5-²H₂]-Bromobenzene

This was prepared from [2,4,6,N,N,N-²H₆]-anilinium bromide (8.0g.) by the method of Akawie et al.⁽¹⁰⁵⁾

Yield 2.1g.(21%); b.p.86-90°/80mm.

1-[3,5-²H₂]-Phenyl-allyl alcohol

The Grignard reagent prepared from [3,5-²H₂]-bromobenzene (2.1g.) and magnesium (320mg.) was treated with acrolein (1ml.). Yield 900 mg.(50%); b.p.62-66°/0.6mm.; n_D^{20} 1.5460; $\nu_{\max}$ 3400, 2260, 1640 and 1585 cm^{-1}

[3,5-²H₂]-Cinnamyl acetate

1-[3,5'-²H₂]-Phenyl-allyl alcohol (850mg.) was refluxed with acetic anhydride (2ml.) for 6 hrs. Yield 750mg.(68%); b.p.82-83°/0.35mm.; n_D^{20} 1.5445; $\nu_{\max}$ 2280, 1735, 1655, 1590, 1585 and 1240 cm^{-1} ; τ 2.79 (aromatic, broadened singlet, 1H), 2.65 (aromatic, broadened singlet, 2H); the mass spectrum shows D₂ 92%, D₁ 8%.

4-Chloro-[2,6,N,N,N-²H₅]-anilinium bromide

p-Chloroanilinium bromide (6.0g.) was heated at reflux with deuterium oxide (6ml.) for 2 days. The water was distilled off and the residue carried to the next stage.

1-Bromo-4-chloro-[2,6-²H₂]-benzene

A suspension of 4-chloro-[2,6,N,N,N-²H₅]-anilinium bromide (6.0g.) in 45% aqueous hydrobromic acid (7ml.) was treated with sodium nitrite (1.95g.) in water (3.5ml.) at 0°. The solution of the diazo salt was added to a boiling solution

of cuprous bromide (2.43g.) in 45% hydrobromic acid (3ml.) over 45 min. so that the product distilled from the reaction mixture in the steam. The required compound was isolated from the distillate by ether extraction. Yield 4.36g. (80%); m.p. 64-65° (ethanol); the mass spectrum shows D_2 62%, D_1 33%, D_0 5%.

4-Chloro-[2,6- 2H_2]-cinnamyl acetate

This was made from 1-bromo-4-chloro-[2,6- 2H_2]-benzene (4.0g.) by the same series of reactions as were used to make the undeuteriated material. Yield 610mg.; b.p. 100°/0.25mm.; n_D^{22} 1.5583; ν_{max} 2280, 1735, 1650, 1580 and 1240 cm^{-1} ; τ 2.73 (aromatic, singlet, 2.4H); mass spectrum shows D_0 4.6%, D_1 33.5% and D_2 61.9%.

o-Bromo-[α,α - 2H_2]-benzyl alcohol

Ethyl o-bromobenzoate (3.5g.) in ether (10ml.) was added to a stirred suspension of lithium aluminium deuteride (360mg., 10% excess) in ether (10ml) over 30 min. After refluxing for 3 hrs., water (1ml.) and 10% aqueous sulphuric acid (15ml) were added to the cooled reaction mixture. The ethereal solution was washed with water, dried and evaporated down to a solid. Yield 2.76g. (95%); m.p. 78-79° (ethanol); ν_{max} 3300, 2200, 2080, 1590, 1565 and 755 cm^{-1} (lit⁽¹⁵⁵⁾ m.p. 80°)

o-Bromo-[α,α - 2H_2]-benzyl tosylate

o-Bromo-[α,α - 2H_2]-benzyl alcohol (2.75g.) was added to a 53.3% dispersion of sodium hydride (0.67g.) in ether (20ml.) and the mixture was refluxed for 20 hrs. The mixture was

cooled in ice-salt mixture and tosyl chloride (2.76g.) in ether (20ml) was added over $\frac{1}{2}$ hr. After 2 hrs. at room temperature, the product was centrifuged and the supernatant solution concentrated to 25 ml. The solution was cooled to -78° and the tosylate filtered off and dried in vacuo.

Yield 3.6g. (72%); m.p. $79-80^{\circ}$ ($60-80^{\circ}$ petroleum ether);

$\nu_{\max}$ 2200, 1595, 1565, 1450, 1365, 1180, 855 and 755 cm^{-1}

o-Bromo- $[\text{}^2\text{H}_3]$ -toluene

The tosylate (3.6g.) in ether (50ml.) was added to lithium aluminium deuteride (450mg.) in ether (25ml.) over 1 hr. After a further 2 hrs., water (2ml.) and 10% aqueous sulphuric acid (20ml.) were added. The ethereal solution was separated, washed with water, dried and distilled.

Yield 1.30g. (71%); b.p. $178-179^{\circ}$; n_D^{20} 1.5540; $\nu_{\max}$ 2250, 1595 and 755 cm^{-1}

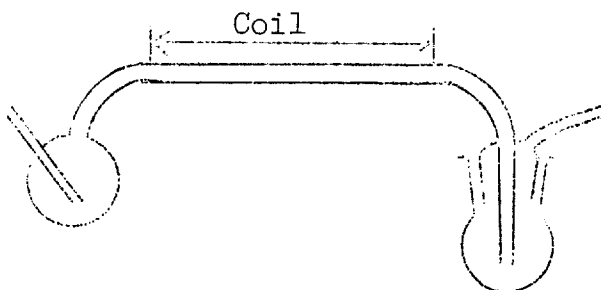
o- $[\text{}^2\text{H}_3]$ -Methylcinnamyl acetate

This was prepared from o-bromo- $[\text{}^2\text{H}_3]$ -toluene (1.25g.) by the same reaction sequence as the undeuteriated compound.

Yield 520mg.; b.p. $89-90^{\circ}/0.5\text{mm.}$; n_D^{20} 1.5340; $\nu_{\max}$ 2220, 1735, 1650, 1600 and 750 cm^{-1} ; the n.m.r. spectrum has no signal at $\tau 7.66$ due to the aromatic methyl group.

Pyrolysis of Cinnamyl Acetate

A diagram of the pyrolysis apparatus, which is made from silica, is shown below.



The heating coil is of Vacron 80/20 wire, resistance 100 ohms, covered with asbestos paper and then asbestos cord. The power is supplied by a Variac variable transformer which has been calibrated against a thermocouple inserted in the pyrolysis tube, so that temperature can be related to voltage.

Cinnamyl acetate (8g.) was distilled from the flask into the pyrolysis tube which was maintained at 750° . The pressure was 1mm. and the time taken was 1 hr. The pyrolysate was collected in the receiver at -78° . The pyrolysate was stirred with moistened sodium bicarbonate and then taken up in ether, washed with water and dried over sodium sulphate. The solution was distilled and the fraction b.p. $30-180^{\circ}/20\text{cm}$. collected (4.1g.). This was separated on a Pye series 105 preparative gas chromatograph, using a 30 ft. methyl silicone gum E30 column at 150° and compounds A and C were obtained in 95% purity.

Compound A. (Styrene): Molecular weight 104 (mass spec.);

$\nu_{\max}$ 1630, 1600, 1580, 1500, 1000, 920, 785 and 705 cm^{-1} ;
 $\lambda_{\max}$ (ethanol) 246 μ .; τ 3.36 (double doublet, $J=17.5$ and 10.5 Hz, 1H), 4.39 (double doublet, $J=17.5$ and 1.5 Hz, 1H), 4.87 (double doublet, $J=10.5$ and 1.5 Hz, 1H), 2.7-3.0 (multiplet, 5H)
 Compound C. (Indene): molecular weight 116 (mass spec.);
 $\nu_{\max}$ 1605, 1465, 775, 720 and 700 cm^{-1} ; $\lambda_{\max}$ (ethanol) 249 μ .
 τ 2.6-3.0 (multiplet, 4H), 3.22 (double triplet, $J=6$ and 2 Hz, 1H), 3.57 (double triplet, $J=6$ and 2 Hz, 1H) and 6.70 (triplet, $J=2$ Hz, 2H). Retention time on gas chromatography is identical to that of indene.

Compound B (phenylacetylene) was only obtained as a mixture with styrene (30% phenylacetylene, 60% styrene). The mass spectrum of this mixture had a large peak at m/e 102 which was not present in the mass spectrum of styrene, hence B has a molecular weight of 102. Retention time of B is identical to that of an authentic sample of phenylacetylene

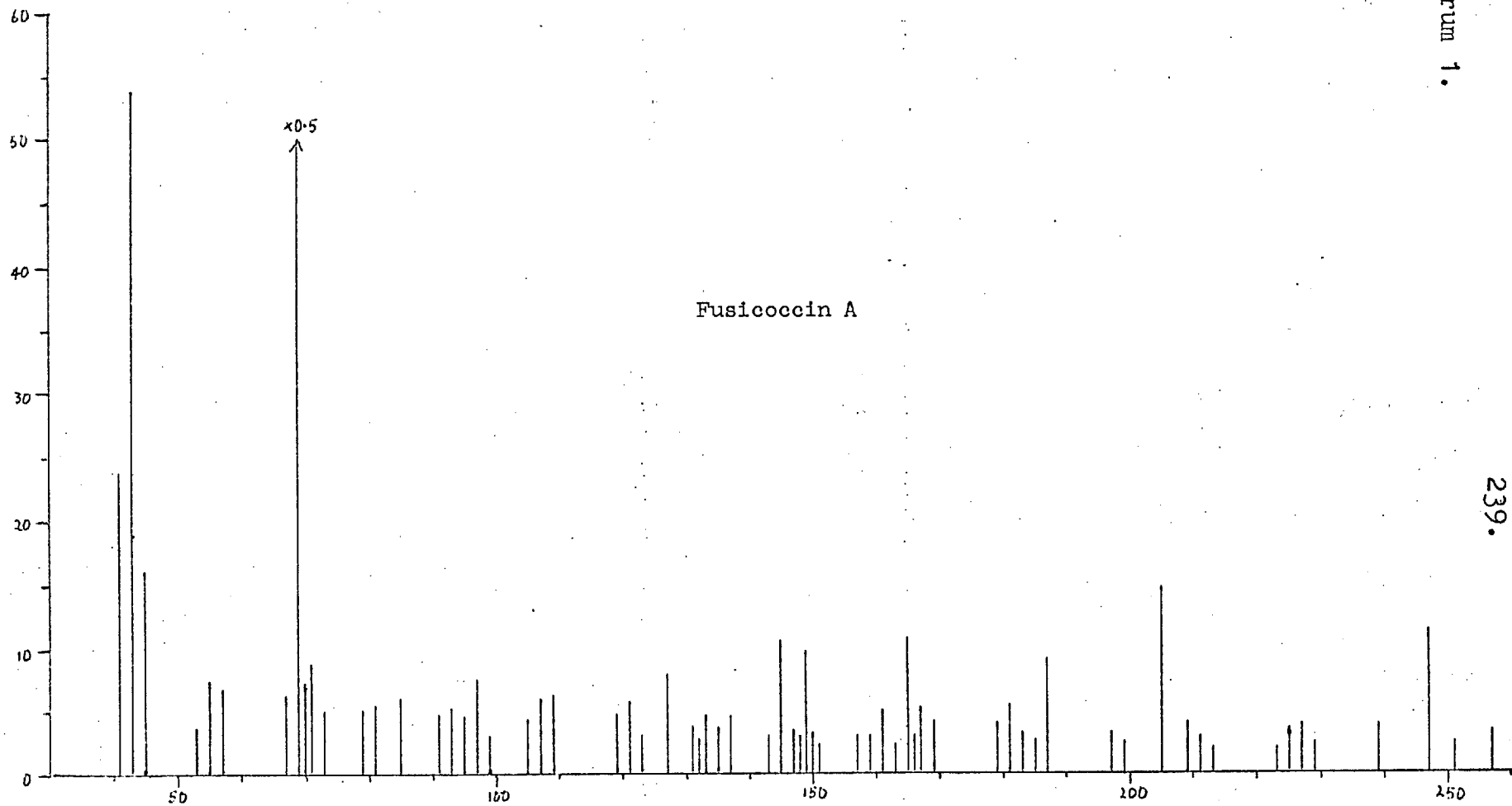
The two compounds D were only obtained as a 1:1 mixture, and physical data for this is as follows: molecular weight (mass spec.) 130; $\nu_{\max}$ 3070, 2920, 1620, 1470, 960, 930, 840, 795, 785, 760, 725 and 700 cm^{-1} ; $\lambda_{\max}$ (ethanol) 254 μ . The n.m.r. spectrum has signals in the range τ 2.6-7.8, therefore there are no methyl groups. This data is consistent with D being a mixture of 1,2 and 1,4-dihydronaphthalenes.

After standing for a week, naphthalene was isolated from the pyrolysate by gas chromatography at 160°; m.p. 79-80°; mol.wt. 128 (mass spec.); $\nu_{\max}$ 795 cm^{-1} ; $\lambda_{\max}$ 267, 276, 284, 286 μ .; τ 2.23 (multiplet) and 2.56 (multiplet) in 1:1 ratio.

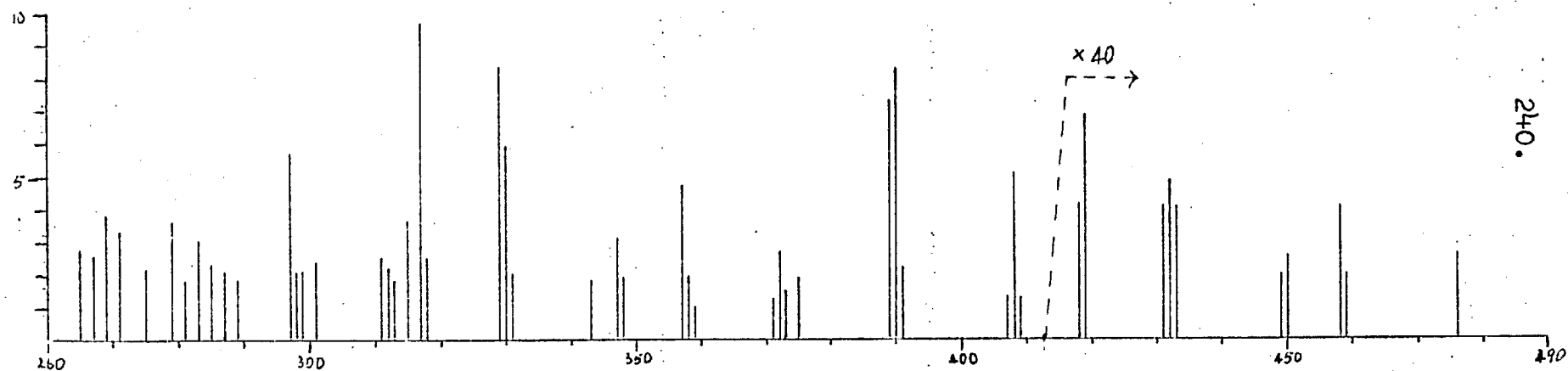
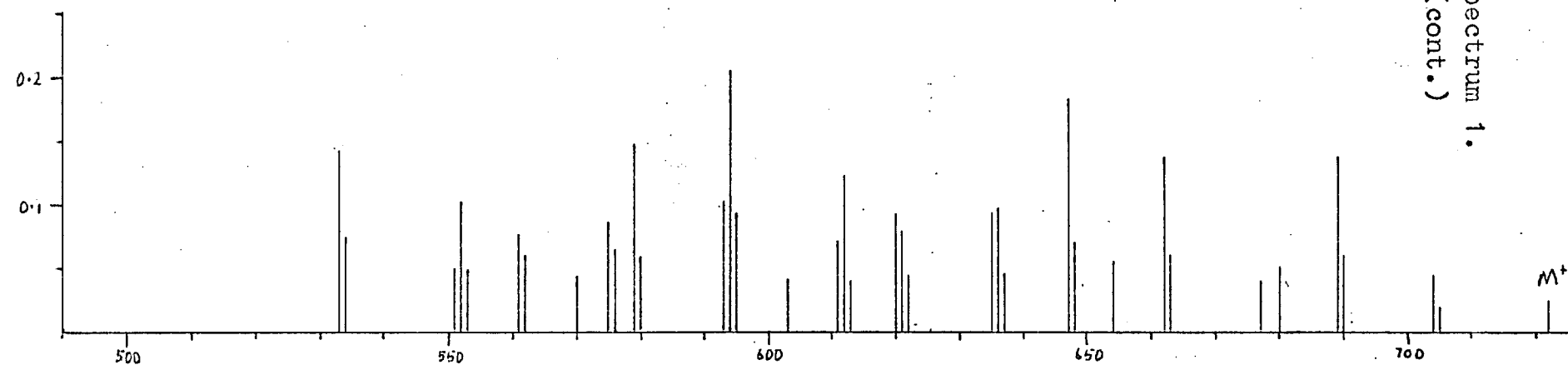
3,3 Mass Spectra

Mass spectra were obtained on an A.E.I. MS9 double focusing mass spectrometer. The ion beam energy was 70eV unless otherwise stated. Glycosides were introduced into the source by the direct insertion (probe) technique, at the minimum temperature that produced a sufficient vapour pressure of sample (120-220°). Allylic acetates were introduced through a heated glass inlet system (hot box).

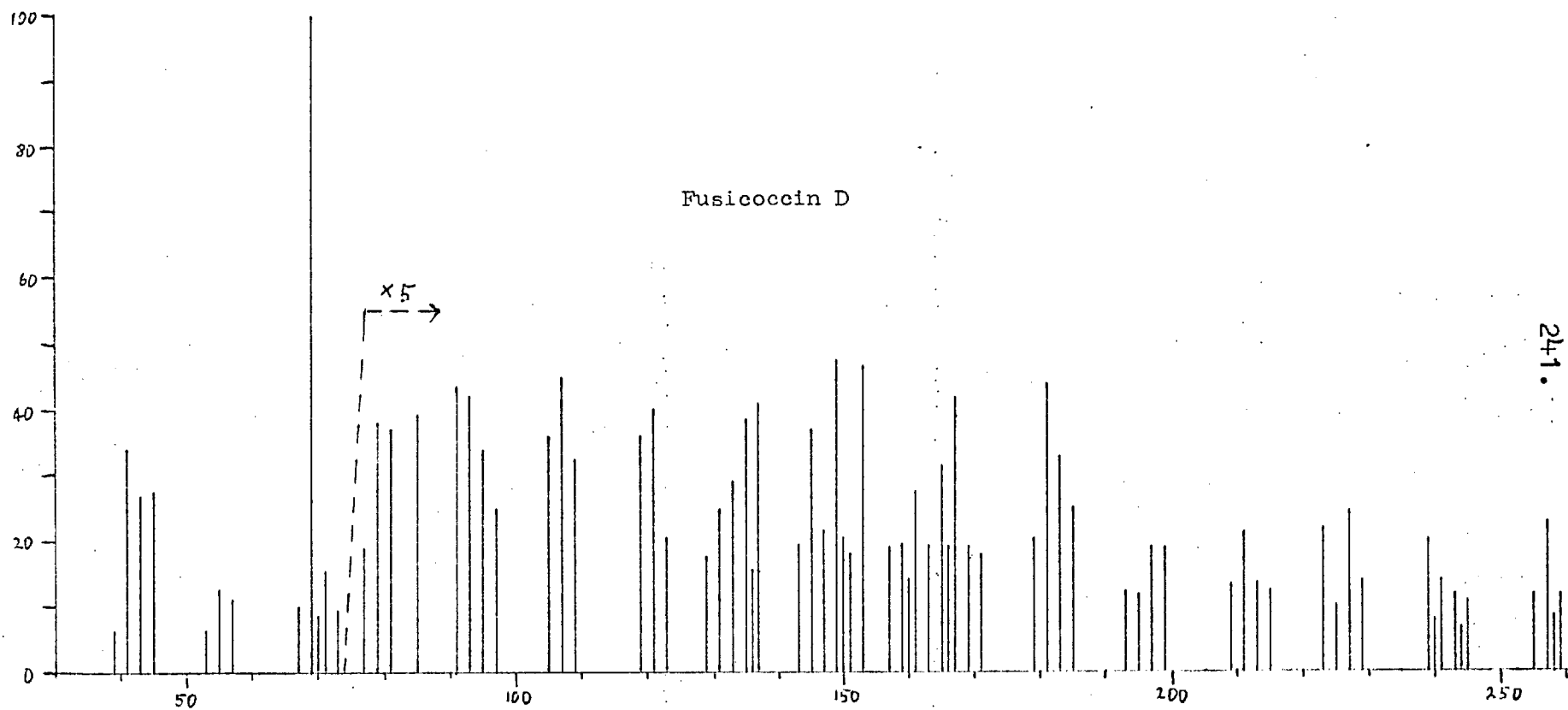
In the line diagrams a peak which is referred to as x n, is drawn n times larger than it actually is.



Mass spectrum 1.
(cont.)

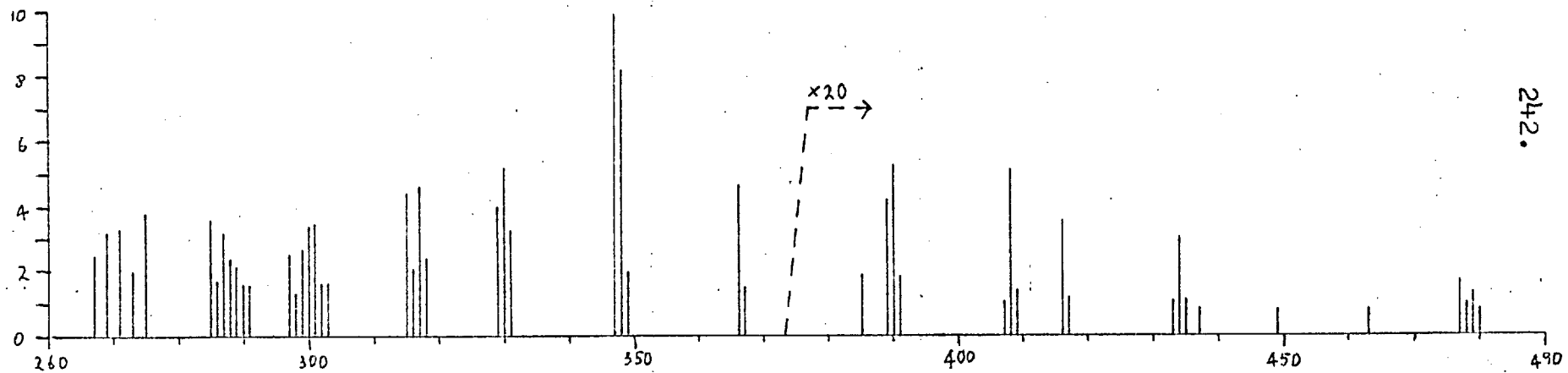
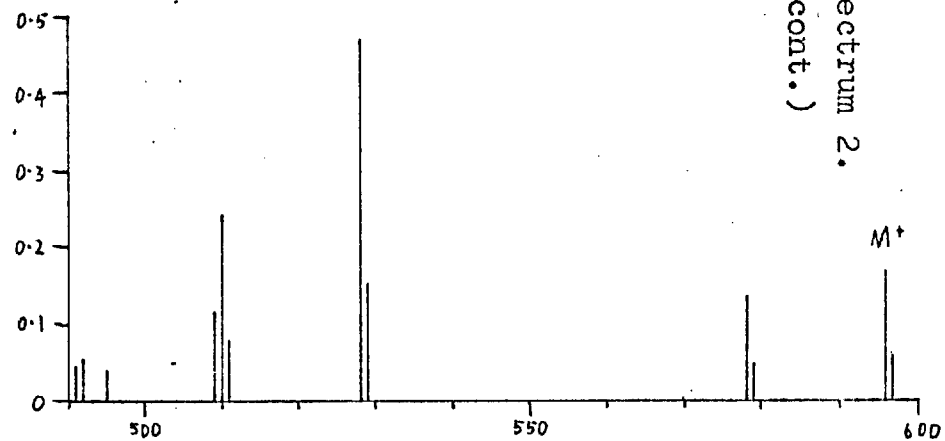


Mass spectrum 2.

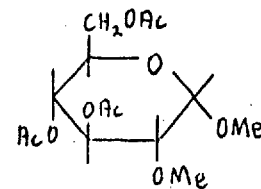
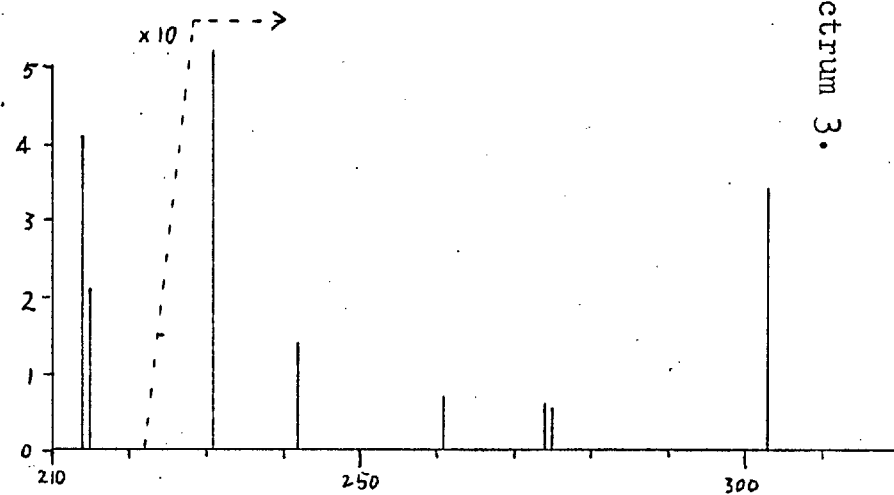
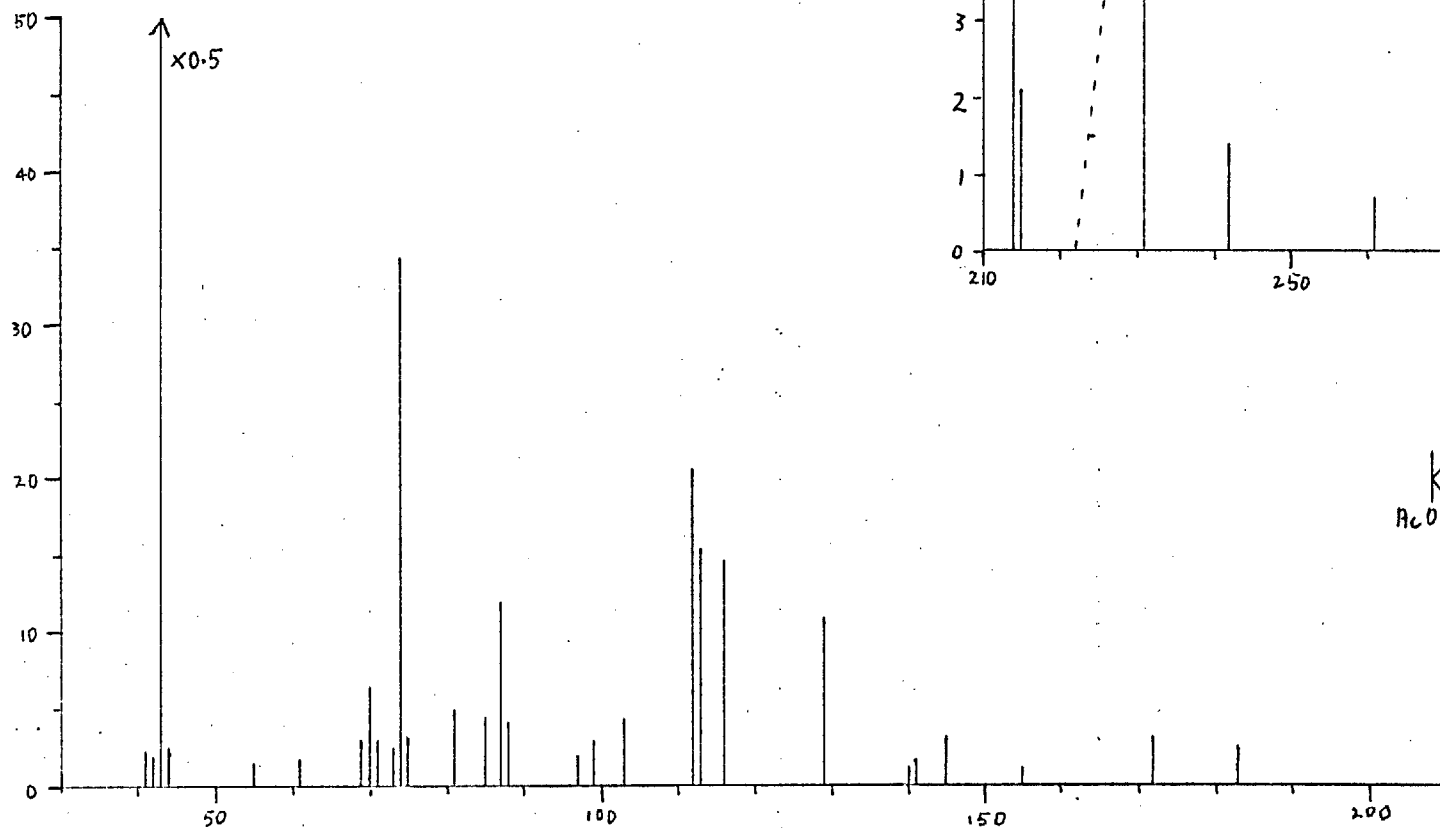


241.

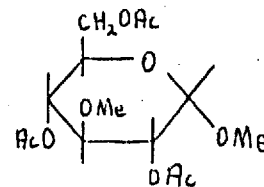
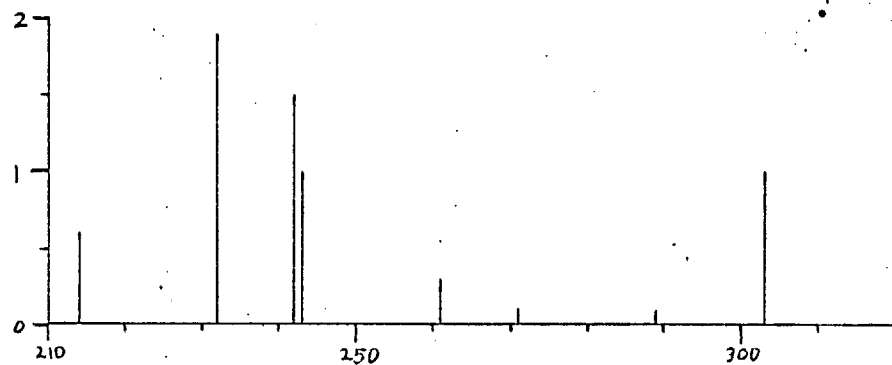
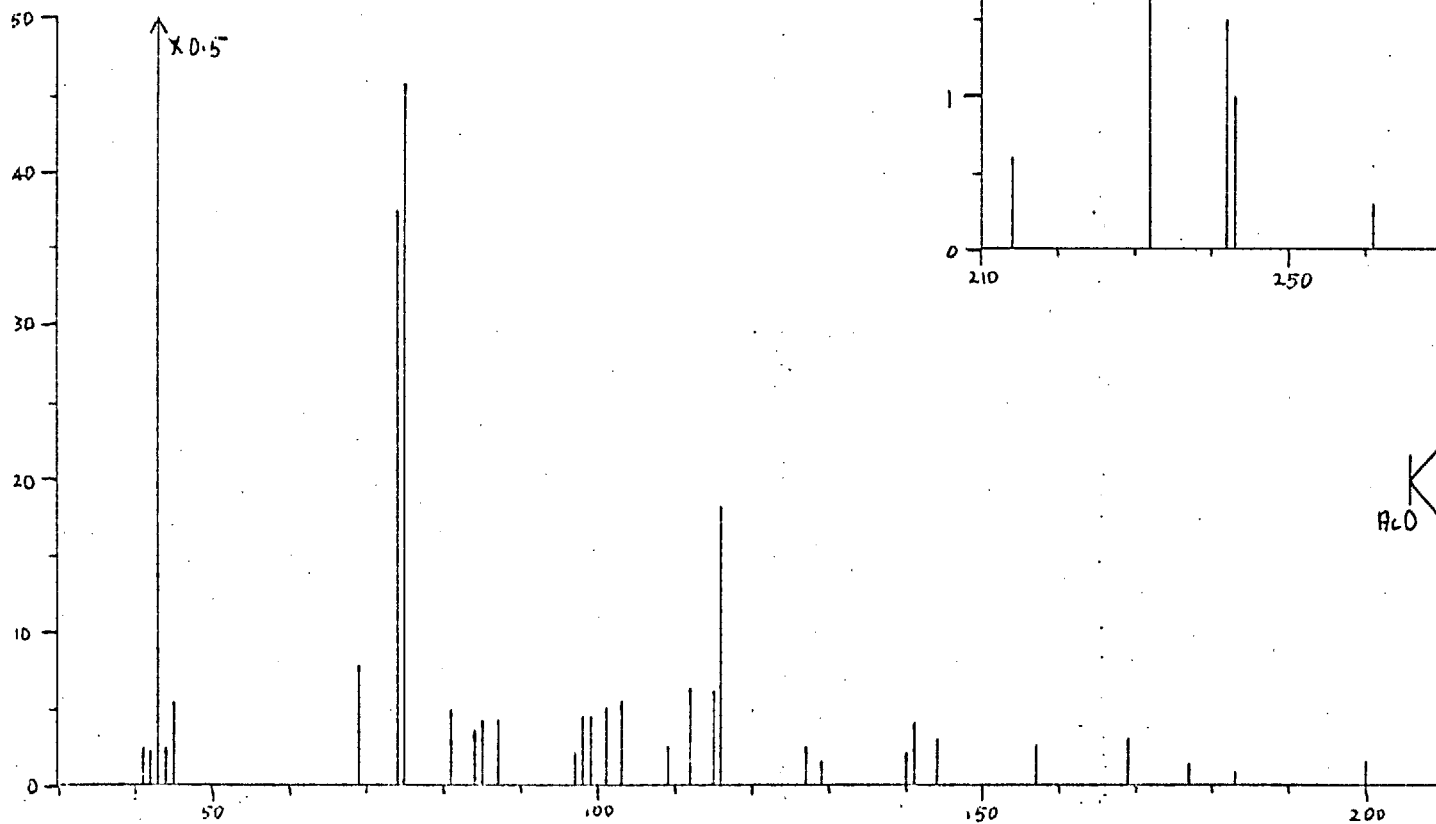
Mass spectrum 2.
(cont.)



Mass spectrum 3.



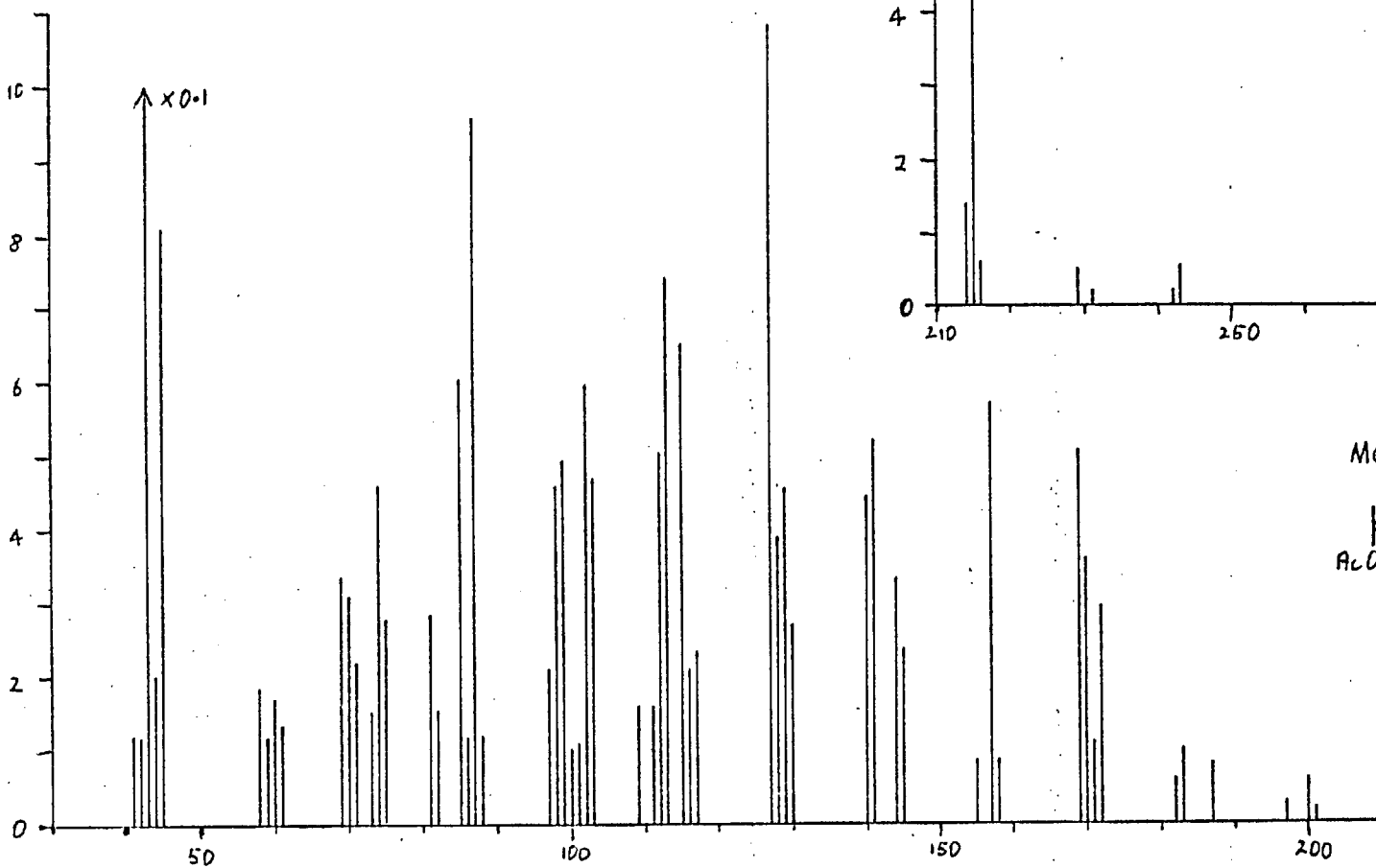
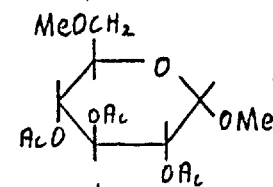
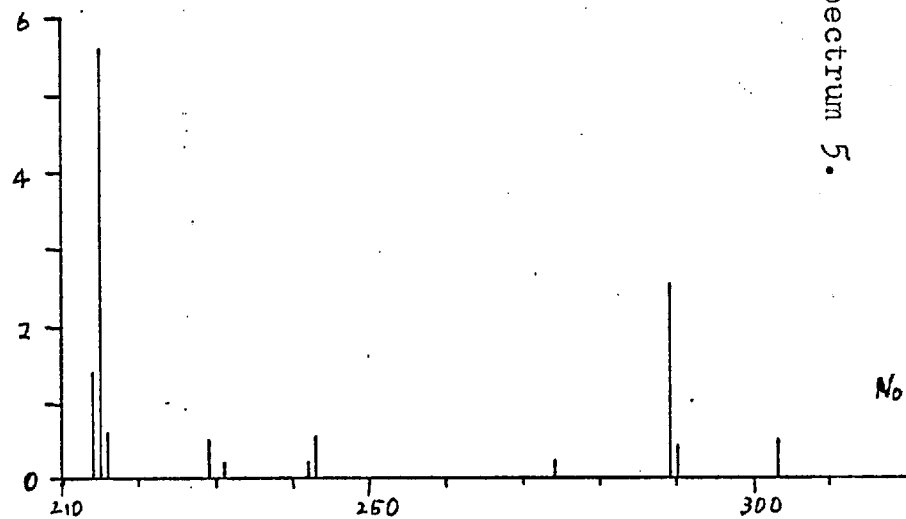
Mass spectrum 4.



Mass spectrum 5.

$N_0 M^+$

245.

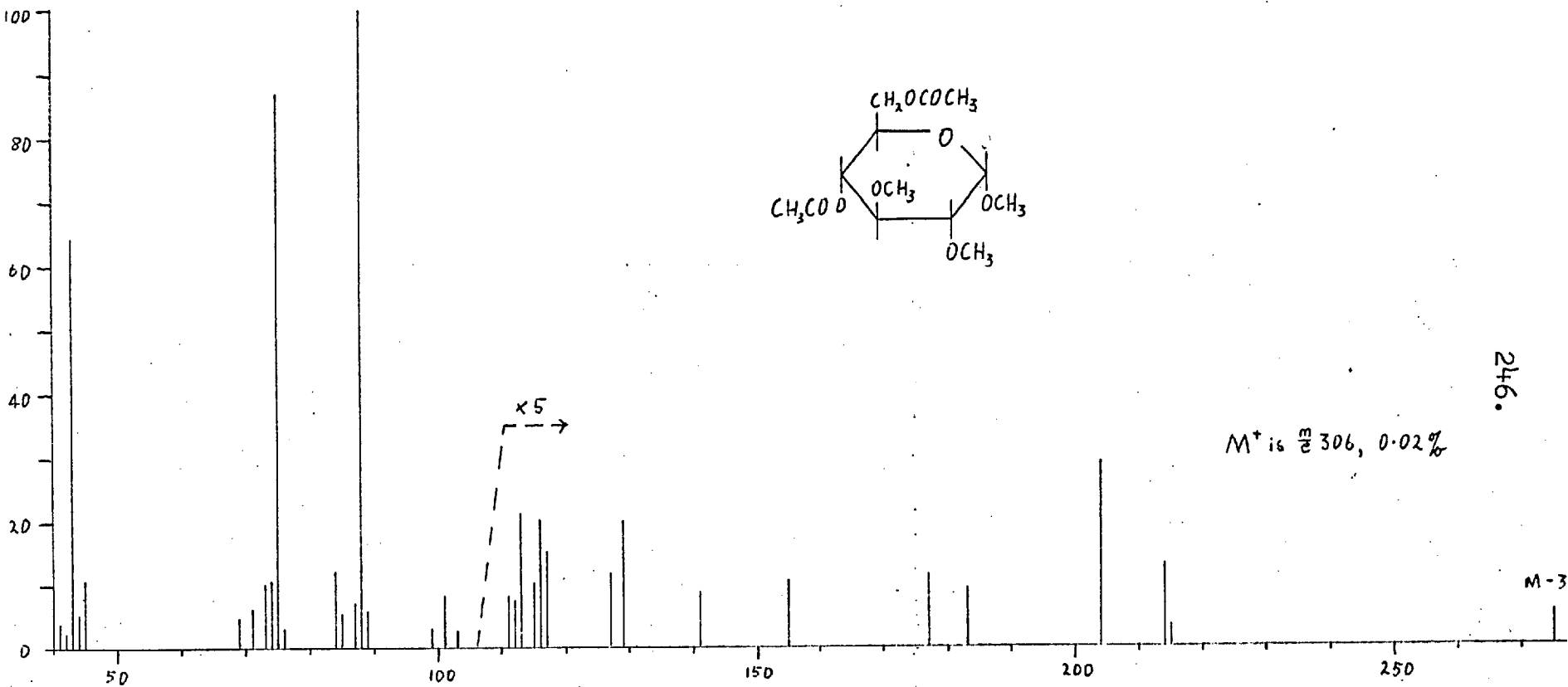
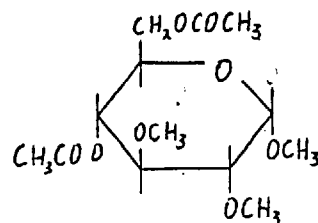


Mass spectrum 6.

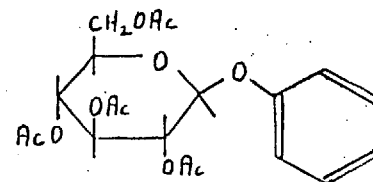
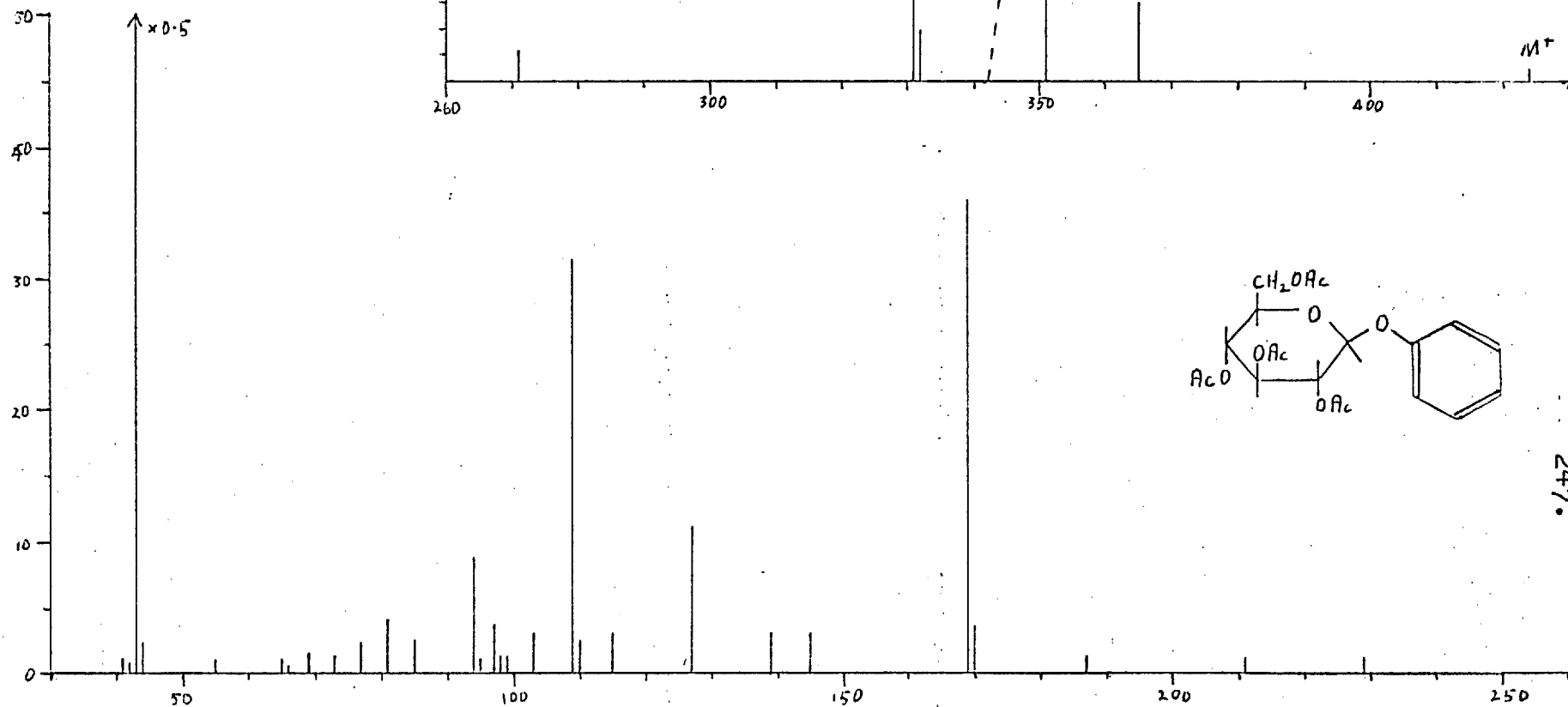
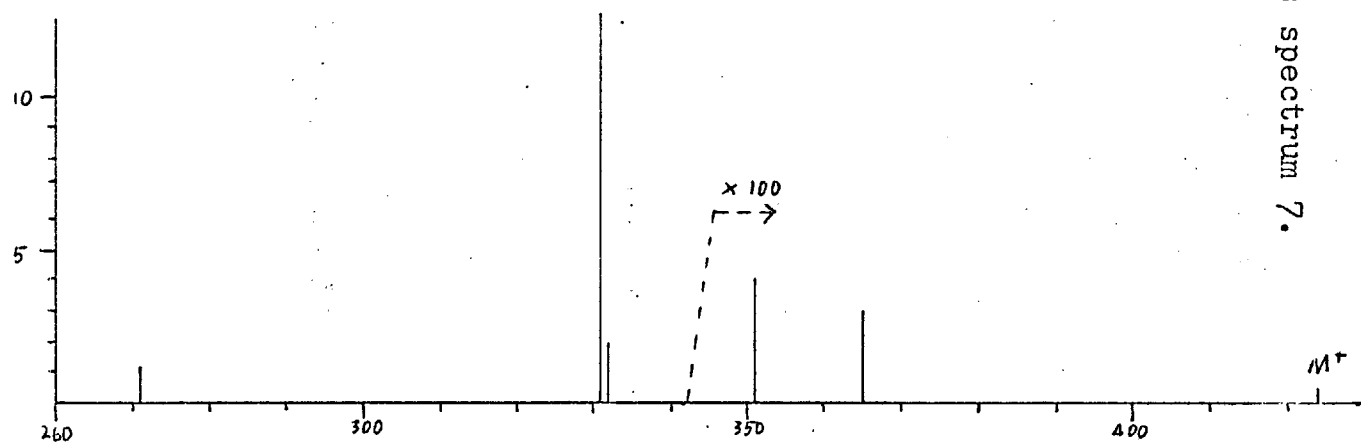
246.

M^+ is m/z 306, 0.02%

M-31

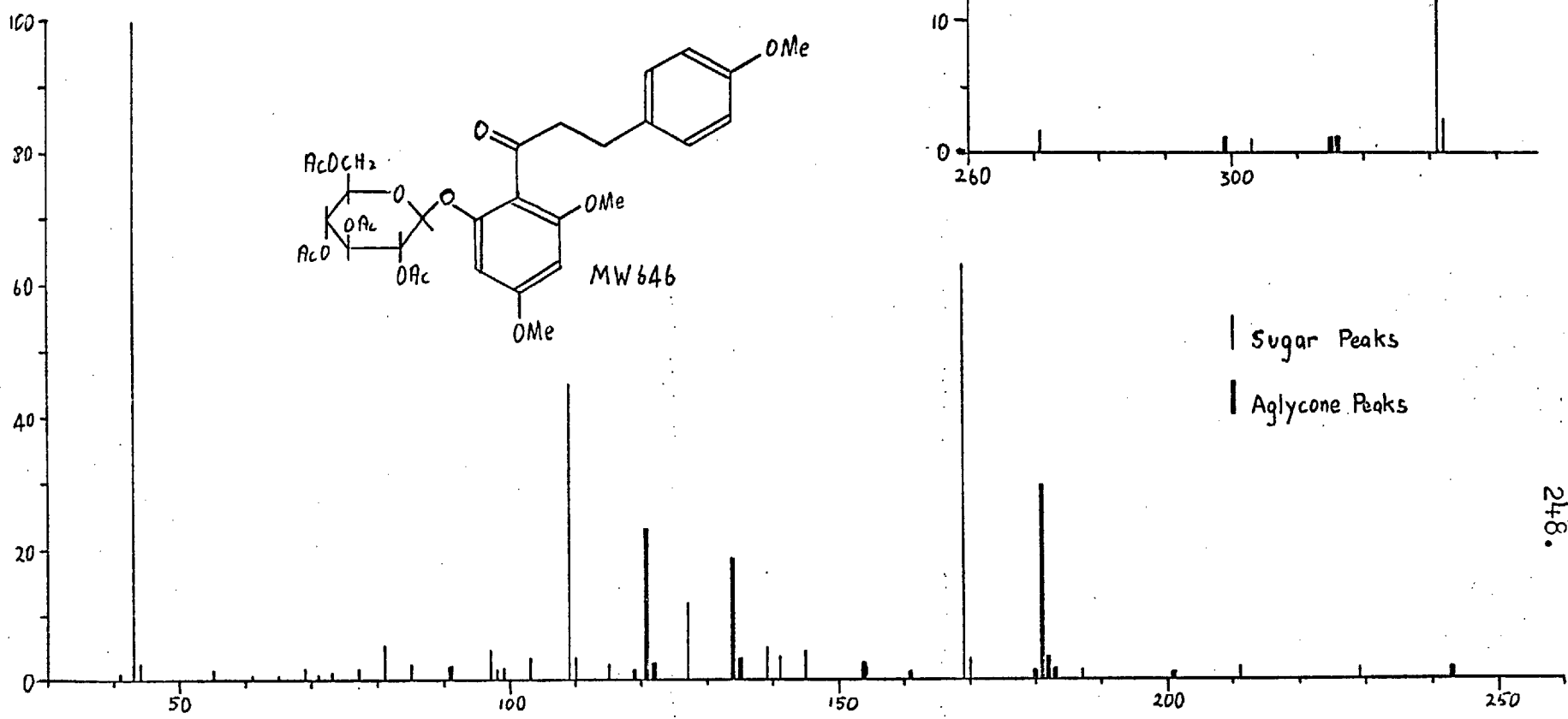
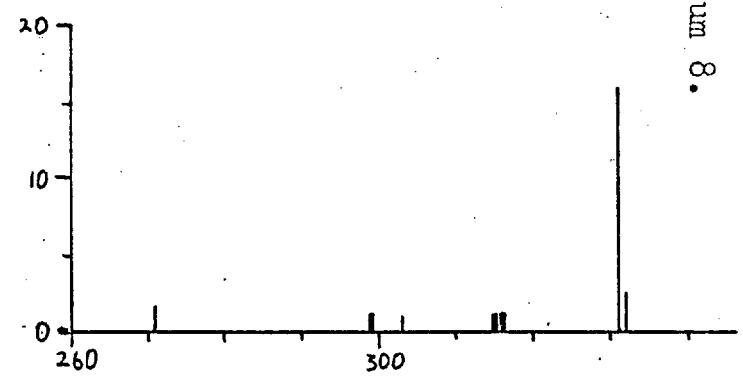
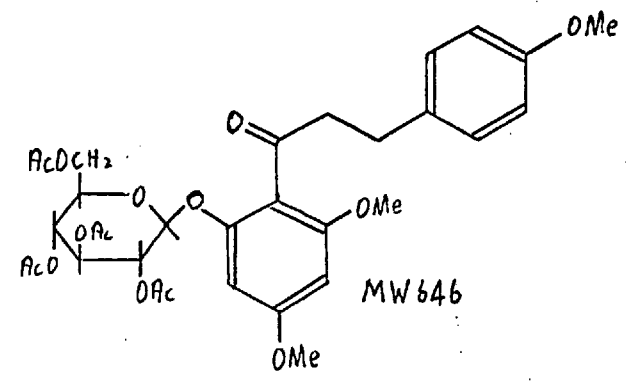


Mass spectrum 7.



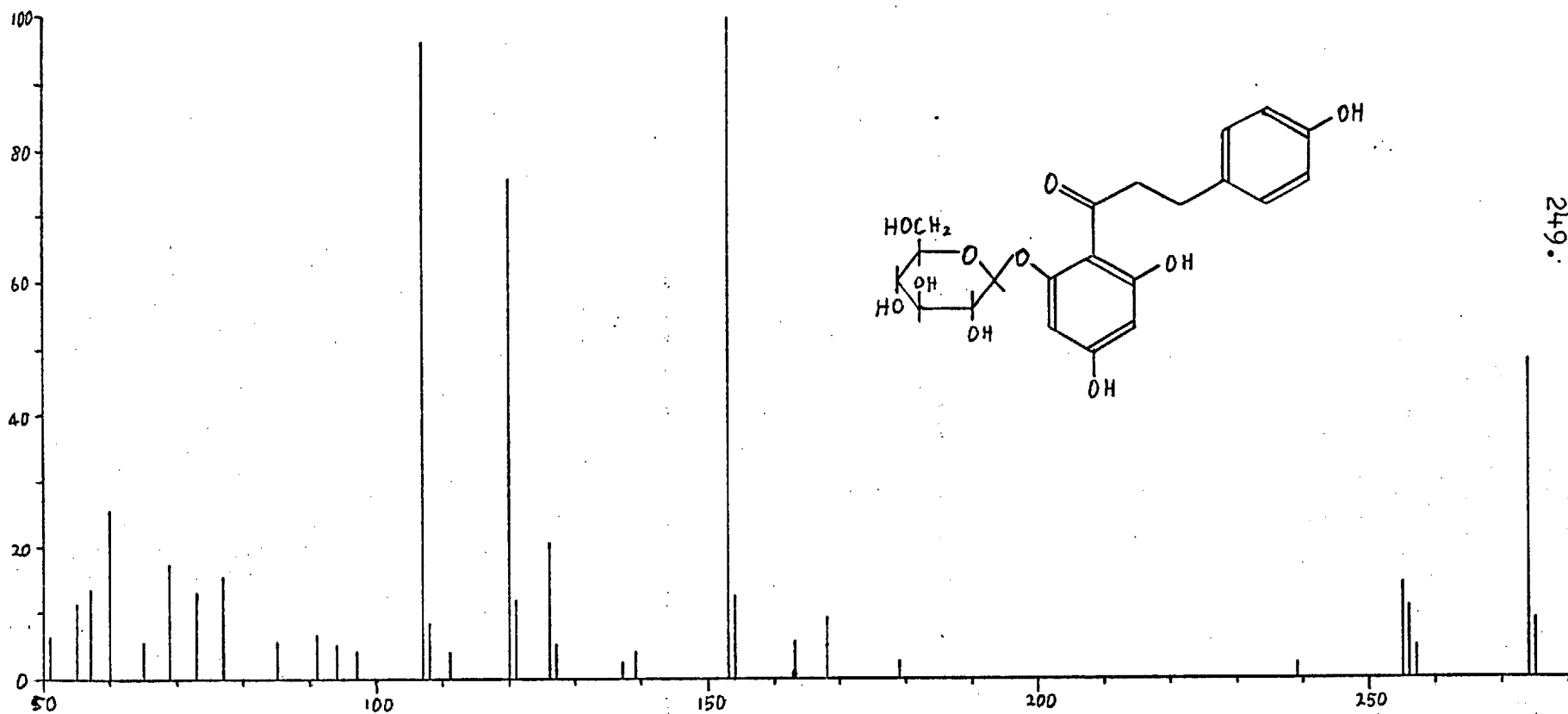
247.

Mass spectrum 8.

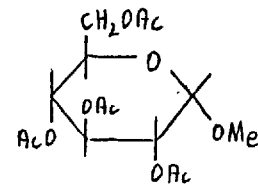
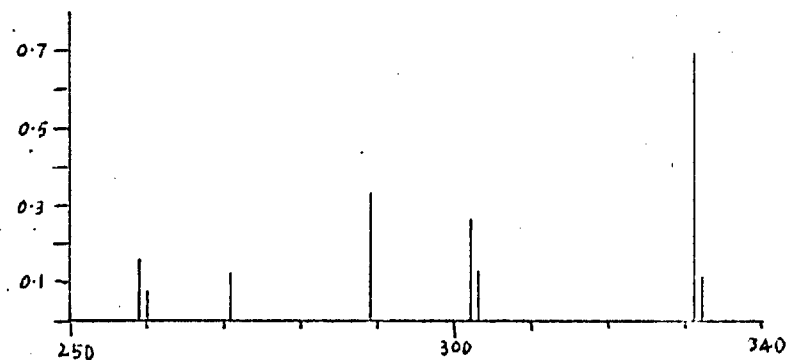
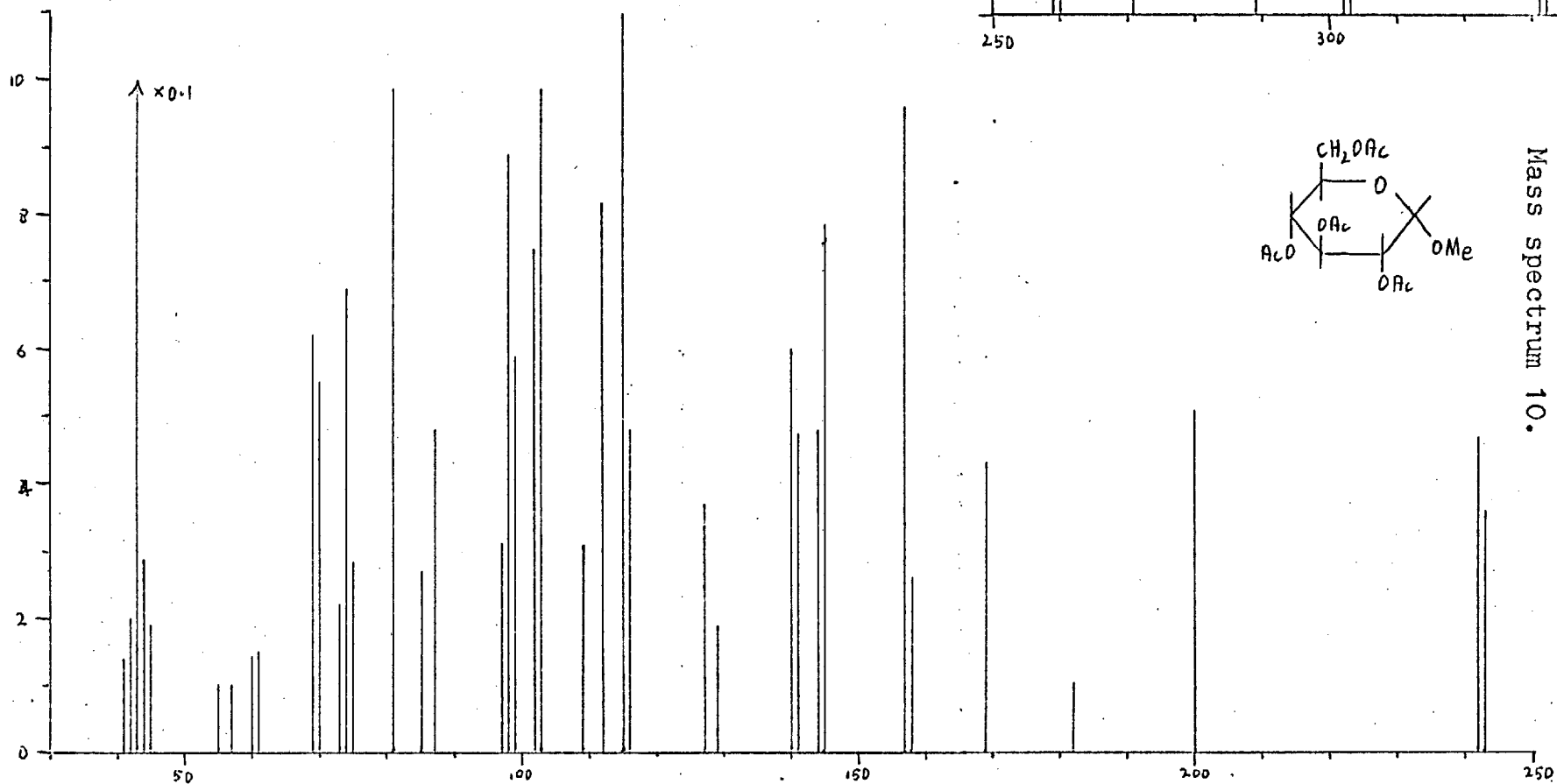


248.

Mass spectrum 9.

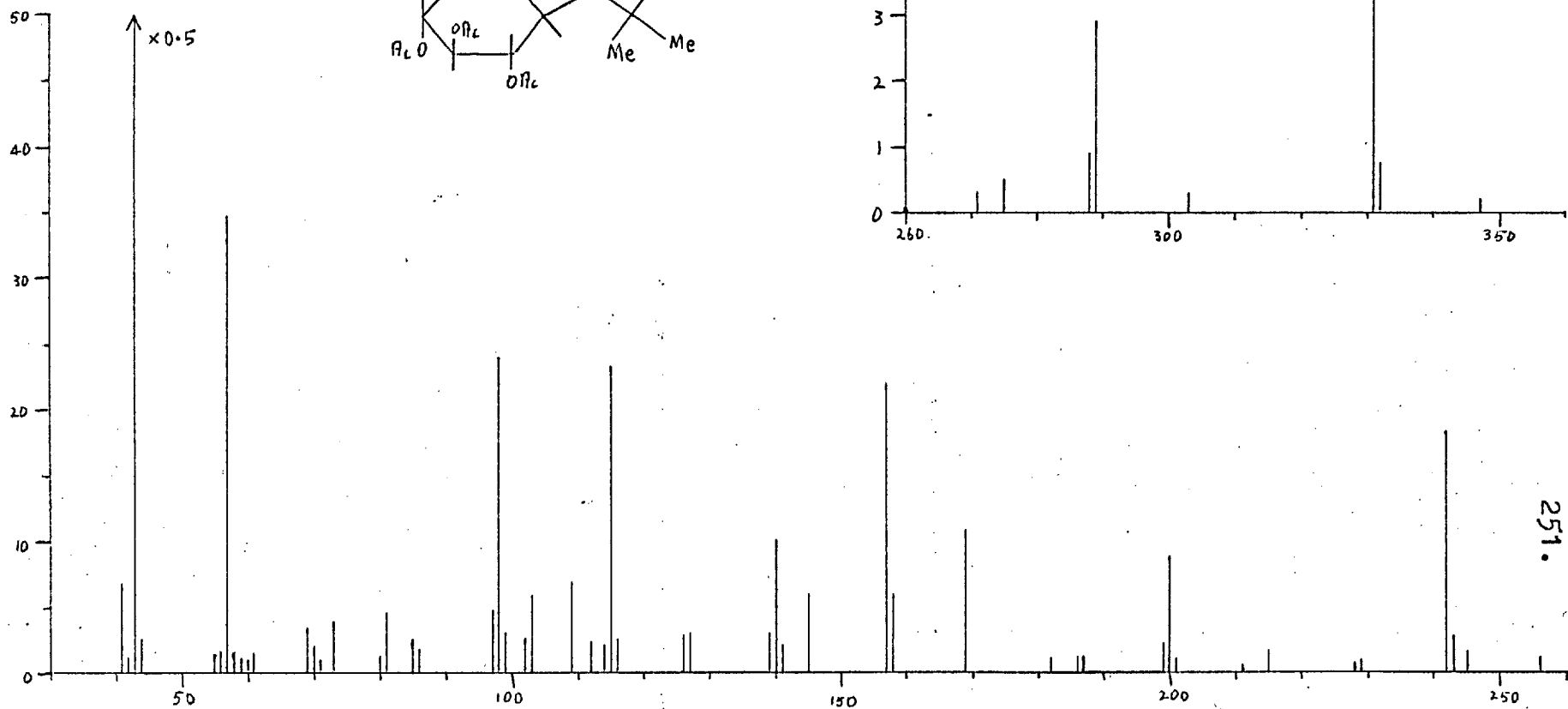
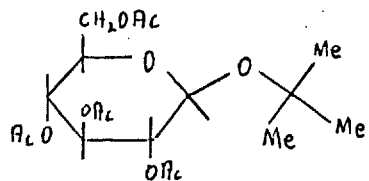


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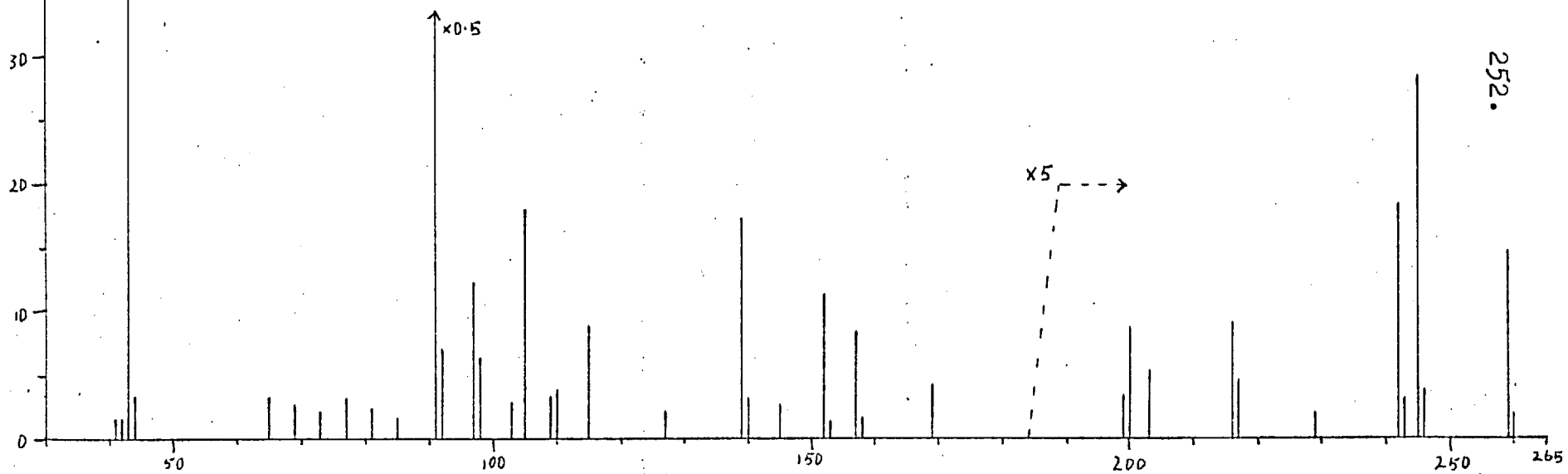
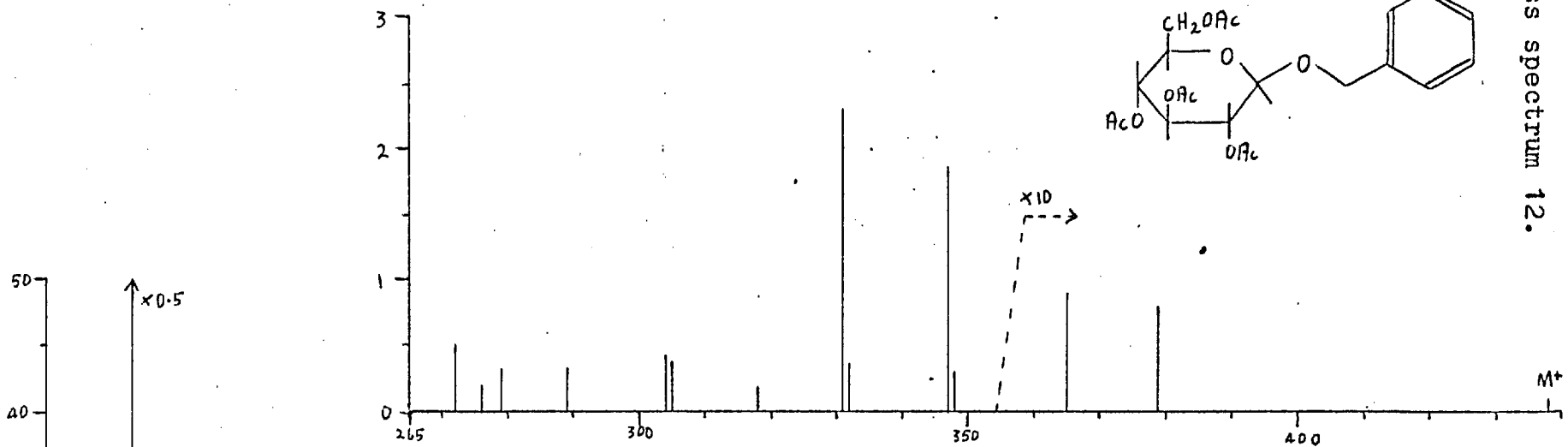
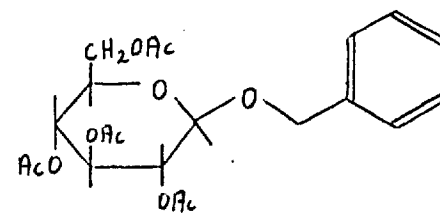


Mass spectrum 10.

Mass spectrum 11.

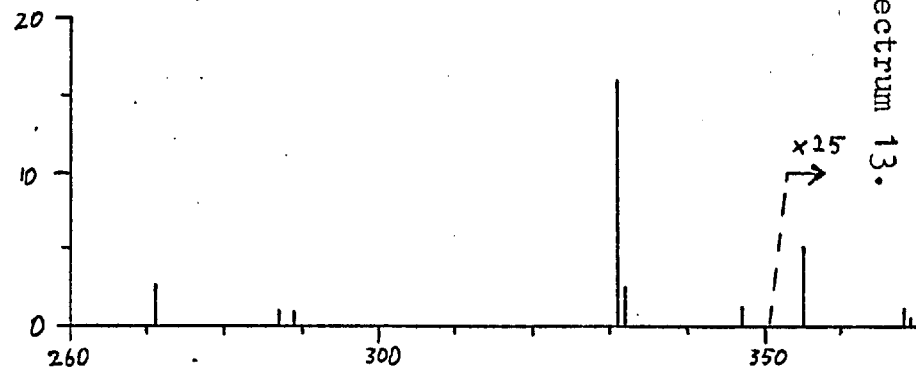


Mass spectrum 12.

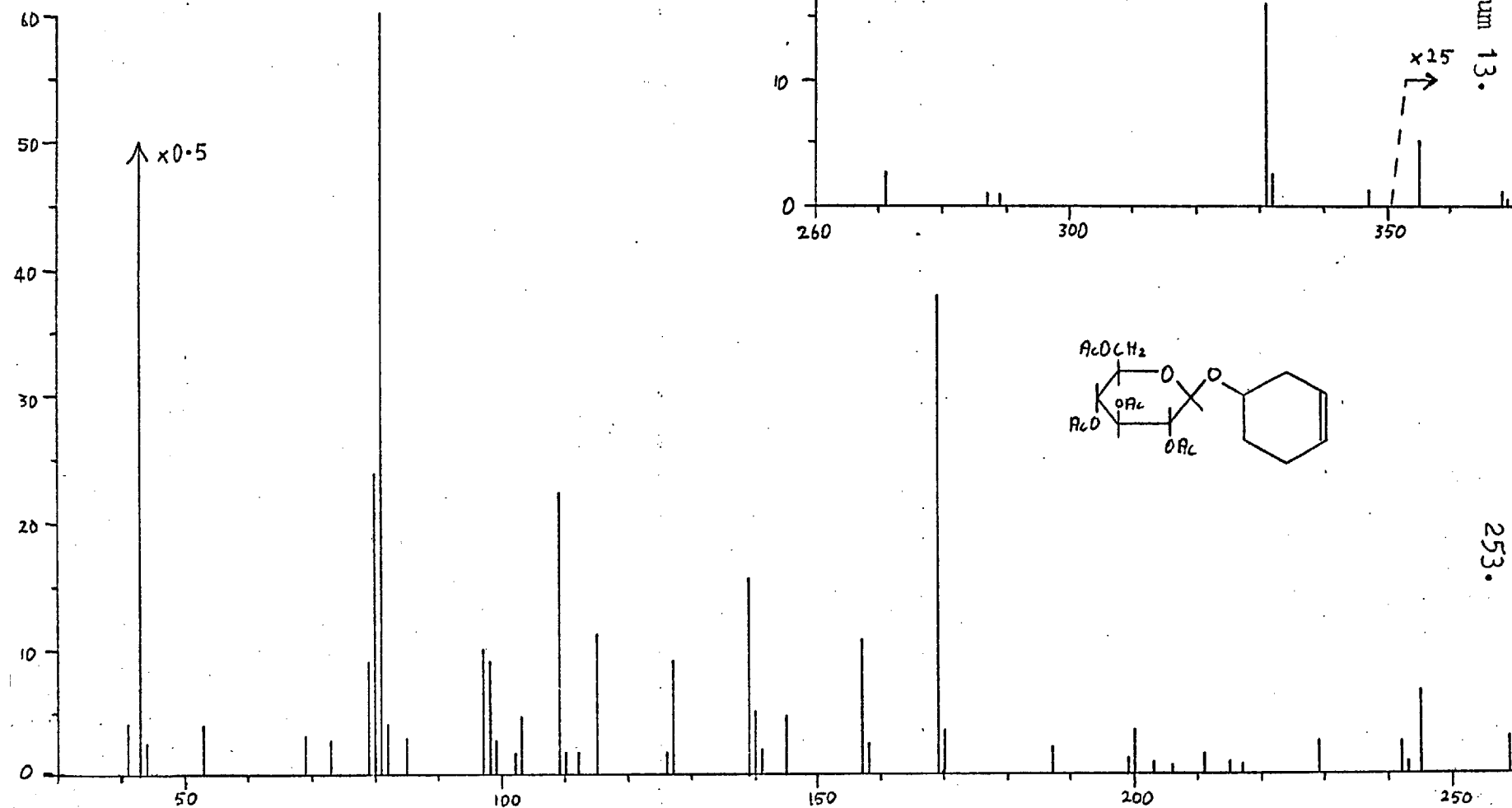
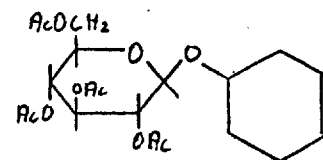


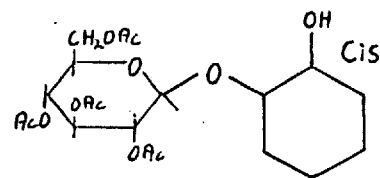
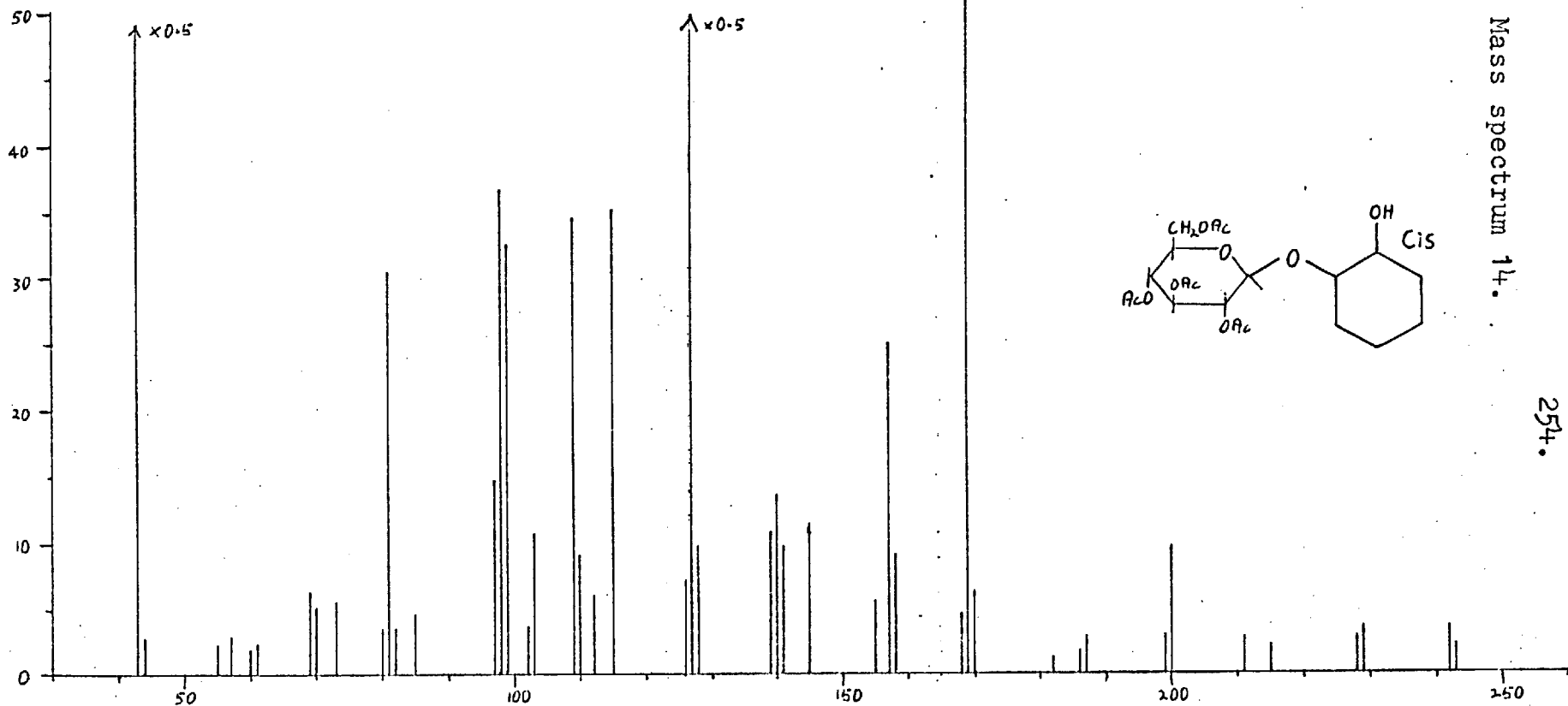
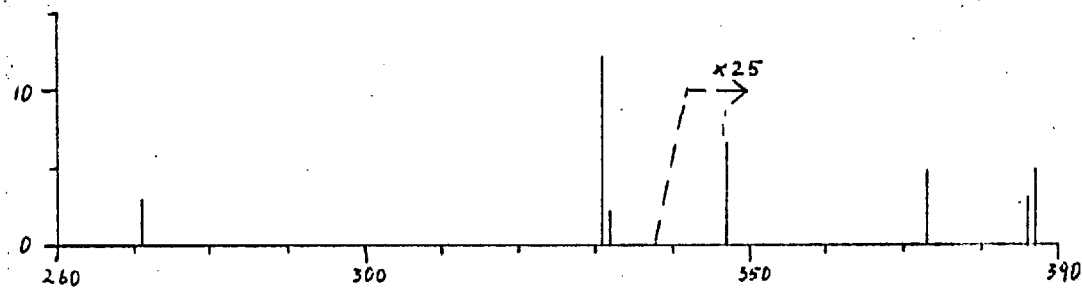
Mass spectrum 13.

$N_0 M^+$
0.428

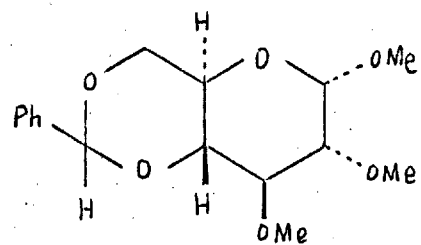


253.

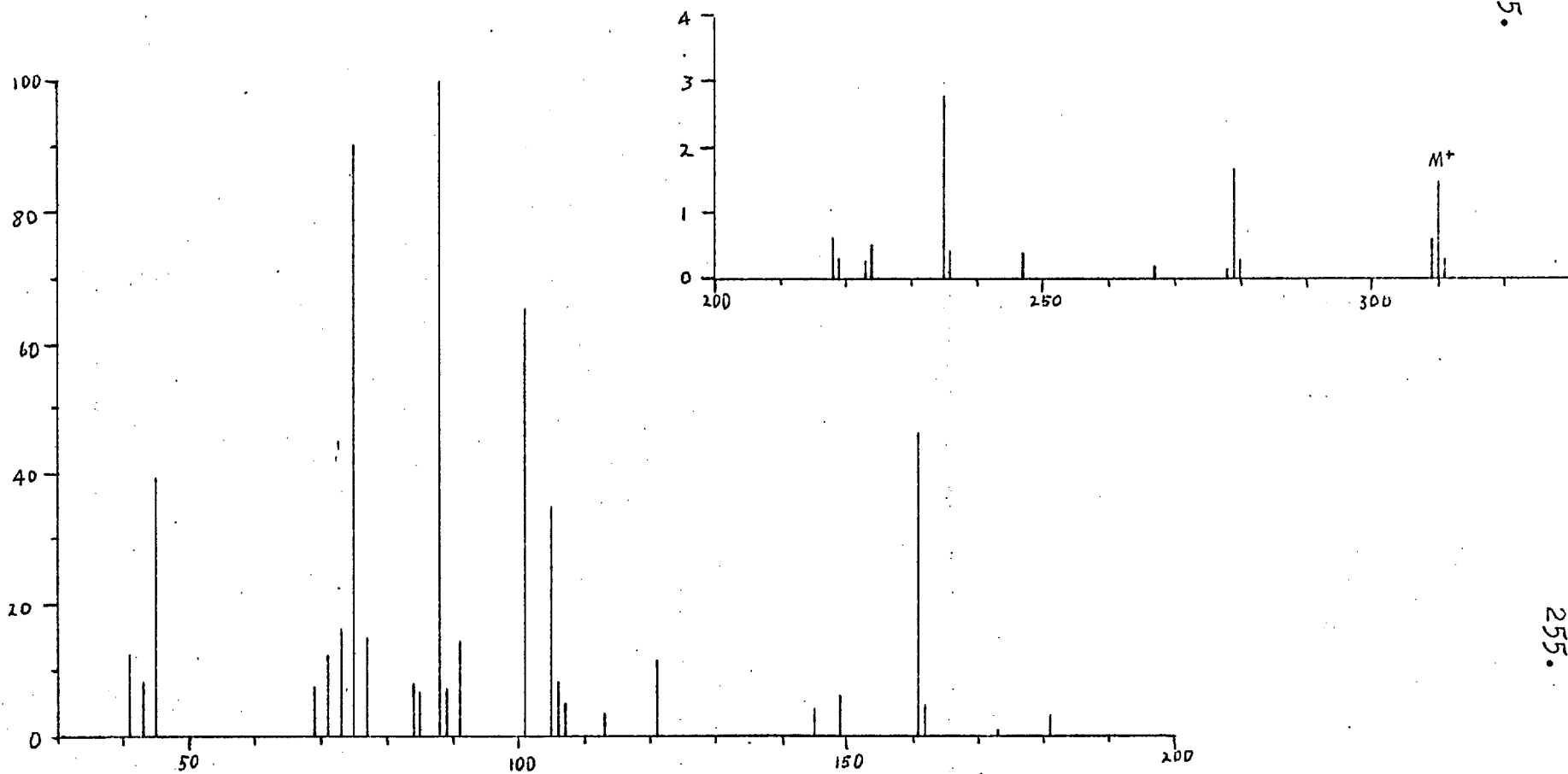




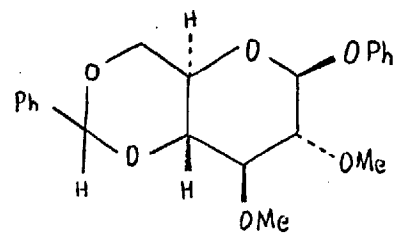
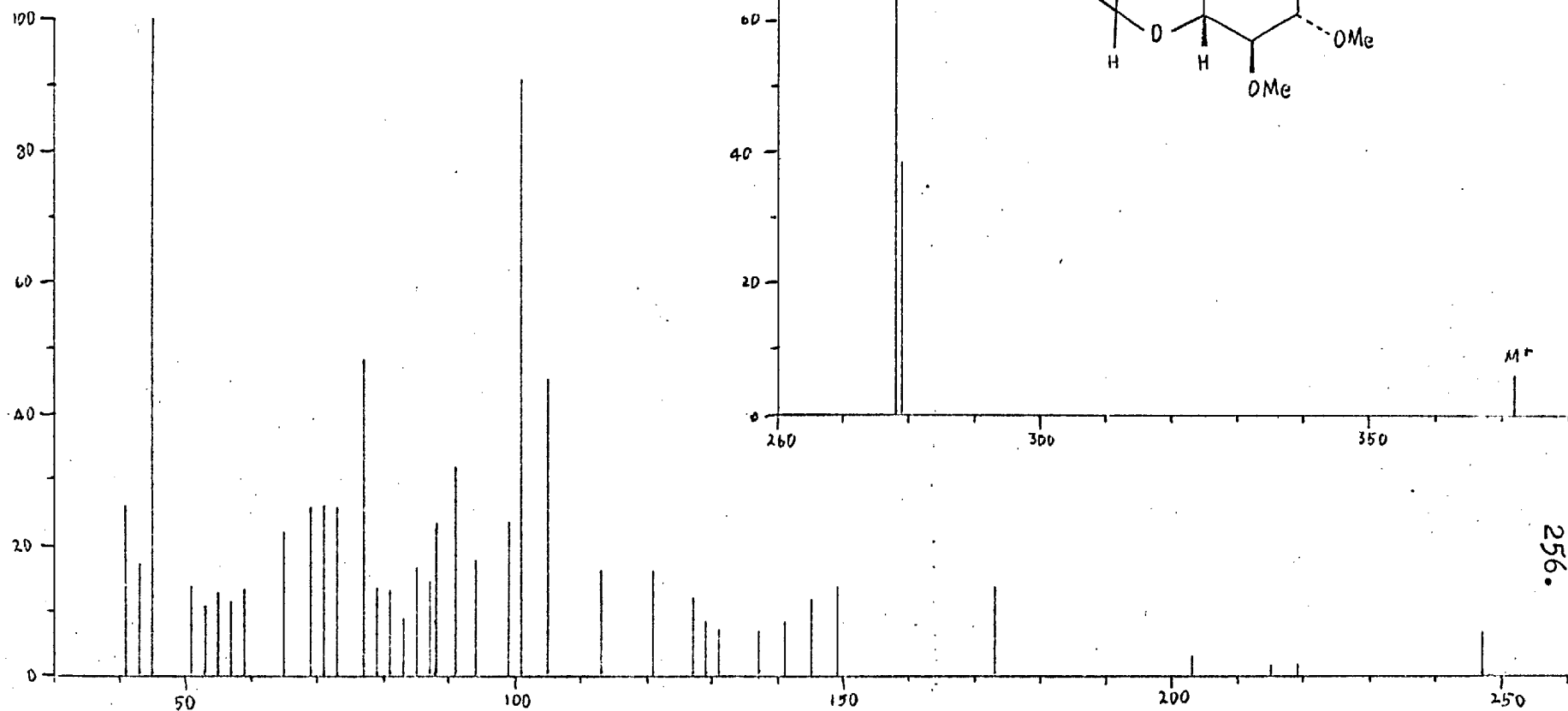
Mass spectrum 14.



Mass spectrum 15.

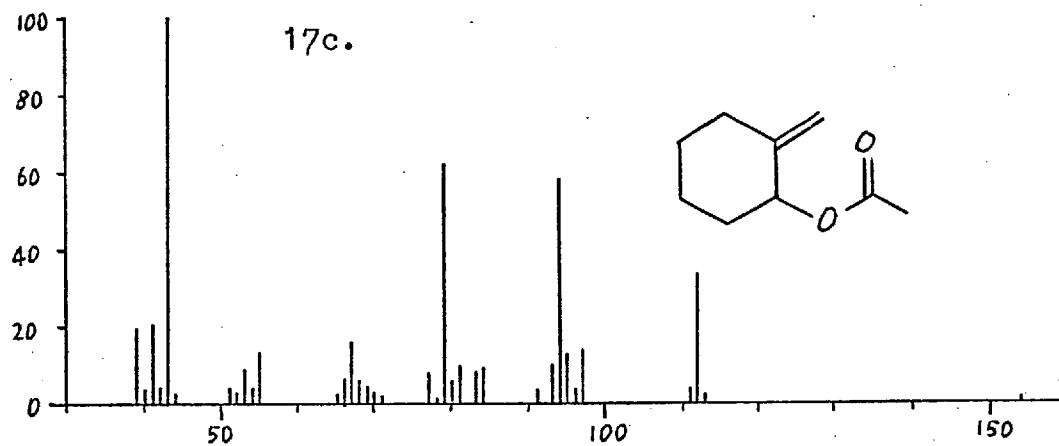
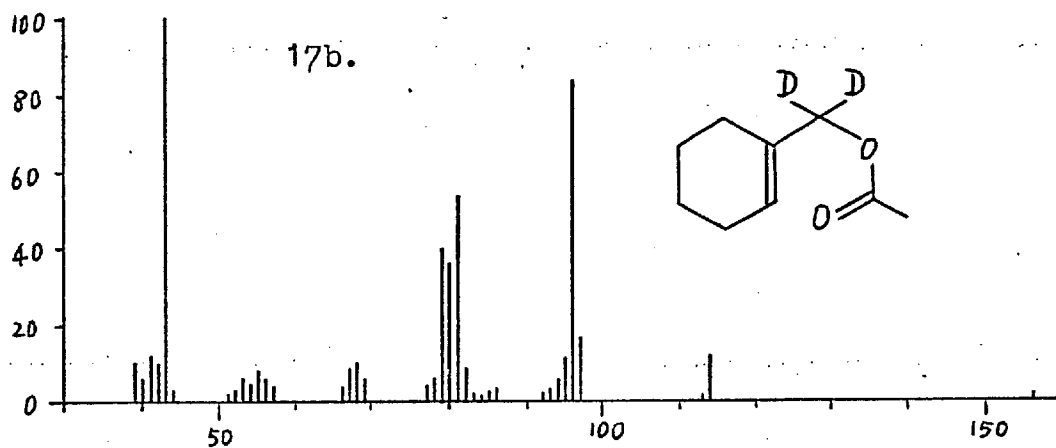
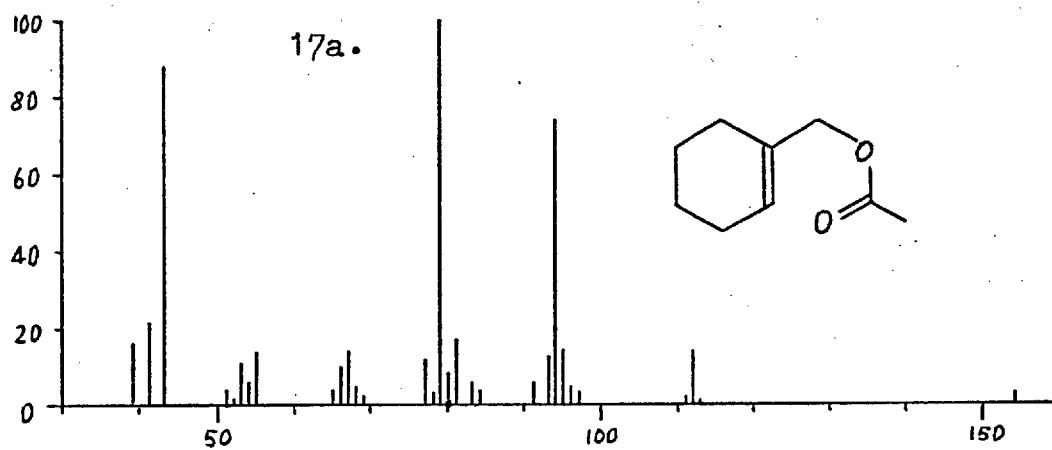


Mass spectrum 16.

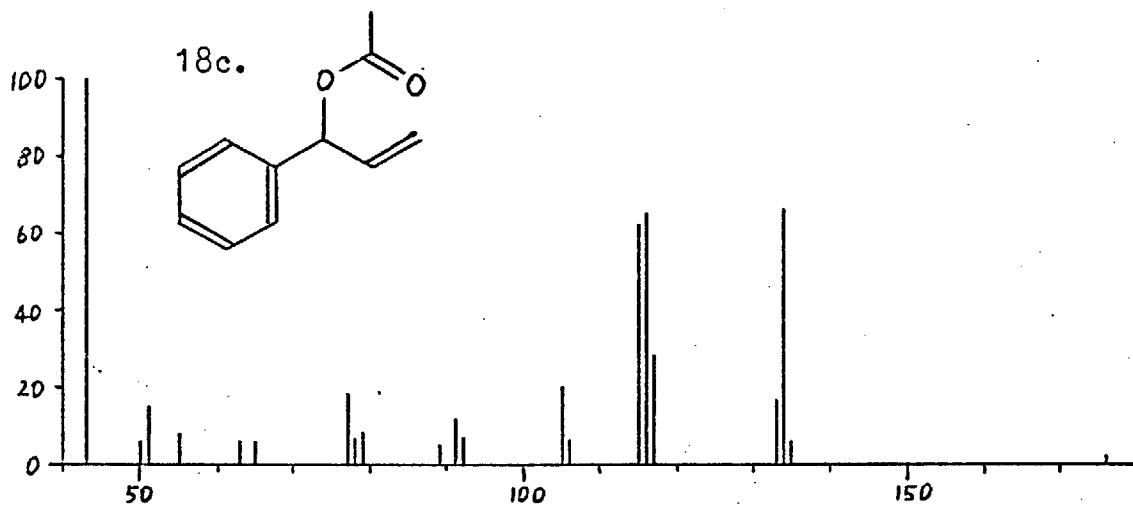
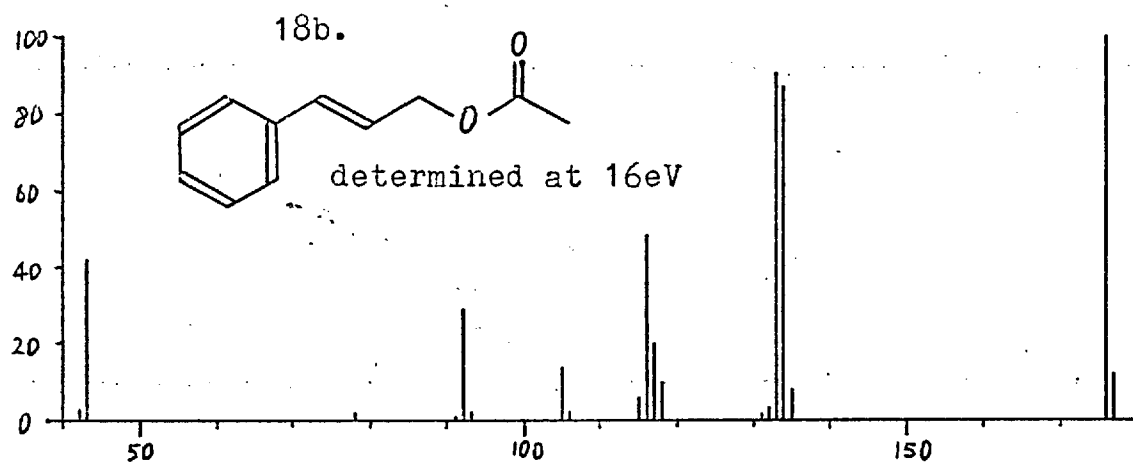
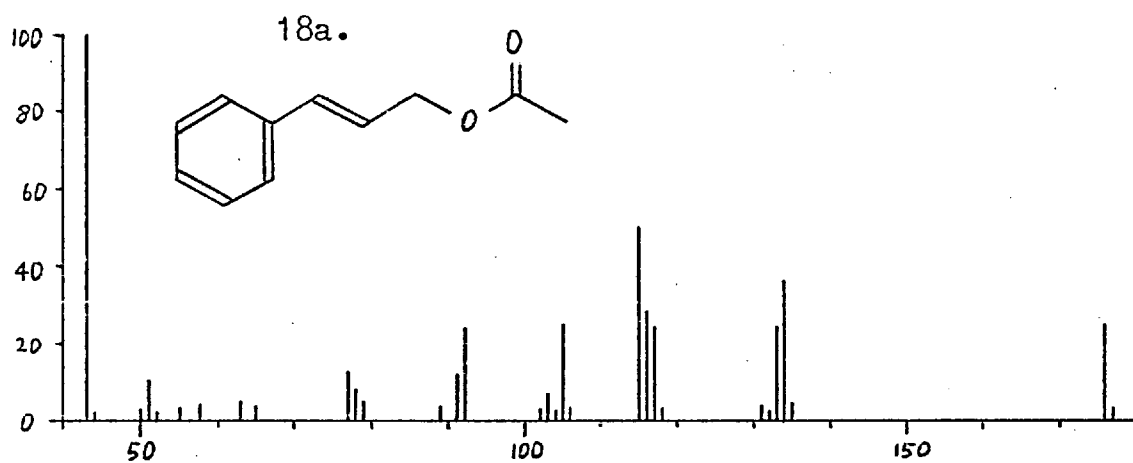


256.

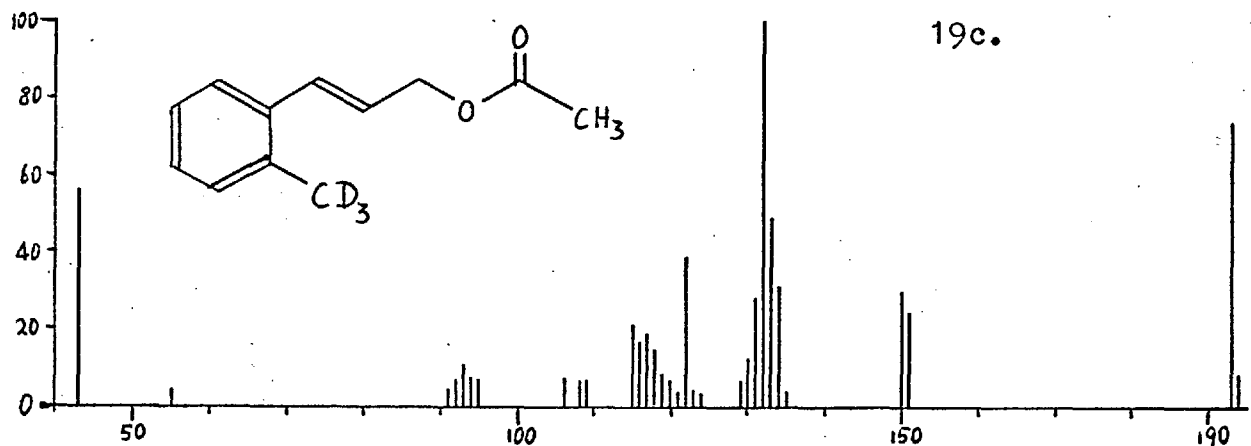
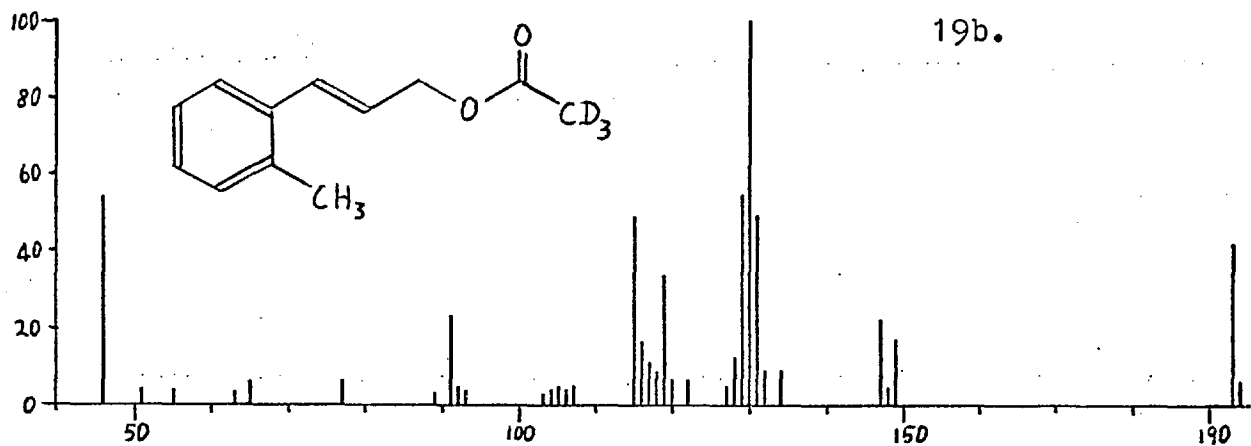
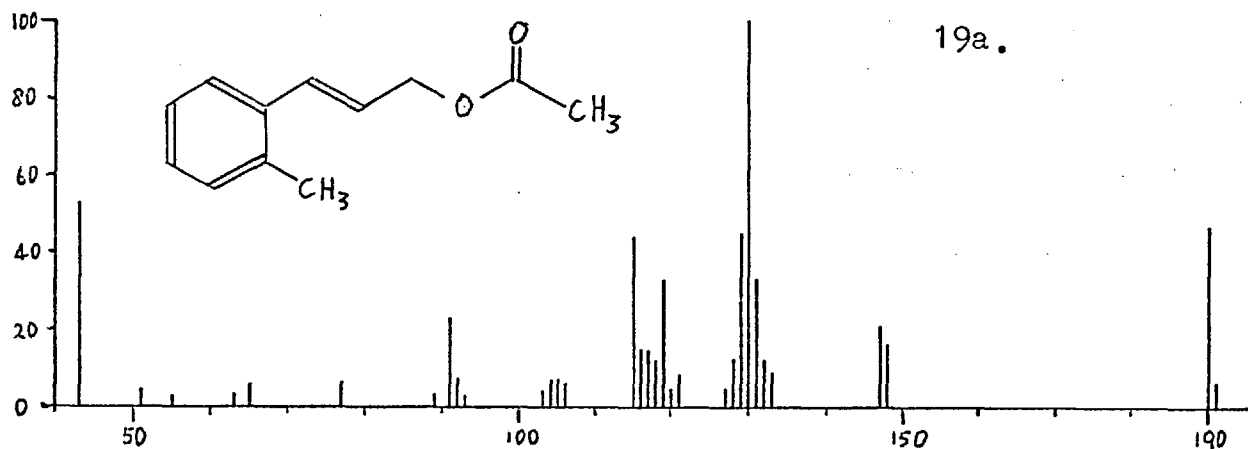
Mass spectrum 17.



Mass spectrum 18.



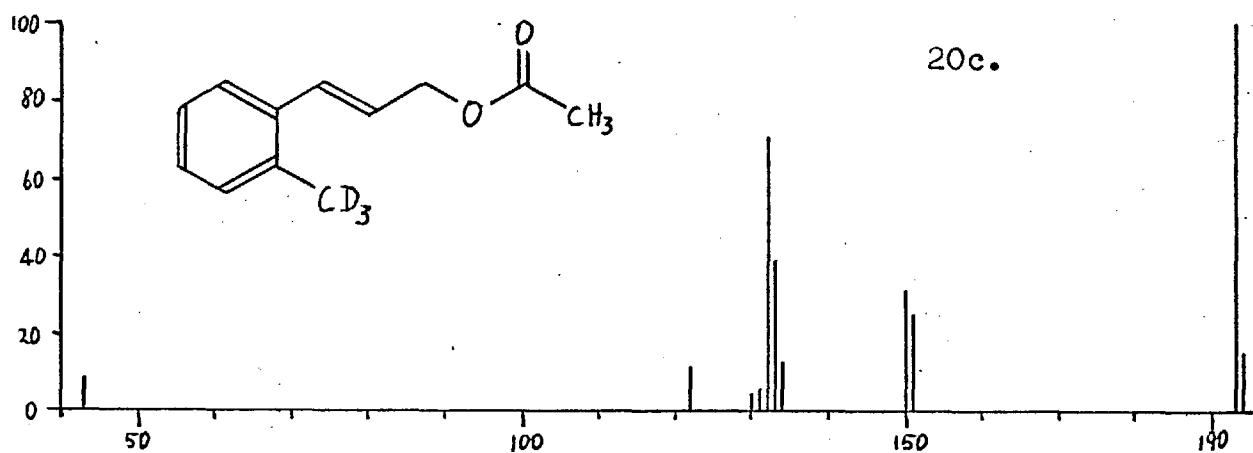
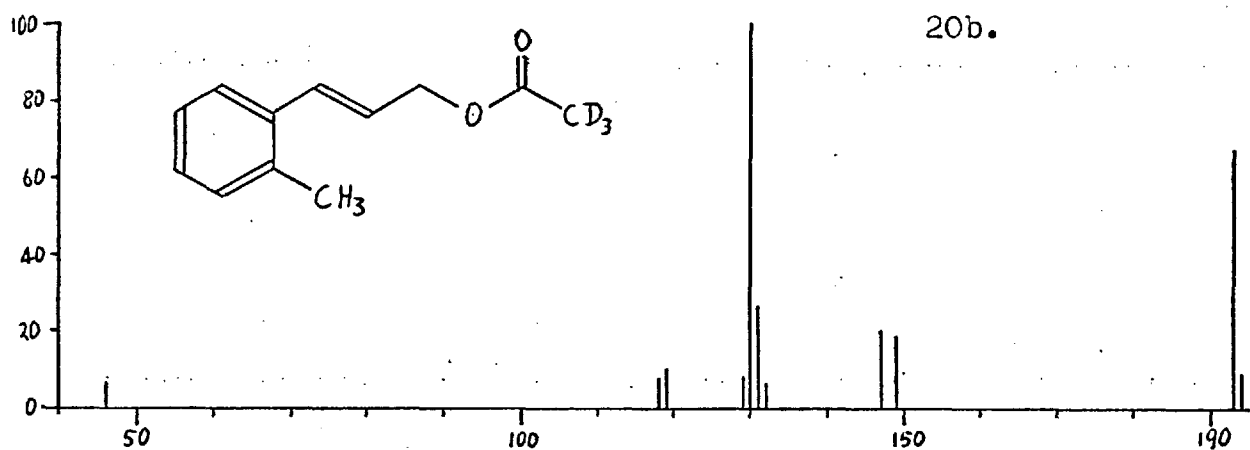
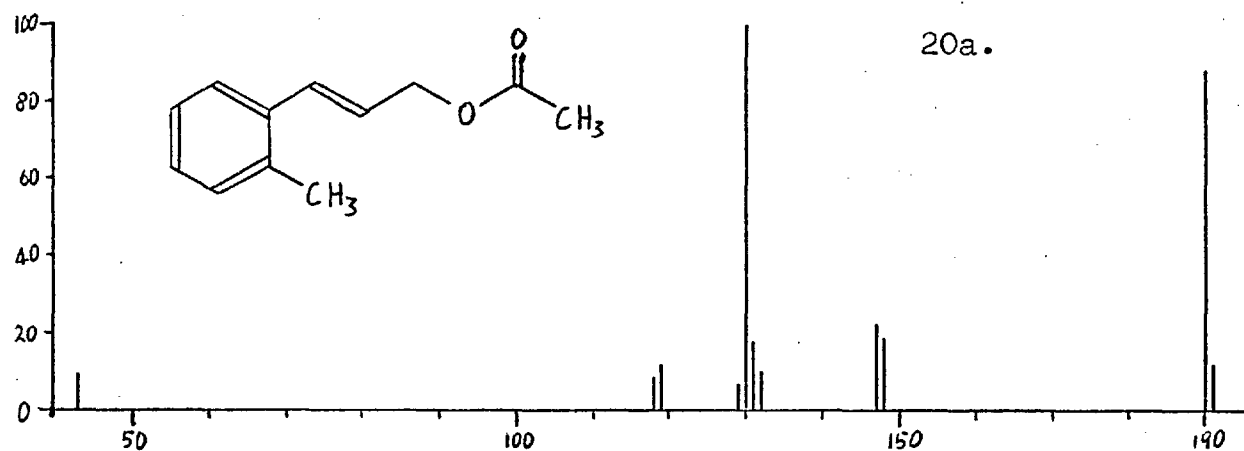
Mass spectra determined at 70eV



Hot Box temp. 100°C

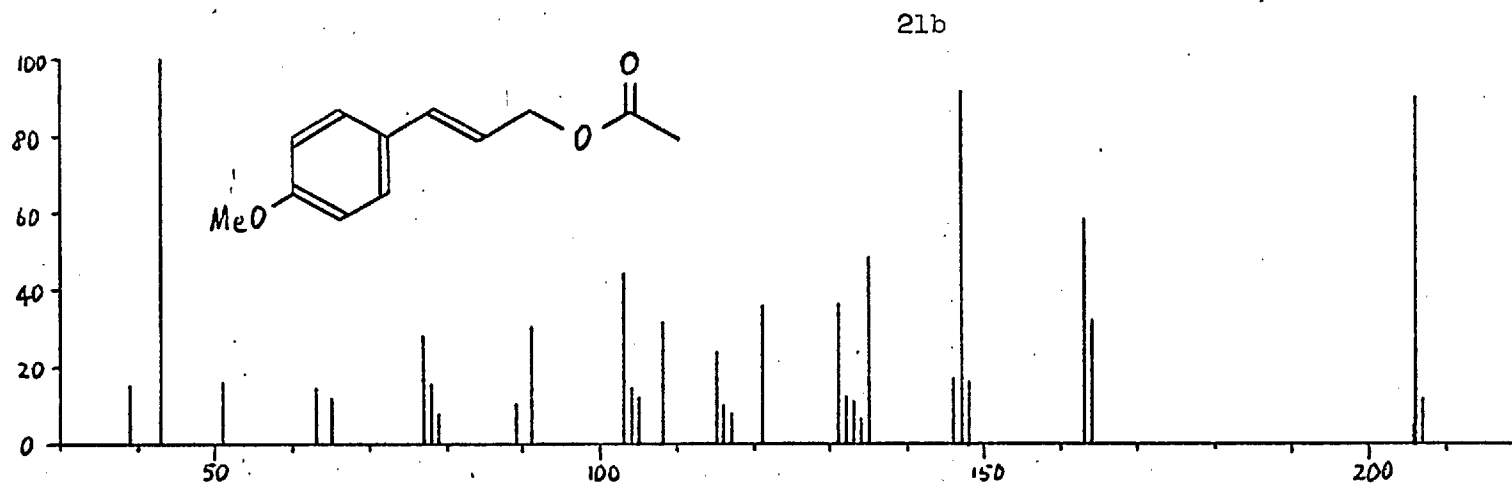
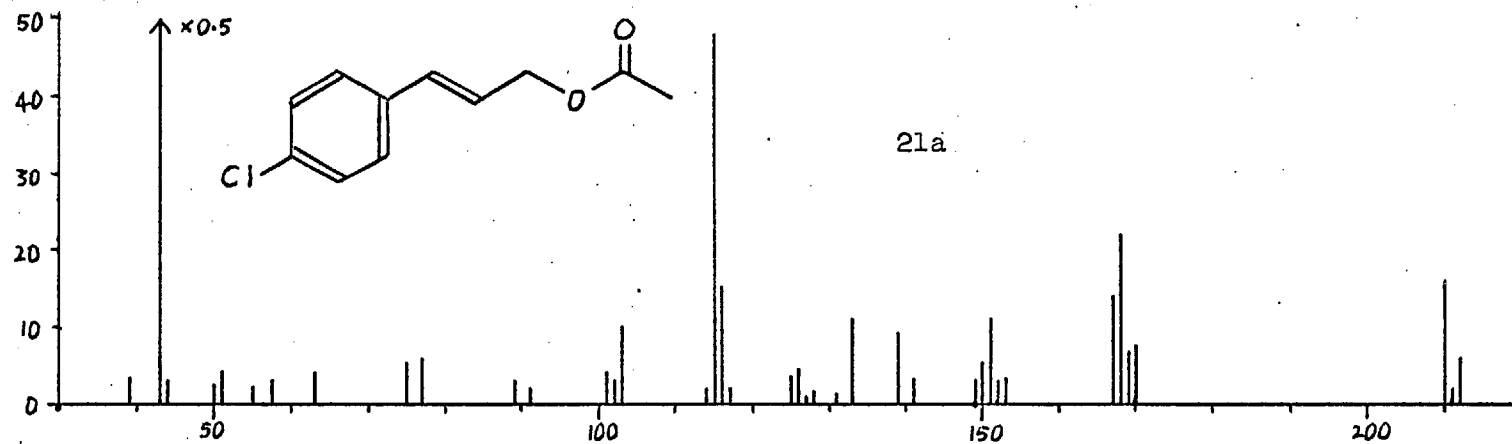
Source temp. 140°C

Mass spectra determined at 16eV

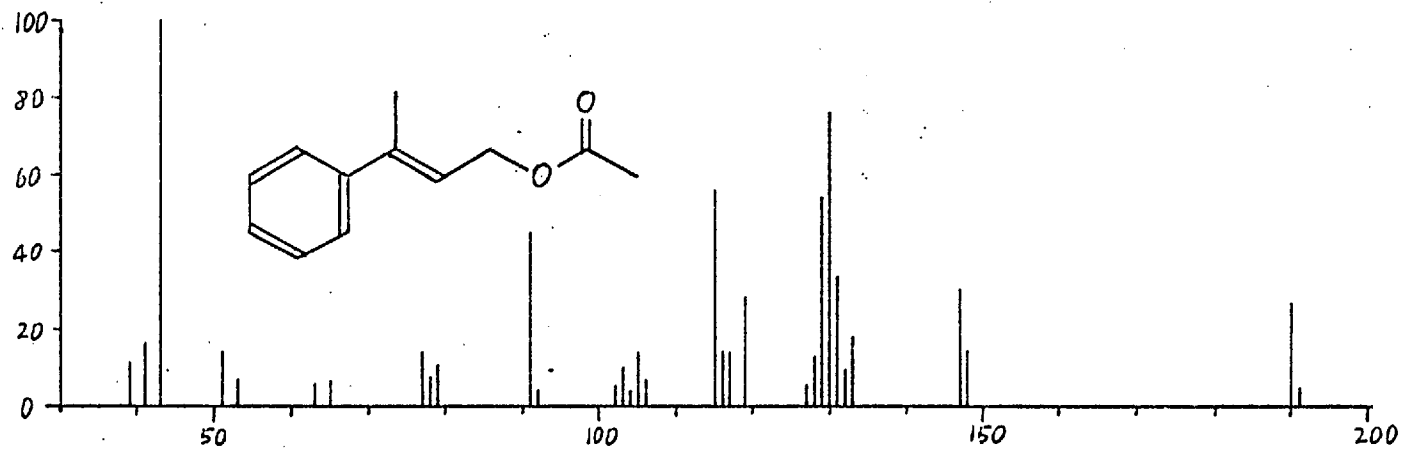


Hot box temp. 100°C

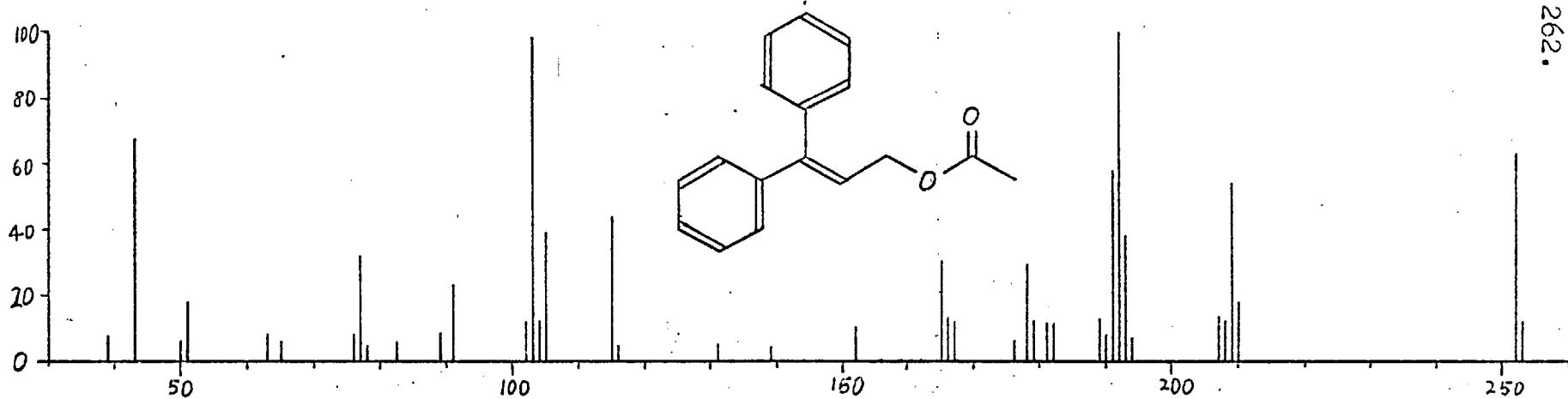
Source temp. 140°C



Mass spectrum 22.



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