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MASS-SPECTROMETRIC CHARACTERIZATION OF 3,6-ANHYDRO-GALACTOSE DERIVATIVES

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ABSTRACT

The mass spectra of various 3,6-anhydrogalactose derivatives, including derivatives of 3,6-anhydro-4-*O*-β-D-galactopyranosyl-D-galactose (carrabiose), have been studied. It has been shown that mass spectrometry confirms unequivocally the presence of the 3,6-anhydro ring in the compound investigated and allows the elucidation of the nature of the C-1 functional group, as well as the nature and position of substituents at C-2 and C-4 in the 3,6-anhydro sugar derivative.

INTRODUCTION

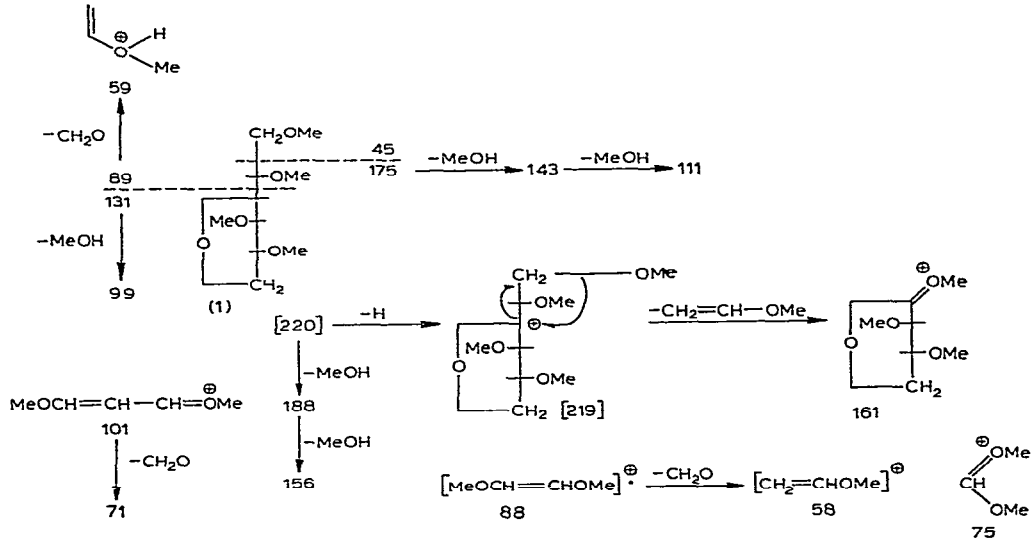
In the study of the red-algae polysaccharides, it is necessary to identify 3,6-anhydrogalactose derivatives. Since their identification by chemical means is unsatisfactory in many respects, mass-spectrometric studies have been undertaken. Mass spectrometry has been successfully used for the investigation of various carbohydrate derivatives[1]. The mass spectra of 3,6-anhydrohexopyranosides have been reported in several publications[2–4]. However, the cleavage of natural polysaccharides results in the formation of derivatives of *aldehydo*-3,6-anhydrogalactose, usually acetals or dithioacetals. Hydrolysis, followed by reduction or oxidation of the aldehyde groups, gives rise to the corresponding derivatives of 3,6-anhydrogalactitol or 3,6-anhydrogalactonic acid. Therefore, the mass spectra of all of these types of compounds [as methyl ethers, trimethylsilyl (TMS) ethers, or acetates], as well as of their naturally occurring 2-*O*-methyl derivatives and the corresponding disaccharides having a D-galactopyranosyl residue at position 4, have been investigated.

MASS SPECTRA OF 3,6-ANHYDROGALACTITOL DERIVATIVES

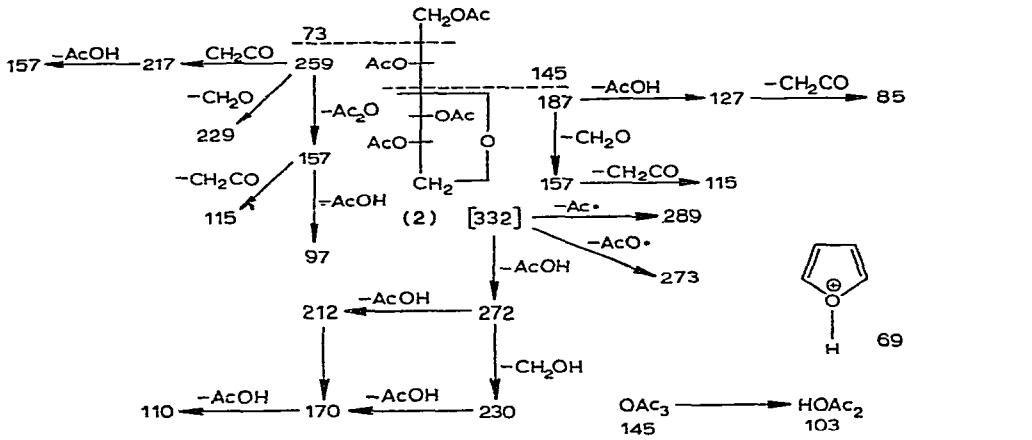
The following 3,6-anhydrogalactitol derivatives have been studied: 3,6-anhydro-1,2,4,5-tetra-*O*-methyl-D-galactitol (**1**), 1,2,4,5-tetra-*O*-acetyl-3,6-anhydro-L-galactitol (**2**), 1,4,5-tri-*O*-acetyl-3,6-anhydro-2-*O*-methyl-L-galactitol (**3**), hepta-*O*-methylcarrabiitol (**4**), and hepta-*O*-(trimethylsilyl)carrabiitol (**5**).

The mass-spectrometric fragmentation of 3,6-anhydro-D-galactitol tetramethyl ether (**1**) (Scheme 1, Fig. 1) involves processes which are characteristic for

alditol methyl ethers[5]. The primary cleavage of the C-1–C-2 or C-2–C-3 bonds gives rise to ions with *m/e* 175 and 45, or 131 and 89, respectively. The primary fragments and the molecular ion lose methanol or formaldehyde to form secondary fragments (*m/e* 188, 156, 143, 111, 99, 59, etc.). In addition, the mass spectrum of the substance shows the presence of peaks at *m/e* 101, 88, and 75, characteristic for all the carbohydrate methyl ethers studied[1]. No detailed investigation of the pathways of their formation has been undertaken in this case. The fragment with *m/e* 161 is likely to



SCHEME 1[a]



SCHEME 2

[a]The figures on this and subsequent schemes are to denote the *m/e* values of the corresponding ions The *m/e* values for the ions that are not observed in a spectrum, are enclosed in square brackets.

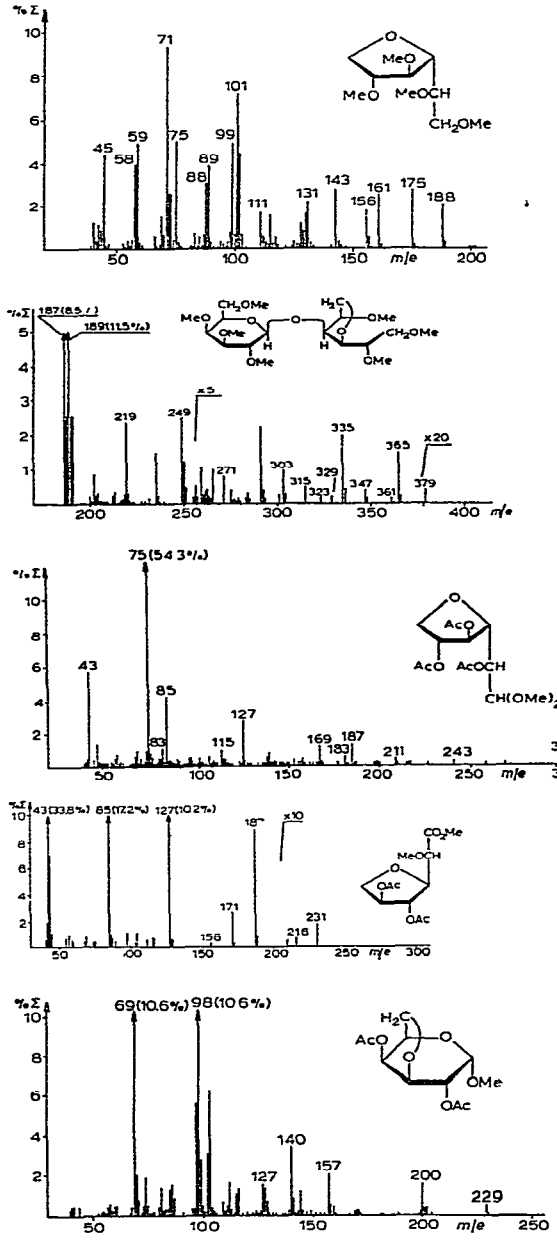


Fig. 1. Mass spectrum of 3,6-anhydro-1,2,4,5-tetra-*O*-methyl-D-galactitol (1).

Fig. 2. Mass spectrum of hepta-*O*-methylcarrabiitol (4).

Fig. 3. Mass spectrum of 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal (7).

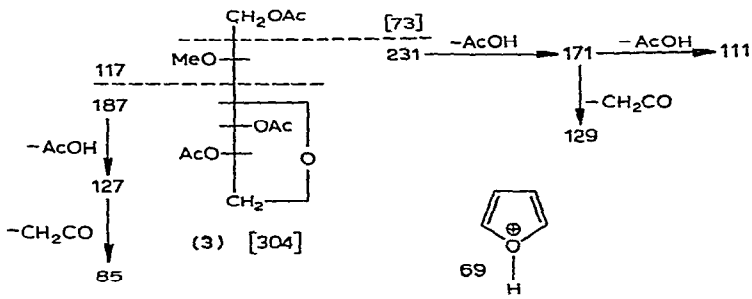
Fig. 4. Mass spectrum of methyl 4,5-di-*O*-acetyl-3,6-anhydro-2-*O*-methyl-L-galactonate (13).

Fig. 5. Mass spectrum of methyl 2,4-di-*O*-acetyl-3,6-anhydro-α-D-galactopyranoside (15).

result from a rearrangement involving CH_3O-group migration. An analogous rearrangement has been reported for alditol methyl ethers[5]. Thus, the mass spectrum of **1** allows its unambiguous identification as a 3,6-anhydrohexitol derivative.

The fragmentation of 3,6-anhydro-L-galactitol tetra-acetate (**2**) is similar to that of other alditol acetates[6]. Formation of all the main peaks in the mass spectrum is explained by the cleavage of the C-1–C-2 and C-2–C-3 bonds and loss of acetic acid or ketene from the fragments formed or directly from the molecular ion. The most-characteristic series of intense peaks starting with *m/e* 187 is connected with the presence of the 3,6-anhydro ring. The origin of the main peaks in the mass spectrum of **2** is shown in scheme 2.

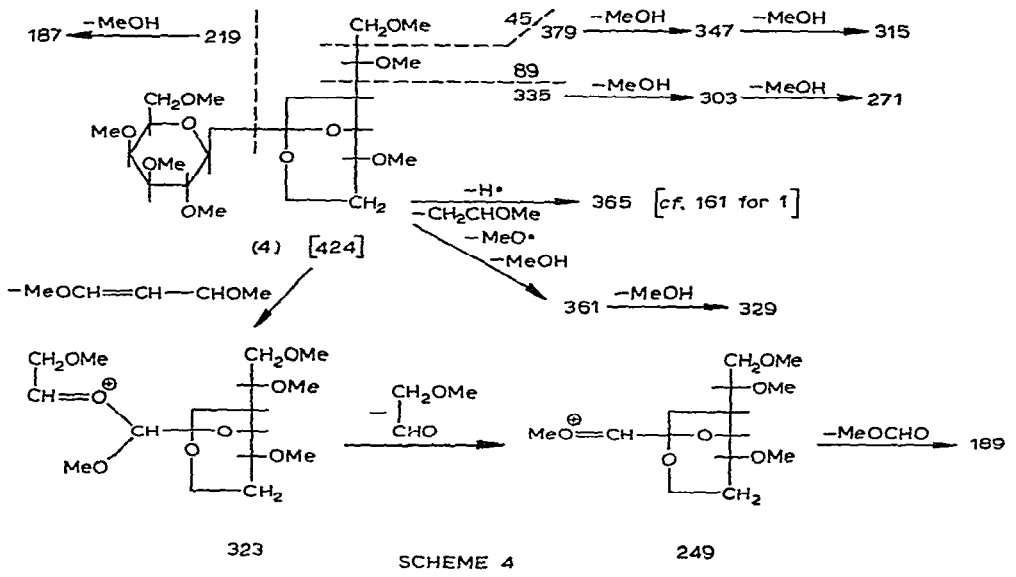
As expected by analogy with the partially methylated alditol acetates[7], the main fragmentation route in the mass spectrum of 3,6-anhydro-2-*O*-methyl-L-galactitol tetra-acetate (**3**) is the cleavage of the C-2–C-3 bond, resulting in formation of the ion with *m/e* 117. In addition, as in the mass spectrum of **2**, there is a series of intense peaks at *m/e* 187, 127, and 85, due to the presence of the 3,6-anhydro ring. Thus, the mass spectrum of **3** confirms the position of an *O*-methyl group at C-2 (Scheme 3).



SCHEME 3

It is evident from Fig. 2 and Scheme 4 that the mass-spectrometric fragmentation pattern of carrabiitol heptamethyl ether (**4**) is similar in many respects to that of **1**, since the mass spectrum of **4** shows the peaks corresponding to cleavage of C-1–C-2 and C-2–C-3 bonds in the 3,6-anhydrogalactitol moiety. The resulting fragments, which contain the tetra-*O*-methyl-D-galactopyranosyl residue, give peaks shifted by 204 towards larger mass. Furthermore, the mass spectrum of **4** exhibits the peaks due to fragmentation of the galactose moiety. This process is similar to that in other disaccharides[8]. Thus, the mass spectrum of **4** unequivocally shows the presence of the monosaccharide substituent (peaks at *m/e* 219, 187), the position of its linkage to the "aglycon" (*m/e* 335)*, and the presence of a 3,6-anhydro ring (*m/e* 189 and so on) in the latter.

*The (1→5)-linkage is excluded, since, in the naturally occurring 3,6-anhydrogalactose derivatives, HO-5 is involved in the pyranose ring.



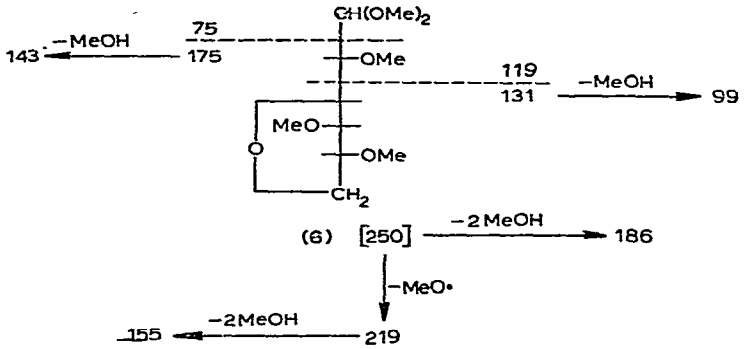
SCHEME 4

The mass spectrum of the TMS-derivative (**5**) of carrabiitol is, in general, analogous to that of **4**. For example, cleavage of the C-1–C-2 and C-2–C-3 bonds in the 3,6-anhydro-D-galactitol moiety produces ions with *m/e* 727 and 103, and 625 and 205, respectively. Loss of the galactosyl residue and subsequent loss of TMS-OH produce the ions with *m/e* 451 and 361, etc. Additionally, in the high-mass region, there are peaks at *m/e* 815 ($M-CH_3$) and 725 ($M-CH_3-$TMS-OH), which allow unambiguous determination of the molecular weight of the substance.

MASS SPECTRA OF DERIVATIVES OF 3,6-ANHYDROGALACTOSE DIMETHYL ACETAL AND RELATED COMPOUNDS

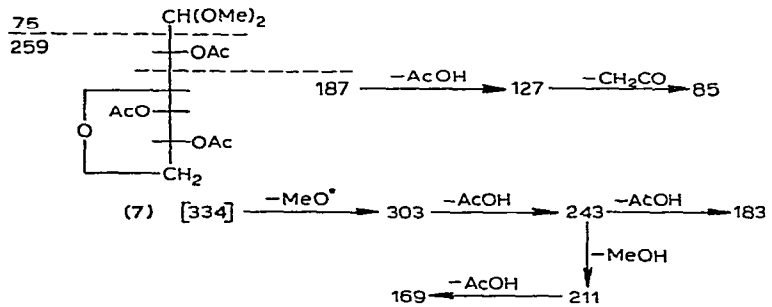
The dimethyl acetals of 3,6-anhydro-2,4,5-tri-*O*-methyl-D-galactose (**6**), 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose (**7**), hexa-*O*-methylcarrabiose (**8**), and hexa-*O*-acetylcarrabiose (**9**); the diethyl dithioacetals of 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose (**10**) and 3,6-anhydro-2,4,5-tri-*O*-(trimethylsilyl)-D-galactose (**11**); the methyl esters of 2,4,5-tri-*O*-acetyl-3,6-anhydro-L-galactonic acid (**12**) and 4,5-di-*O*-acetyl-3,6-anhydro-2-*O*-methyl-L-galactonic acid (**13**); and methyl 3,6-anhydro-2,4-di-*O*-methyl-α-D-galactopyranoside (**14**) and methyl 2,4-di-*O*-acetyl-3,6-anhydro-α-D-galactopyranoside (**15**) have been studied.

The mass spectra of the dimethyl acetals (**6–9**) show a characteristically intense peak at *m/e* 75. This peak allows dimethyl acetals to be distinguished from other 3,6-anhydrogalactose derivatives. As in the case of the 3,6-anhydrogalactitol derivatives, the presence of a 3,6-anhydro ring leads to series of ions, which correspond to the initial cleavage of the C-2–C-3 bond (for methyl ether **6**, at *m/e* 119 and 131, with the fragments formed therefrom by loss of methanol; for acetate **7**, at *m/e* 187, with the fragments derived therefrom by loss of AcOH or CH_2CO). In addition, the mass



SCHEME 5

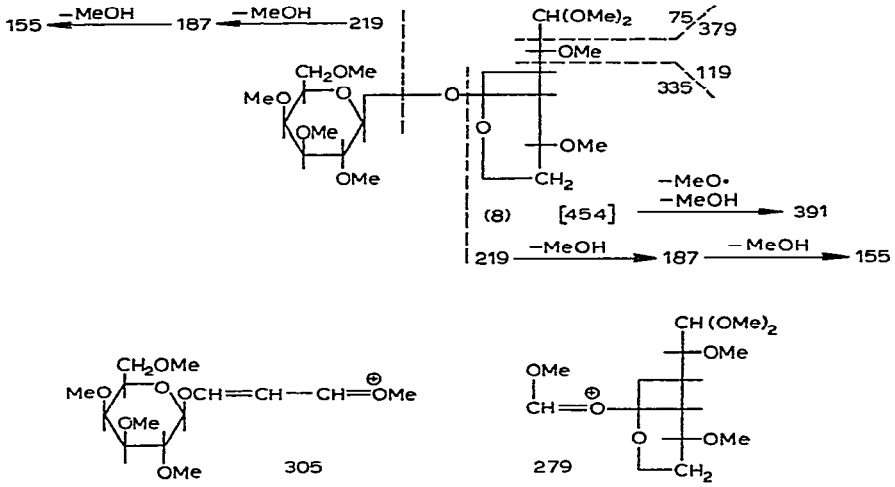
spectra of dimethyl acetals contain a series of ions starting with the loss of MeO$^{\bullet}$ from the molecular ion. The resulting fragments allow the determination of the molecular weight of the substances. Scheme 5 shows the fragmentation pattern for the methyl ether **6**, and Scheme 6 that for the acetate **7**; its mass spectrum is shown in Fig. 3.



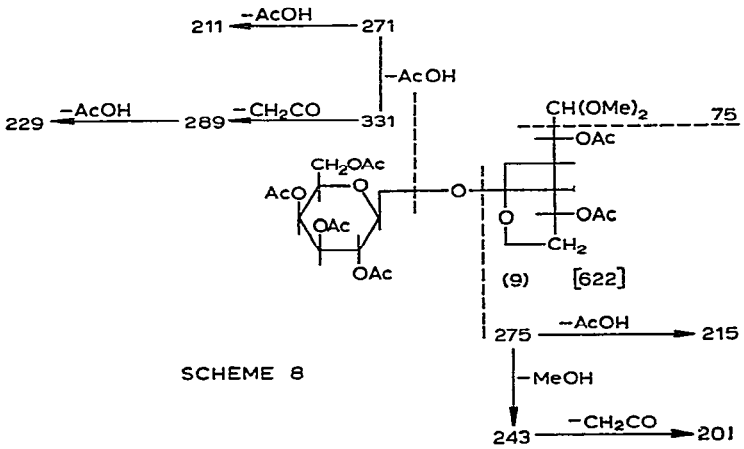
SCHEME 6

The mass spectra for the disaccharide dimethyl acetals **8** and **9**, apart from a very intense peak at *m/e* 75, show the peaks demonstrating the presence of a hexopyranose moiety (*m/e* 219 for methyl ether **8** and 331 for acetate **9**; *cf.* Schemes 7 and 8), and also the presence of a 3,6-anhydrohexose moiety (*m/e* 219 for **8**, and 275 for **9**). The fragments (*m/e* 335 and 119) resulting from **8** due to the cleavage of the C-2–C-3 bond indicate that the monosaccharide substituent is linked to C-4 of the 3,6-anhydro-D-galactose moiety.

The fragmentation of the diethyl dithioacetals **10** and **11** is similar to that of the dimethyl acetals. The peak at *m/e* 135 proves the presence of the diethyl dithioacetal group. For **10**, it is the main peak in the spectrum, but for **11** its intensity is slightly diminished. As follows from Scheme 9, acetate **10** gives rise to a standard series of peaks at *m/e* 187, 127, and 85, of rather high intensity, which indicate the presence of the 3,6-anhydro ring. In addition, the spectrum contains peaks corresponding to the



SCHEME 7



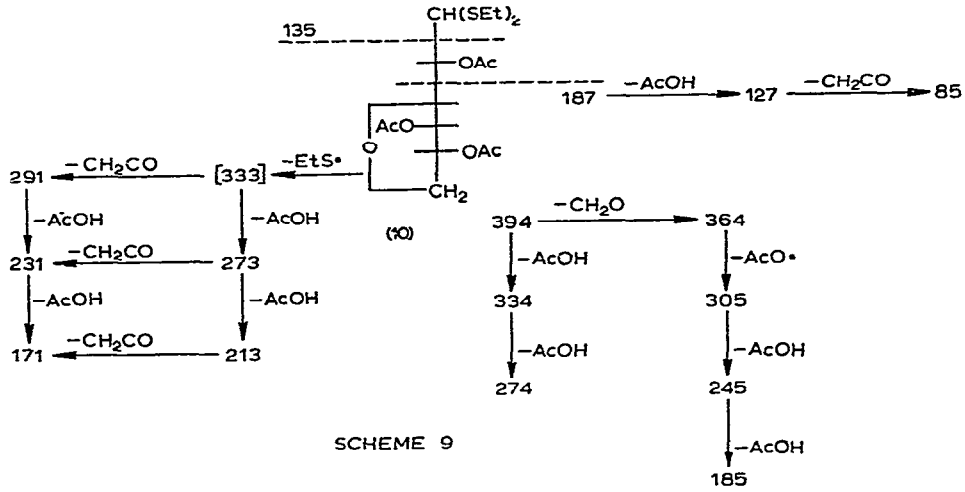
SCHEME 8

initial loss of the EtS group from the molecular ion and subsequent loss of AcOH or CH_2CO from the resulting fragment. The primary cleavage in the trimethylsilyl derivative **11** is the same as that in the acetate **10**, although the intensity of many characteristic peaks is lower. The primary fragments further lose TMS-OH.

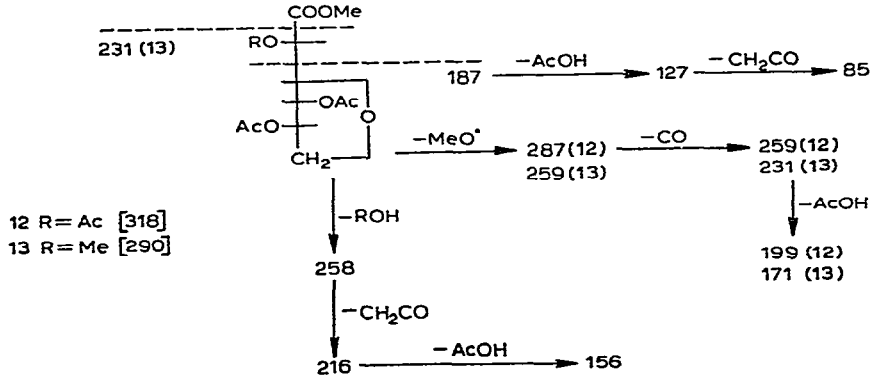
Unlike acetals, the fragmentation of 3,6-anhydrogalactonic acid derivatives **12** and **13** proceeds with charge localization mainly at C-3, so that rather intense peaks (*m/e* 187, 127, 85) characteristic of the presence of the 3,6-anhydro ring are produced. The position of the methoxyl group at C-2 in **13** follows from the series of peaks at *m/e* 231 (M−COOMe) and 171, which have not been observed for acetate **12** (Fig. 4). The formation of other fragments in the mass spectra of these compounds is shown in Scheme 10.

The mass spectra of 3,6-anhydrogalactose derivatives possessing pyranoid structures are quite different from those of the aldehyde derivatives described above.

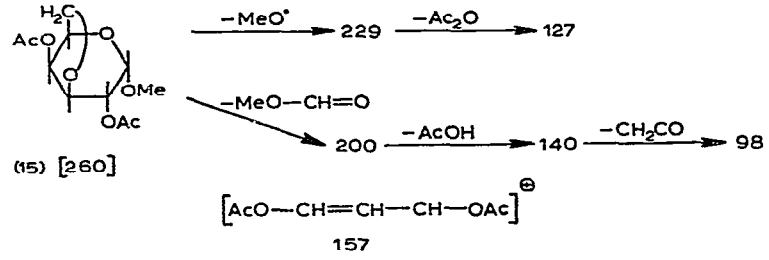
Methyl 3,6-anhydro-2,4-di-*O*-methyl-α-D-galactopyranoside (**14**) gives a mass spectrum almost identical with that[3] of methylated methyl 3,6-anhydrogluco-pyranoside. As seen from the fragmentation pattern of acetate **15** (Scheme 11), its mass spectrum (Fig. 5) shows peaks for the fragments characteristic of pyranoid forms of the acetylated sugar derivatives[9].



SCHEME 9



SCHEME 10



SCHEME 11

It is evident, from the above data, that the 3,6-anhydro ring in 3,6-anhydrogalactose acetals, 3,6-anhydrogalactitol, or 3,6-anhydrogalactonic acid derivatives can be easily detected by means of mass spectrometry, due to the formation of ions corresponding to the cyclic part of the molecule (*m/e* 131 for methyl ethers, 187 for acetates). The presence of dimethyl acetal or diethyl dithioacetal groupings gives rise to very intense peaks at *m/e* 75 and 135, respectively. For derivatives substituted at position 2 or 4, including the corresponding disaccharides, the nature and position of the substituent can be easily established from the fragments arising from cleavage of the C-2–C-3 bond. The mass spectra of derivatives of 3,6-anhydrogalactopyranose are very different from those of the aldehyde, alditol, or acid derivatives, but do show the presence of the 3,6-anhydro ring.

EXPERIMENTAL

The mass spectra were obtained with an MX-1303 mass spectrometer. The substances were introduced through a heated inlet at 150°. Disaccharides were introduced directly into the ion source at 70°.

Synthetic samples of 3,6-anhydro-D-galactose dimethyl acetal[10], 3,6-anhydro-D-galactose diethyl dithioacetal[11], and methyl 3,6-anhydro-α-D-galactopyranoside[10] were used for preparation of derivatives. 3,6-Anhydro-D-galactitol, carrabiitol, and carrabiose dimethyl acetal were isolated from κ-polysaccharide[12] of the red alga *Tichocarpus crinitus* (Gmel.) Rupr. 3,6-Anhydro-L-galactitol and 3,6-anhydro-L-galactonic acid were obtained by partial hydrolysis of agar followed by reduction (KBH_4) or oxidation (Br_2) and subsequent hydrolysis, and 3,6-anhydro-2-*O*-methyl-L-galactitol and 3,6-anhydro-2-*O*-methyl-L-galactonic acid were obtained from the agar-like polysaccharide[13] of *Rhodomela larix* (Turn.) C. Ag.

Methylation was performed with methyl iodide–sodium hydride–methyl sulphoxide[14], and acetylation with acetic anhydride–pyridine. The trimethylsilyl derivatives were obtained by the action of Me_3SiCl and $(Me_3Si)_2NH$ in pyridine[15], and the methyl esters by the action of methanolic hydrogen chloride. The substances were purfied by t.l.c. on silica gel with chloroform–acetone and identified by g.l.c. (Pye–Argon chromatograph; poly(neopentylglycol succinate) or silicone SE-30 on Chromosorb W).

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