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MASS-SPECTRAL FRAGMENTATIONS OF PERACETYLATED DIALDOSE DIANHYDRIDES*

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ABSTRACT

Mass spectra of peracetylated dialdose dianhydrides were measured and fragmentation pathways were discussed. Four characteristic peaks, at *m/e* 316, 303, 288, and 275, are effective in the identification of dialdose dianhydrides.

INTRODUCTION

Mass spectrometry is now a conventional method for the structural analysis of various kinds of carbohydrates. Extensive studies on the monosaccharides have revealed common rules of fragmentation[1–3], and recent studies have focused on the application of these rules to oligosaccharides and polysaccharides. The fragmentation of oligosaccharides generally takes place in such a way that the sugar residues that constitute them are degraded independently[2–5]. However, the mass spectra of the peracetylated dialdose dianhydrides examined in this study are rather complex, and cannot be assigned by simple application of the common rules for the monosaccharides, because the two sugar residues are not degraded independently, but in relationship with each other, due to the presence of the fused-ring system. Therefore, analysis of the mass spectra of the dialdose dianhydrides serves as a new tool for the identification of these compounds.

We now report on studies of the mass-spectrometric behavior of peracetylated dialdose dianhydride derivatives, especially on the structures of the fragment ions having relatively large values of *m/e*.

RESULTS AND DISCUSSION

Because of the complexity of permethylated derivatives, due to the abundant degradations of the pyranose ring[2,3], peracetylated dialdose dianhydrides were

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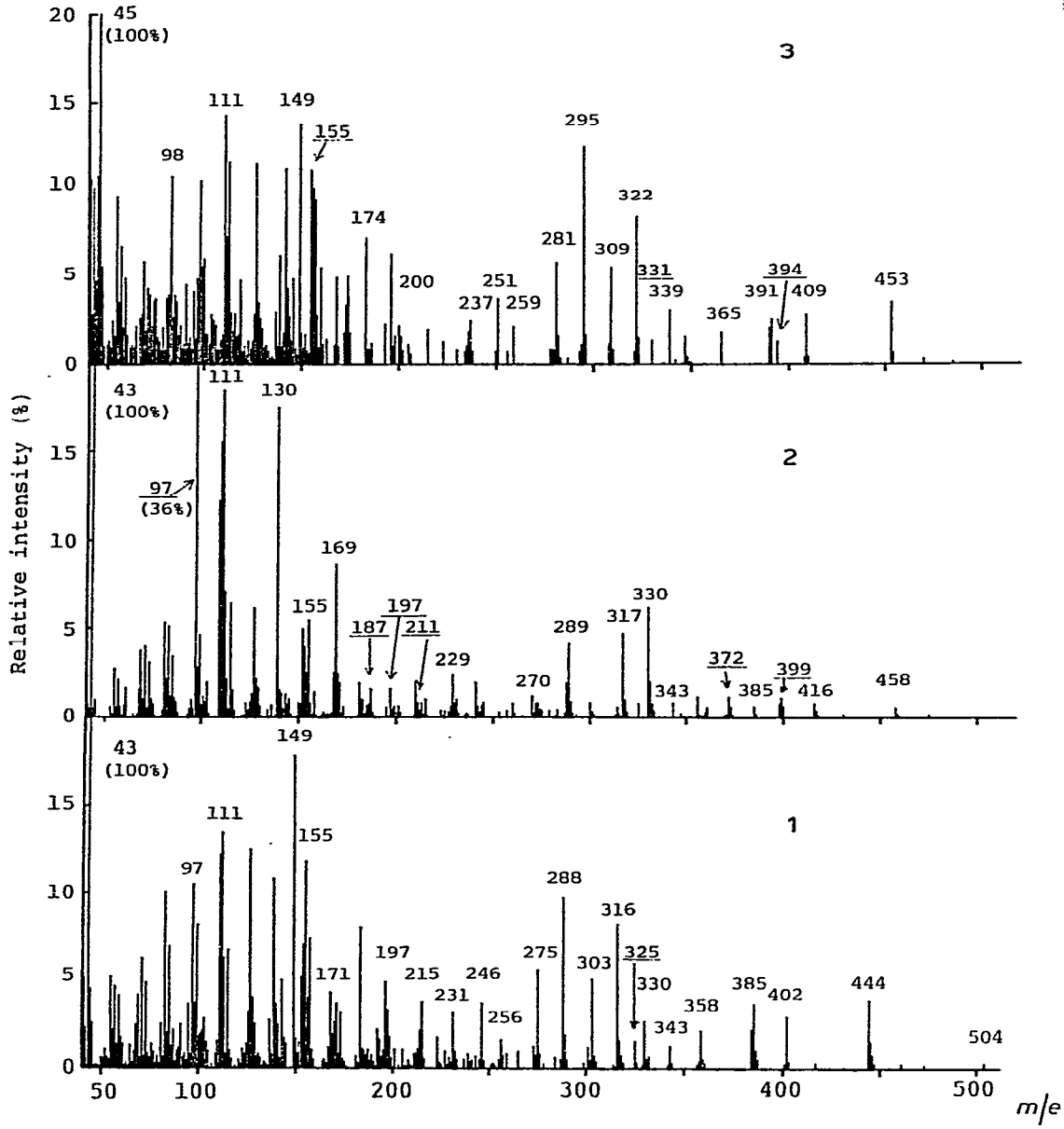
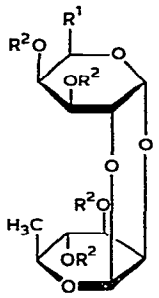


Fig. 1. Mass spectra of **1**, **2**, and **3**.

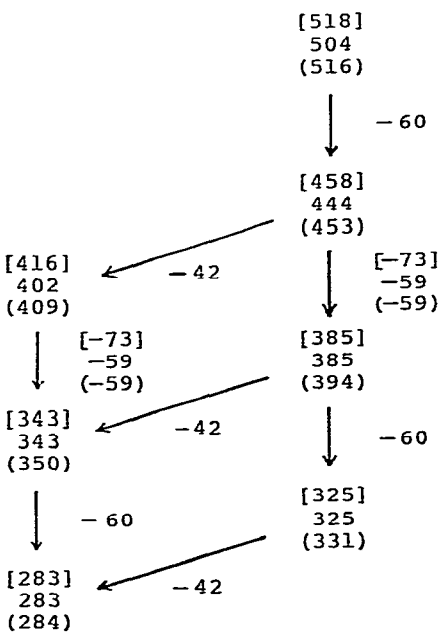
analyzed by mass spectrometry. These were the 1,2′:1′,2-dianhydride (**1**) of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate; its reduction product, namely, the 1,2′:1′,2-dianhydride (**2**) of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and 3,4,6-tri-*O*-acetyl-α-D-galactopyranose; and the per(deuterioacetyl)ated derivative (**3**) of **1**. The mass spectra of **1**, **2**, and **3** are given in Fig. 1.



1 $R^1 = CO_2Me$, $R^2 = COCH_3$
2 $R^1 = CH_2OCOCH_3$, $R^2 = COCH_3$
3 $R^1 = CO_2Me$, $R^2 = COCD_3$

The peak for the molecular ion was observed only in the spectrum of **1**. The intensities of the peaks that appeared in the high-mass regions were relatively low, and the peak for the aA_1 type of fragmentation at the glycosidic center was not found. These observations were in marked contrast to those made on the spectra of the peracetates of (1→2)-linked disaccharides, in which the aA_1 fragments had great intensities[6]. The spectrum of **1** had four characteristic peaks, of *m/e* 316, 303, 288, and 275, that proved effective in the identification of dialdose dianhydrides.

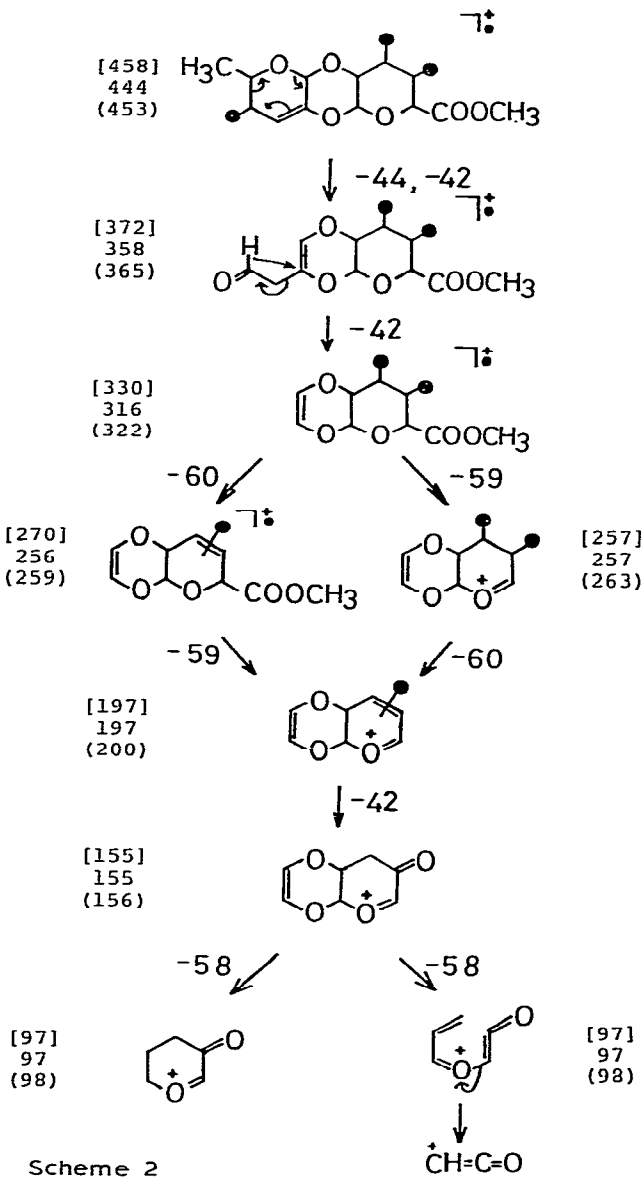
The fragmentation pathways of **1** could be summarized into four classes, and the structures of the fragment ions were determined by comparison with the spectra of **2** and **3**.



Scheme 1

The first class of fragmentation pathways of **1** could be explained as in Scheme 1. The values of *m/e* of the fragment ions of **2** and **3** are given in brackets and paren-

theses, respectively. This pathway was the one in which the fragment ions were generated by successive eliminations of acetic acid and ketene, as is common with other peracetylated derivatives[1]. The elimination of acetic acid from the molecular ion gave the ion of *m/e* 444, and this newly formed fragment-ion was degraded to the *m/e* 385 ion by the loss of 59 mass units. This process of degradation was achieved by elimination of the methoxycarbonyl group at C-5 of the residue in the form of $\dot{C}OOCH_3$ radical. The observation that the loss of mass units in this process was

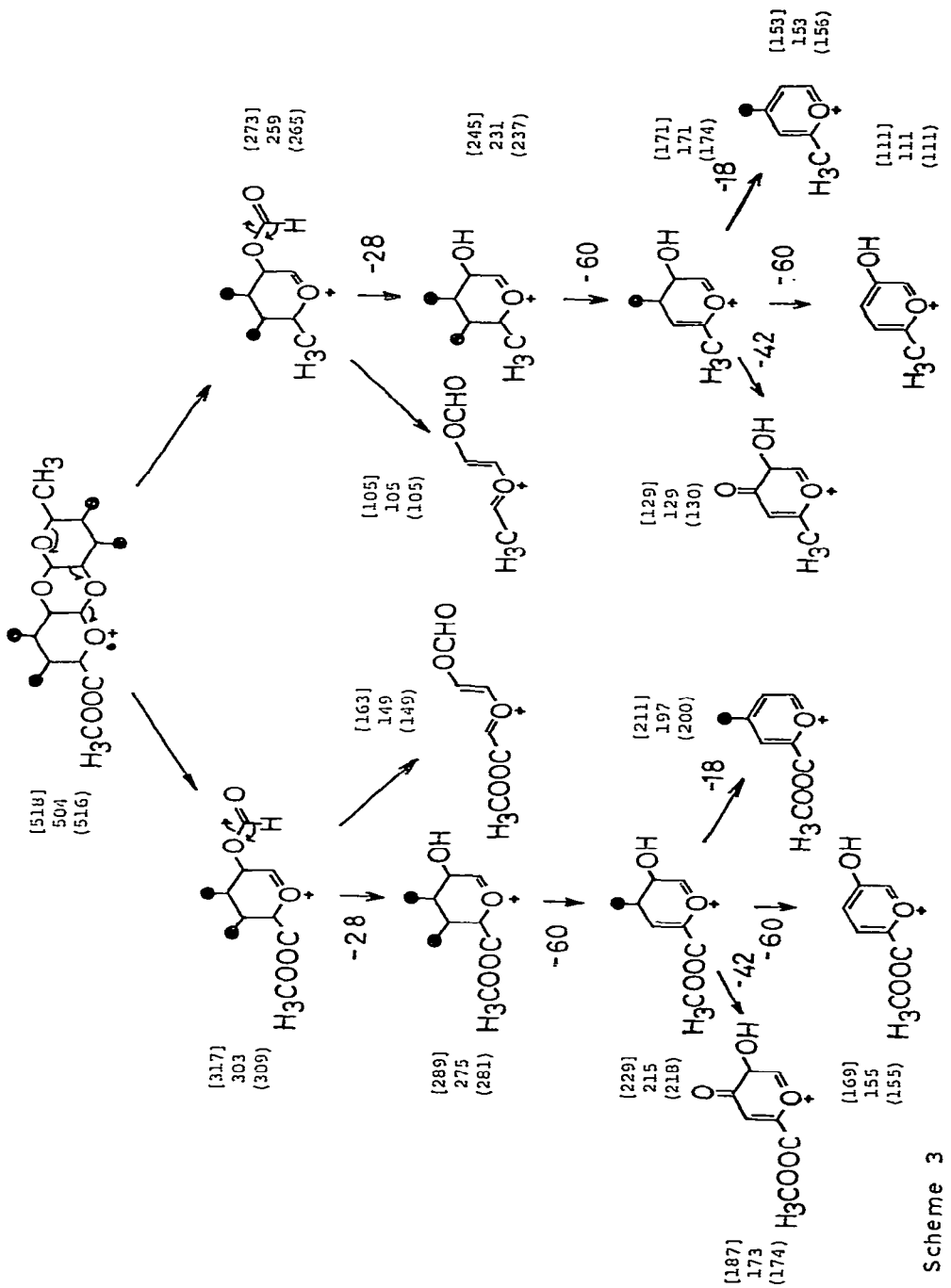


Scheme 2

59 in the spectrum of **3** showed that the fragment released was not an acetoxyl group but a methoxycarbonyl radical. In the spectrum of **2**, the decrease of the value of *m/e* was 73 ($\dot{C}H_2OAc$ radical), supporting the presumed release of a methoxycarbonyl radical from the *m/e* 444 ion. The release of a methoxycarbonyl radical was also observed in the step from the *m/e* 402 ion, generated by the elimination of ketene, to the *m/e* 343 ion. The fragment ion that was converted into the *m/e* 283 ion by the loss of acetic acid could be generated from the ion of *m/e* 385 by the elimination of ketene. The series of fragment ions of *m/e* 325, 283, and 241 were generated by successive eliminations of acetic acid and ketene. These pathways of fragmentation were those in which the backbone structure of the pyranose rings was retained throughout the reaction, and proved effective in determination of the size of the rings. The formulas of the fragment ions are written in completely planar forms in the succeeding Schemes, because the configurations of sugars have little influence on their mass spectra. The symbol (—●) represents an acetoxyl group.

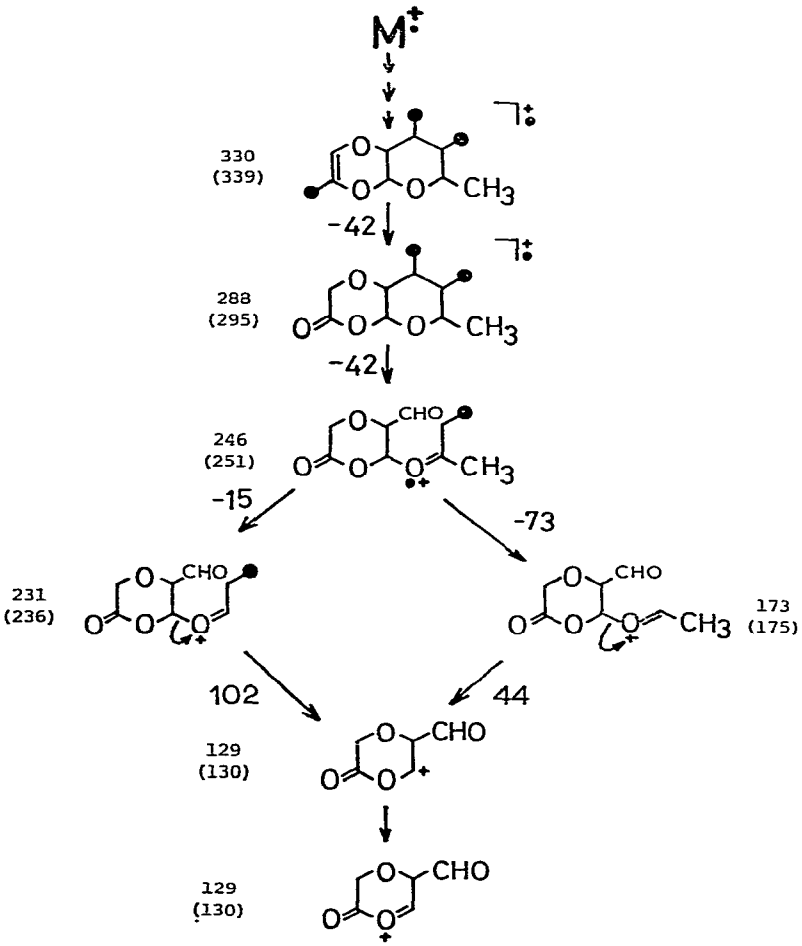
The second class of fragmentation pathways (see Scheme 2) started from the ion of *m/e* 444. The elimination of CH_3CHO by a retro-Diels–Alder reaction led to an unstable ion, of *m/e* 400, which was immediately converted into the *m/e* 358 ion by the loss of ketene. This fragmentation took place only at the rhamnose residue, not at the galacturonic acid residue, because the fragment ions having *m/e* 314 and 272 were not observed in the spectrum of **1**. The elimination of ketene from the *m/e* 358 ion gave the *m/e* 316 ion. In the spectrum of **3**, the decrease of the value of *m/e* in this reaction (*m/e* 365→322) was not 44 but 43, showing that the elimination of ketene was caused by the shift of the hydrogen atom of the aldehyde group. The resulting *m/e* 316 ion, and the *m/e* 330 ion in the spectrum of **1**, had large intensities and were characteristic for the peracetates of dialdose dianhydrides. The ion of *m/e* 316 could be distinguished from the aA_1 ions (*m/e* 317) of the peracetates of methyl glycosides of methyl aldobiouronates[6], and the ion of *m/e* 330 in the spectrum of **2** could also be distinguished from the ions (*m/e* 331) of peracetates of disaccharides of aldohexoses. This difference increased to 5 mass units in the spectra of per(deuterioacetyl)ated derivatives; therefore, the fragment ion of *m/e* 316 was effective for detecting the structure of dialdose dianhydrides. Further fragmentations of this ion were caused by the loss of acetic acid, methoxycarbonyl radical, and ketene, to give the *m/e* 155 ion, and then this was degraded to the *m/e* 97 ion by a retro-Diels–Alder reaction. These pathways were completely supported by the spectra of **2** and **3**.

The third class of fragmentation pathways involved successive, radical transfers. Molecular structures proposed for the fragment ions belonging to this class are given in Scheme 3, where the arrows written in the formulas represent the transfer of radicals (⇀) and electron pairs (→) in the major pathways. Among four peaks for the fragment ions of *m/e* 316, 303, 288, and 275, having relatively large intensities in the high-mass region of the spectrum of **1**, only one peak for the *m/e* 316 ion was included in the foregoing two pathways; but the pattern constituted by these four peaks was remarkable, and was important for the analysis of the fragmentation path-


[518]
504
(516)
[317]
303
(309)
[289]
275
(281)
[273]
259
(265)
[245]
231
(237)
[229]
215
(218)
[211]
197
(200)
[187]
173
(174)
[171]
171
(174)
[169]
155
(155)
[163]
149
(149)
[153]
153
(156)
[129]
129
(130)
[111]
111
(111)
[105]
105
(105)
-28
-60
-42
-18

Scheme 3

ways of the dialdose dianhydride, in order to determine the molecular structures of the three residual ions. In the spectrum of **2**, the peak corresponding to the *m/e* 303 ion in the spectrum of **1** was found at *m/e* 317. The shift by 14 mass units showed that the fragment ion contained a methoxycarbonyl group. The shift, by 6 mass units, of the *m/e* 303 ion in the spectrum of **3** showed that this fragment ion contained two acetoxyl groups, and that the fragmentation process did not involve the elimination of ketene. The difference between the values of *m/e* of the molecular ion and the fragment ion was 201. These facts showed that the large fragment containing a large part of the rhamnose residue was eliminated from the molecular ion *via* the third class of fragmentation pathway, namely, radical transfers. The molecular ion lost an $OHC–CH(OAc)–CH(OAc)–\dot{C}HCH_3$ radical by radical transfers. In the case in which radical transfers took place at the galacturonic acid residue, the resulting fragment-ion was *m/e* 259, but the intensities of this fragment ion and succeeding

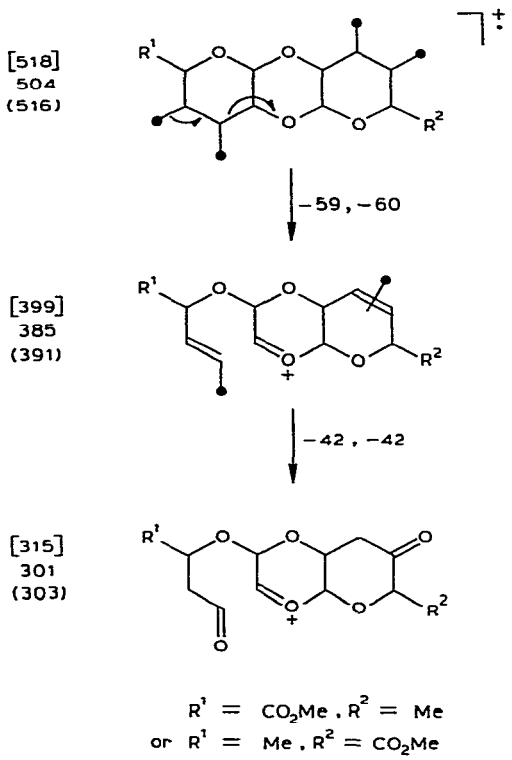


Scheme 4

fragment-ions were markedly small; therefore, the frequency of radical transfers at the galacturonic acid residue was very low. A retro-Diels–Alder reaction on the residual, galacturonic acid residue of the ion of *m/e* 303 gave the $H_3COOCH=\overset{+}{O}\text{-}CH=CH\text{-}OCH_3$ ion (*m/e* 149), which led to the ion $C^+H=CH\text{–}OCHO$ (*m/e* 71). On the other hand, the shift of the hydrogen atom of the aldehyde group of the *m/e* 303 ion, accompanied by the elimination of a CO molecule, generated the stable, dihydropyran ion of *m/e* 275. The fragment-ion of *m/e* 215, which was derived from the *m/e* 275 ion by the elimination of acetic acid, was situated on the branch point for the ions of *m/e* 197, 173, and 155. These fragment ions were respectively generated by the release of water, ketene, and acetic acid. The ion of *m/e* 259, which was generated by the radical transfers at a galacturonic acid residue, was degraded by a pathway analogous to that of the *m/e* 303 ion. These conclusions were completely supported by the spectra of **2** and **3**.

The fourth class of fragmentation pathway involved the *m/e* 288 ion. This pathway was characteristic for the spectrum of the dialdose dianhydrides (see Scheme 4). The fragment ion had 295 mass units in the spectrum of **3**, showing that two acetoxyl groups still remained in the fragment ion, and that one of the two acetoxyl groups which were eliminated from the molecular ion was released as ketene. The fragment ion of *m/e* 288 was presumed to be derived by the elimination of one ketene molecule from the ion of *m/e* 330; this was the only one in the high-mass region of the spectrum of **1** that was not included in the three classes of fragmentation pathway. The value of *m/e* for the peak corresponding to the *m/e* 330 ion in the spectrum of **1** increased by 9 in that of **3**; this showed that three acetoxyl groups were present in this fragment ion, and constituted solid support for the presumption regarding the elimination of ketene. Although the difference between the values of *m/e* of the molecular ion and this fragment ion was large (174), only one acetoxyl group was released. This fact showed that rearrangement of acetoxyl groups took place, because acetoxyl groups in the molecular ion were separated into two adjacent groups by the dioxane ring formed among two glycosidic bonds, and it was deemed improbable that only one acetoxyl group was eliminated from the molecular ion without rearrangement. From this discussion, the molecular structure for the fragment ion of *m/e* 330 that is illustrated in Scheme 4 was proposed. The process of the formation of this ion was not clear, but this process should contain the shift of one of two acetoxyl groups on the galacturonic acid residue towards C-2. It is more probable that the acetoxyl group that participated in the rearrangement reaction was the one attached to C-3 of the galacturonic acid residue. This process is similar to that in which the acetoxyl group on C-2 shifts towards C-1 in the peracetylated glucopyranoses[7]. Had this fragmentation process containing acetoxyl-group rearrangement taken place at the rhamnose residue of the molecular ion, the fragment ion of *m/e* 374 (*m/e* 383 for **3**), and succeeding fragment-ions, should have been observed in the spectrum of **1**, but these fragment ions were not detected. Despite the fact that the fragment ion of *m/e* 288 was the largest peak in the high-mass region of the spectrum of **1**, the fragment peak of *m/e* 302 (equivalent to the *m/e* 288 ion in **1**) was absent

from the spectrum of **2**. The ion of *m/e* 330 was observed in the spectrum of **2**, but almost all of this peak corresponded to the *m/e* 316 ion in the spectrum of **1** and, therefore, the peak corresponding to the ion of *m/e* 330 for **1** was very small. These observations showed that the methoxycarbonyl group participated strongly in this rearrangement reaction. The fragment ion of *m/e* 288, derived from the *m/e* 330 ion by the elimination of ketene, lost one more ketene molecule, to give the ion of *m/e* 246, and this ion generated the *m/e* 129 ion by the loss of $-OCH(CH_3)-CH_2OAc$ by the two different pathways given in Scheme 4.



Scheme 5

Another (minor) pathway of fragmentation was included in the spectrum (see Scheme 5). The fragment ion of *m/e* 444 was degraded to *m/e* 385 by the loss of 59 mass units. Although this step has already been clarified as a release of methoxycarbonyl radical, the peak for *m/e* 391 (in addition to the peak for *m/e* 394) was observed in the spectrum of **3**. The decrease of the value of *m/e*, namely, 62, showed that the release of acetyl radical from C-2 of the galacturonic acid or rhamnose residue corresponded to this step. The resulting fragment-ion, *m/e* 343, was degraded to *m/e* 301 by the loss of ketene.

EXPERIMENTAL

1,2′:1′,2-Dianhydride (**1**) *of 3,4-di-*O*-acetyl-β-*L*-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-*D*-galactopyranuronate.* — Compound **1** was prepared from 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose by successive treatment with 2.5% methanolic hydrogen chloride and 1:1 acetic anhydride–pyridine as previously reported[8,9].

1,2′:1′,2-Dianhydride (**2**) *of 3,4-di-*O*-acetyl-β-*L*-rhamnopyranose and 3,4,6-tri-*O*-acetyl-α-*D*-galactopyranose.* — Compound **2** was prepared by reduction of **1** with sodium borohydride, followed by treatment with 1:1 acetic anhydride–pyridine as previously reported[8,9].

1,2′:1′,2-Dianhydride (**3**) *of 3,4-di-*O*-(acetyl-*d_3*)-β-*L*-rhamnopyranose and methyl 3,4-di-*O*-(acetyl-*d_3*)-α-*D*-galactopyranuronate.* — Compound **1** in absolute methanol was deacetylated with sodium methoxide in the usual way, and the product was acetylated with 1:1 $(CD_3CO)_2O$–pyridine. The identity of the recrystallized material (yield 76%) was checked by n.m.r. spectroscopy.

Mass spectrometry. — The mass spectra were recorded by direct introduction of the sample at an ionizing potential of 40 eV. The temperature in the ionizing chamber was in the range of 180–200°. The intensities of the peaks in the spectra (see Fig. 1) are expressed in intensities relative to that of the base peak.

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