

**Mass spectrometry of uronic acid derivatives. XIII.\***  
**Effect of some C-1 substituents upon the mass spectral**  
**fragmentation of methyl 2,3,4-tri-*O*-acetylhexopyranuronates**

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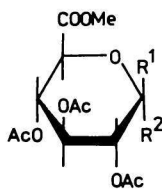
Various C-1 substituted (OMe, OAc, OH, Cl, Br) methyl 2,3,4-tri-*O*-acetylhexopyranuronates have been studied by 70 and 12 eV mass spectrometry. Differences in the fragmentation mechanisms were found by interpreting the spectra of compounds labelled with trideuteriomethoxy, trideuterioacetoxy, and deuterioxy groups, and by metastable transition measurements.

В работе изучены различные С-1 замещенные (ОМе, ОАс, ОН, Сl, Вr) производные метил-2,3,4-три-*O*-ацетилгексопирануранатов с помощью масс-спектрометрии применяя 70 и 12 эВ. При помощи масс-спектров соединений, меченых тридейтерометоксиловой, тридейтероацетоксиловой и дейтероксиловой группами, а также по метастабильным переходам, были найдены отличия в механизме их фрагментации.

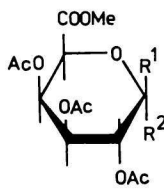
Part IX of this Series [2] dealt with the mass spectral (70 and 12 eV) fragmentation of methyl (methyl *O*-acetyl-*O*-methyl- $\alpha$ -D-glucopyranosid)uronates. The information presented therein may be used as an aid in the identification of methyl (methyl *O*-methylhexopyranosid)uronates, formed normally on methanolysis of methylated, uronic acid-containing substances. However, the obtained information together with that about the fragmentation of acetyl derivatives of neutral monosaccharides [3—9] and uronic acids [10] were found insufficient to unambiguously identify various C-1 substituted methyl 2,3,4-tri-*O*-acetylhexopyranuronates encountered as intermediates in numerous regulated syntheses carried out in this laboratory. Various C-1 substituted compounds (I—IX) were therefore prepared and the effect of the substituents upon the mass spectral fragmentation of the sugar skeleton has been studied

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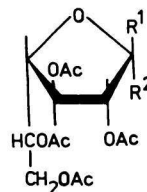
\* For Part XII see Ref. [1].



I-VII



VIII



IX

Compound	R <sup>1</sup>	R <sup>2</sup>	Compound	R <sup>1</sup>	R <sup>2</sup>
I	H	OMe	VI	H, OD	
II	OCD <sub>3</sub>	H	VII	H	Br
III	OAc	H	VIII	H	Cl
IV	H, OCOCD <sub>3</sub>		IX	OMe	H
V	H, OH				

## Experimental

Compound *I* was prepared by conventional acetylation of the corresponding trihydroxy derivative [11]. For preparation of *II* compound *VII* [12] was allowed to react with methanol-*d*<sub>3</sub> under the conditions of Koenigs—Knorr synthesis of glycosides as described [13]. Compound *III* was obtained according to [14]. Compound *IV* was obtained by acetylation of *V* [15] with pyridine—acetic anhydride-*d*<sub>6</sub>. Compound *VI* was obtained by repeated concentration of a solution of *V* in a methanol-*d*<sub>1</sub>—*D*<sub>2</sub>O mixture (10:1) directly in the instrument. The achieved degree of deuteration was 68%. The syntheses of *VIII* [12] and *IX* [16] have been described elsewhere. Compounds *I*, *II*, and *IV* were prepared on a milligram scale only to be used in this study. Pure substances were obtained by purification of the crude reaction products by chromatography.

Mass spectra (70 and 12 eV) were measured with an MCh 1306 instrument. The temperature at the site of evaporation was 40—50°C, and that in the ionizing chamber was 130°C. Metastable transitions were either found in the recorded 70 eV spectra (these are given in the text and the schemes as calculated values, e.g. *m*\* = 151.3), or determined separately using an MS-MT-01 metastable ion detector coupled to the JMS-100 D instrument (the respective ions are marked with an asterisk).

## Results and discussion

### *C-1 Methoxy derivatives*

In order to clarify the mechanism of the fragmentation of methyl (methyl 2,3,4-tri-*O*-acetyl- $\alpha$ -*D*-glucopyranosid)uronate (*I*) its 1-*O*-trideuteriomethyl

analogue *II* was prepared and studied; the  $m/e$  values found in its mass spectrum are given in parentheses. Mass spectrum of *I* is shown in Fig. 1. When for the origin of the formed ions an analogy with the fragments produced in the fragmentation of permethylated monosaccharides is applicable the nomenclature of ion Series according to Kochetkov [17] (capital letters A—K) is used. Series A begins mostly

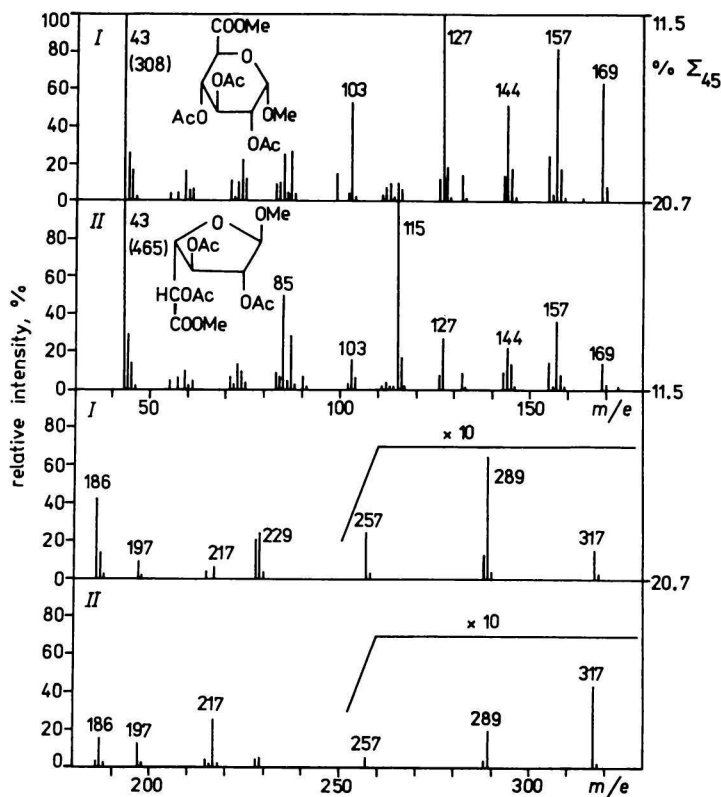
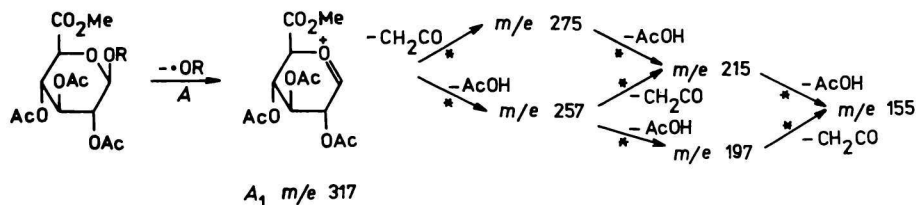
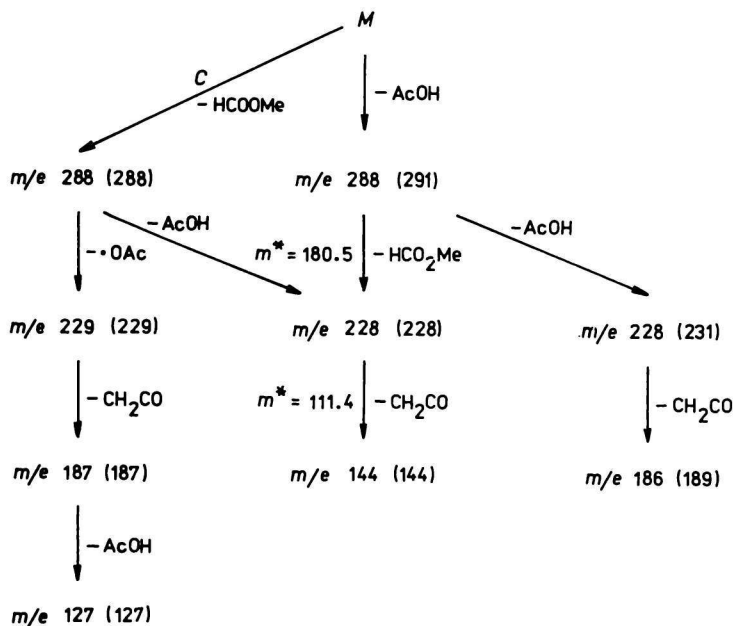


Fig. 1. Mass spectra (70 eV) of methyl (methyl 2,3,4-tri-*O*-acetyl- $\alpha$ -D-glucopyranosid)uronate (*I*) and methyl (methyl 2,3,5-tri-*O*-acetyl- $\beta$ -D-galactofuranosid)uronate (*IX*).

with a cleavage of the glycosidic methoxy group followed by the elimination of acetic acid and ketene (Scheme 1). The presence of  $[M - \cdot\text{OMe} - \text{AcOH}]^+$  ion peaks in the spectrum of *II* at  $m/e$  257 and 260 shows that to some extent ( $\sim 33\%$ ) the methoxy group of the methoxycarbonyl function is also split off. Two explanations have been offered for the origin of  $[M - 60]^{*+}$  ions. According to [8] they represent  $C_1$  ions formed by an elimination of methyl formate. The ions  $[M - 60]^{*+}$  may, however, be formed [18] also as a result of an elimination of acetic acid. In the fragmentation of *I* both types of elimination indicated have been observed (Scheme 2).



Scheme 1

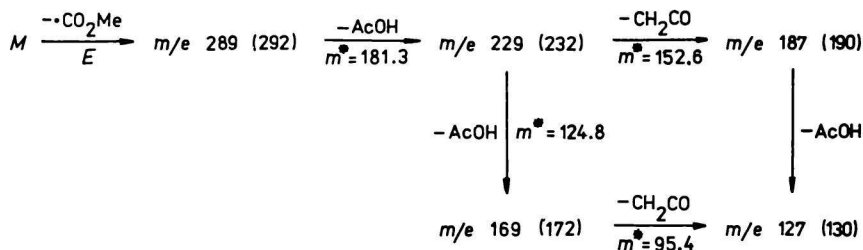


Scheme 2

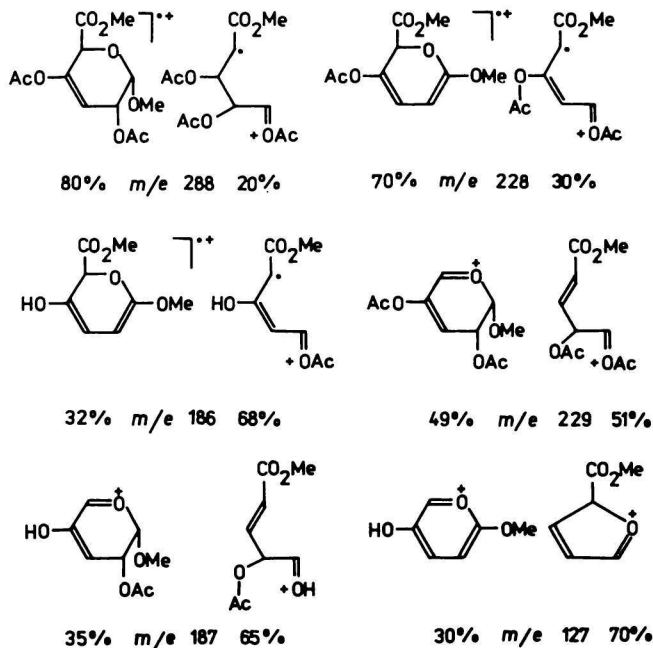
Series *E* commences by splitting off of the methoxycarbonyl group (Scheme 3).

A comparison of the peak intensities found in the spectrum of *I* with those in the spectrum of the labelled analogue *II* shows that some of the ions of the *A* and *C* Series are isomeric mixtures. The most probable structures of the isomeric ions together with their relative contributions are given in Scheme 4. The ions at  $m/e$  157 and 115 are those of *F* Series ( $m^* = 84.3$  for  $157 \rightarrow 115$ ).

The fragmentation of the pyranosides *I* and *II* is accompanied by the formation of furanoid ions appearing at  $m/e$  217 (Fig. 1). The formation of the five-membered-ring ions from pyranose acetates, first observed in the fragmentation of *cis*- and *trans*-2,3-diacetoxy tetrahydropyrans [19], is quite pronounced in the case of 2-deoxy-*L*-arabino-hexopyranose per-*O*-acetate [5, 6, 17]. The phenomenon has been explained by a rather improbable splitting off of a  $\bar{C}H-CH_2-OAc$  particle,



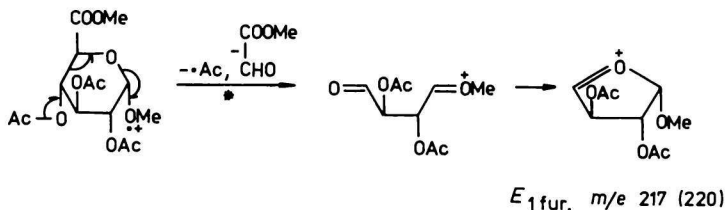
Scheme 3



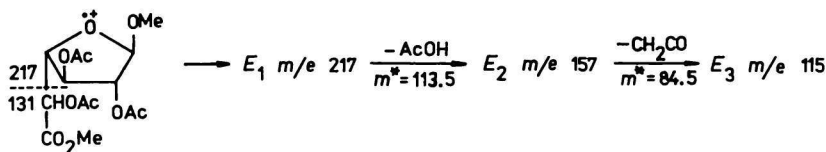
Scheme 4

following the liberation of an  $\cdot\text{OAc}$  radical from C-4. The found metastable transition  $M \rightarrow 217$ , together with the shift of the respective  $m/e$  value in the spectrum of *II*, reflects the fact that the furanoid  $E_1$  ions are formed directly from the molecular ions via "h-rupture" [20–22] (Scheme 5).

A question arose from the fact that furanoid ions were formed in the fragmentation of *I* whether it is possible at all to determine, from the mass spectra alone, the ring size of acetylated uronic acids. For comparison the spectrum of methyl (methyl 2,3,5-tri-*O*-acetyl- $\beta$ -D-galactofuranosid)uronate (*IX*) was measured. It can be seen from Fig. 1 where the spectra of *I* and *IX* are shown that the ions  $E_1$  and  $E_3$  with  $m/e$  217 and 115 formed from the molecular ions of the furanoside *IX* by cleavage of the side chain (Scheme 6) are much more intense than the ions



Scheme 5



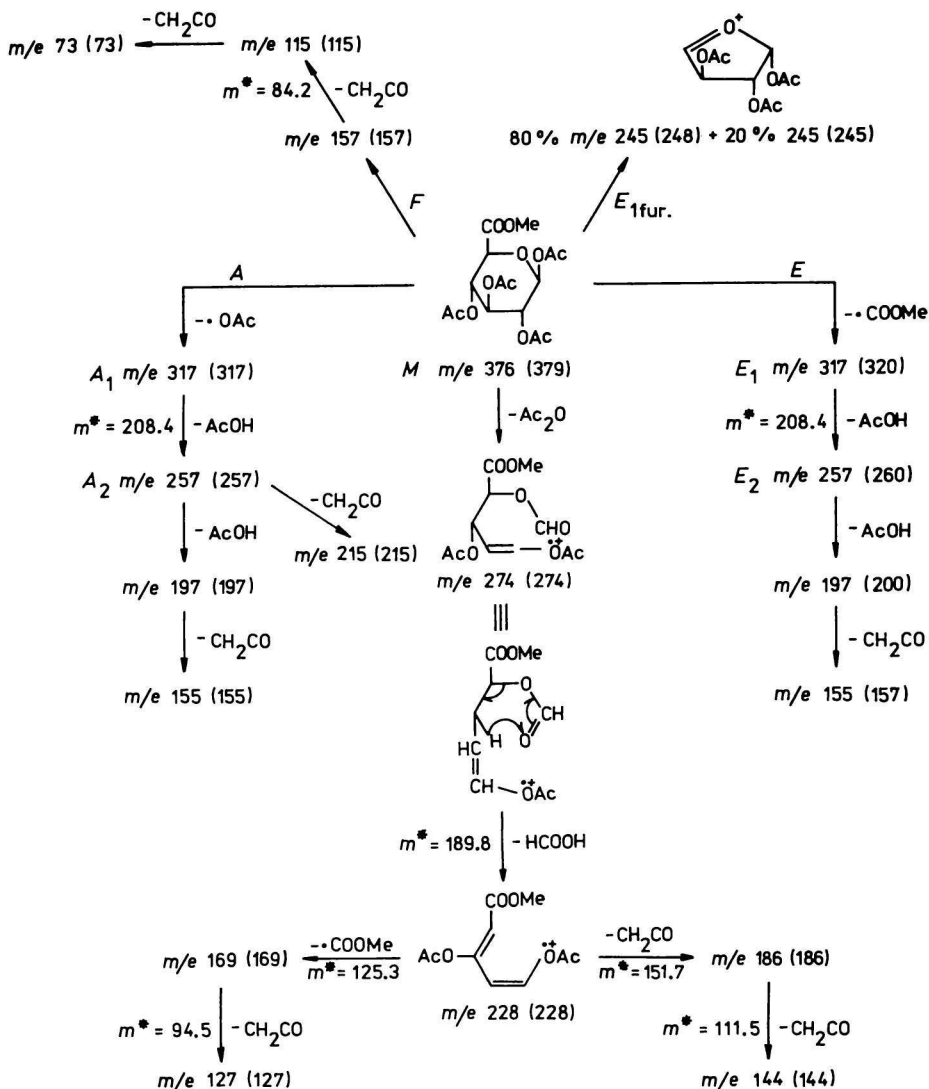
Scheme 6

appearing at the same  $m/e$  value in the spectrum of *I*. On the other hand, the peaks at  $m/e$  157 in the spectrum of the pyranoside *I* are much more pronounced, since they represent both ions of the  $E_2$  and  $F_1$  Series. Although the two isomeric classes of substances (pyranosides and furanosides) can be distinguished from the mass spectra alone, this sort of determination should be done with care, particularly when there is a possibility that the substance in question is a mixture of both. In this case an unambiguous identification requires GC-MS technique.

### C-1 Acetoxy derivatives

The interpretation of the spectrum of methyl 1,2,3,4-tetra-*O*-acetyl- $\beta$ -*D*-glucopyranuronate (*III*) is hampered since the substance contains at each of the five carbon atoms of the pyranoid cycle substituents of the same mass (59 mass units). The spectrum of *III* has been published [10], but since the mechanism of the fragmentation was not elucidated in detail the 1-*O*-trideuterioacetyl derivative *IV* was prepared and studied. From the observed shifts of  $m/e$  values, found metastable transitions, and aided by the information about the fragmentation of *D*-glucose per-*O*-acetate [4, 7] the complete fragmentation mechanism for *III* is now proposed (Scheme 7).

The furanoid  $E_1$  ions are again formed via “*h*-rupture”, as shown in Scheme 2. Since the same substituents are present at C-1 and C-4 the furanoid  $E_1$  ions consist of two ion species distinguishable only in the spectrum of the C-1 labelled substance *IV*.



Scheme 7

### C-1 Hydroxy derivatives

The presence of the free hydroxyl group at C-1 in *V* alters to some extent the mode of disintegration of its molecular ions, compared with the glycoside *I* and the 1-*O*-acetate *III*. The fragmentation mechanism (Scheme 7 and 8) is based on the interpretation of 70 and 12 eV spectra (Fig. 2) and metastable transition measurements. The course of Series A, E, and F is given in Scheme 7. An intense Series commences by the elimination of acetic acid with the participation of the

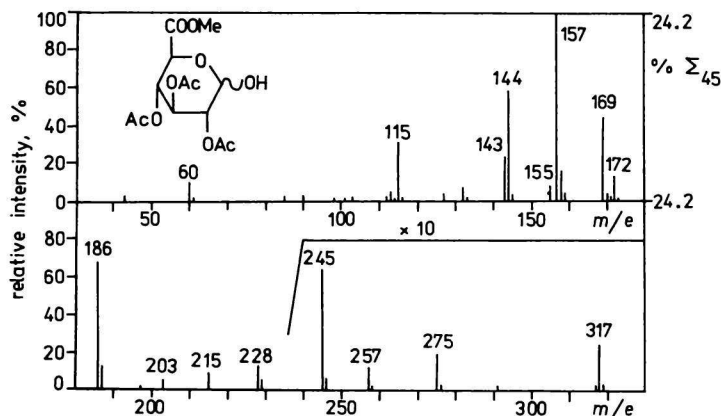
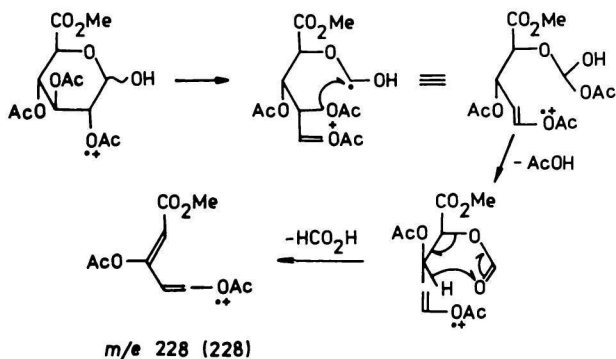
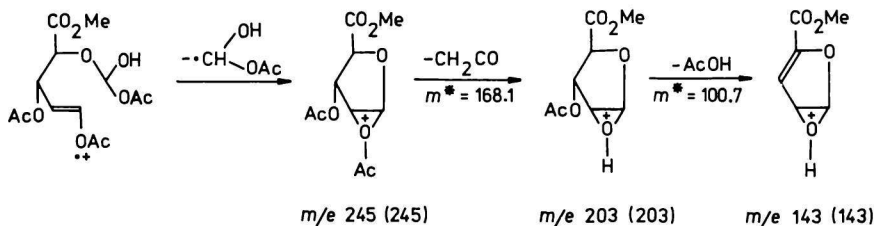


Fig. 2. Mass spectrum (12 eV) of methyl 2,3,4-tri-*O*-acetyl- $\alpha,\beta$ -D-glucopyranuronate (V).

hydrogen atom of the hydroxyl group, followed by the elimination of formic acid (Scheme 8) and two molecules of ketene, to give rise to the final ions at  $m/e$  144 (144 also for VI). As a result of the presence of the electron-withdrawing methoxycarbonyl group the splitting off of a molecule of acetic acid and of the  $\cdot\text{CHO}$ , or an  $\text{AcO}-\dot{\text{C}}\text{H}-\text{OH}$  radical from the rearranged molecular ions occurs simultaneously. In this way furanoid ions containing unaltered C-2, C-4, and C-5 substituents are formed (Scheme 9).



Scheme 8



Scheme 9



*C-1 Halogene derivatives*

The disintegration Series characteristic of the fragmentation of the glycoside *I* occurs also in the fragmentation of methyl 2,3,4-tri-*O*-acetyl-1-chloro-1-deoxy- $\alpha$ -D-galactopyranuronate (*VIII*) and the bromide analogue *VII* (Fig. 3).

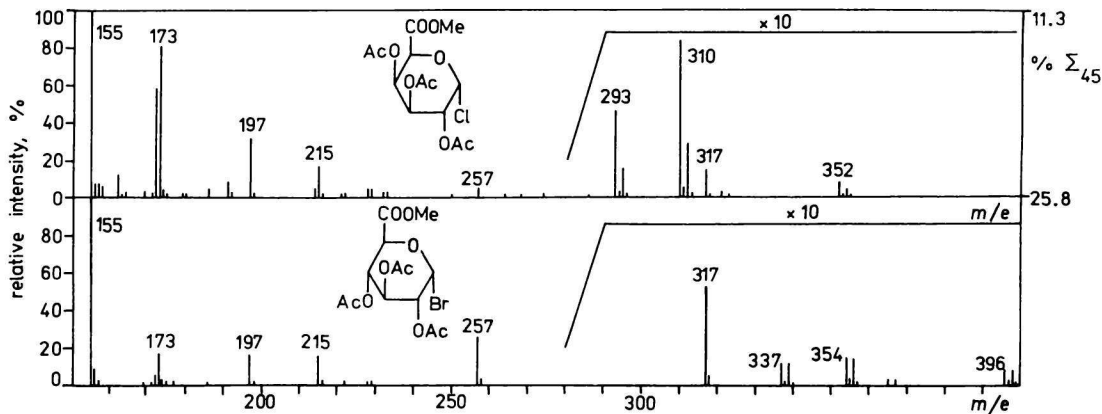


Fig. 3. Partial ( $m/e > 150$ ) mass spectra (70 eV) of methyl 2,3,4-tri-*O*-acetyl-1-chloro-1-deoxy- $\alpha$ -D-galactopyranuronate (*VIII*) and methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy- $\alpha$ -D-glucopyranuronate (*VII*).

A noticeable peak of molecular ions is present not only in the 12 eV but also in the 70 eV spectrum of these substances. The high electron density at the halogen atom and, consequently, high probability of its ionization could be the cause of the rather unique phenomenon occurring in the fragmentation of this class of substances, *i.e.* the splitting off of a molecule of ketene from the molecular ions (\* for  $352 \rightarrow 310$  with *VIII* and \* for  $396 \rightarrow 354$  with *VII*).

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