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MASS SPECTRA OF PERACETATES OF SOME (1→2)-LINKED DISACCHARIDES*

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The mass spectra of two peracetylated disaccharides and the pentaacetate (**1**) of an aldobiouronic acid methyl ester, all having a (1→2)-linkage, were analyzed. The fragmentation pathway common to these compounds could be classified into four groups: (*1*) the aA type, which was the main pathway, (*2*) the abJ type, (*3*) the baA type, and (*4*) the baC type. The manner of fragmentation of peracetate **1** was similar to those of the disaccharides.

INTRODUCTION

During the course of the analysis of the mass spectra of peracetylated disaccharide dianhydrides[1], comparative studies on the peracetates of (1→2)-linked disaccharides, which are among the major components of plant gums and mucilages, were needed; but mass-spectrometric studies on oligosaccharides have been conducted mainly on methylated derivatives, due to their high volatility, and only a few reports are available on the spectra of acetylated oligosaccharides[2–4]. Acetylated derivatives generally have low volatilities, and their spectra are difficult to record by a g.l.c.–m.s. system, and need to be obtained with a direct-inlet system. On the other hand, the spectra of methylated derivatives are rather complex, due to the abundant breakdowns of the sugar rings. In contrast, the mass spectra of acetylated derivatives are rather simple, because the frequency of breakdown of the sugar rings is relatively low; therefore, acetylated derivatives are also useful for the structural analysis of oligosaccharides.

We here deal with the analysis of the fragmentation pathways of peracetylated compounds having a (1→2)-linkage.

RESULTS AND DISCUSSION

The compounds tested in this study were methyl 3,4-di-*O*-acetyl-2-*O*-(methyl

*Mass Spectrometry of Dialdose Dianhydrides. Part II. For Part I, see ref. 1.

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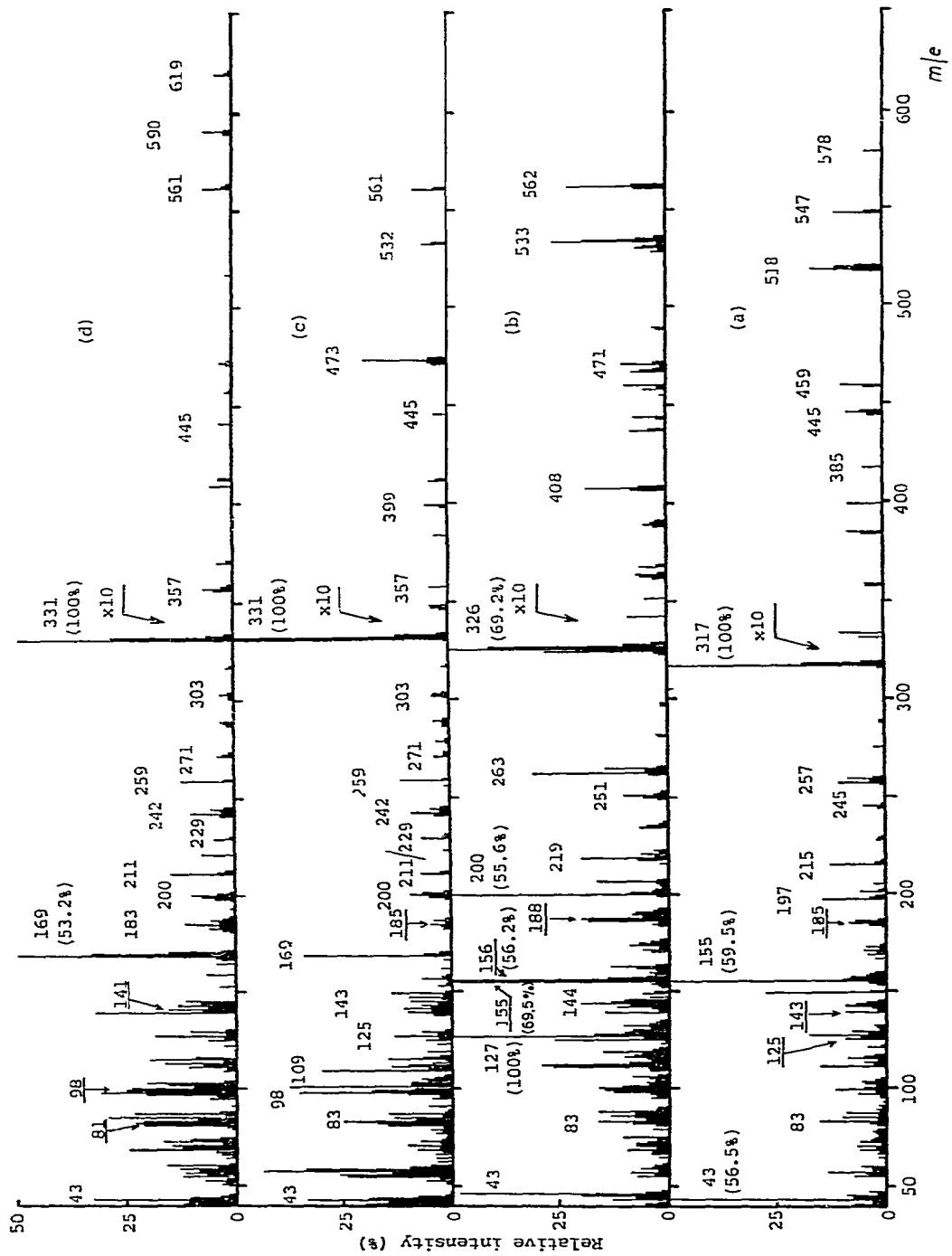
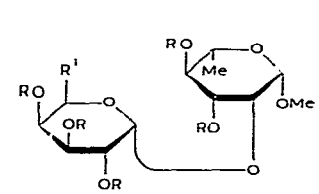


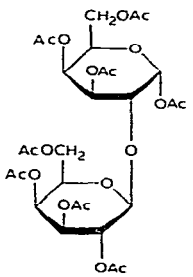
Fig 1. Mass spectra of (a) **1**, (b) **2**, (c) **3**, and (d) **4**.

2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-β-L-rhamnopyranoside (**1**), its per-*O*-(deuteroacetyl)ated derivative (**2**), methyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-L-rhamnopyranoside (**3**), and 1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**4**). The fragmentation pathways were analyzed by comparison of the spectra of these four compounds (see Fig. 1). The mass spectra of these compounds were very similar to each other, showing that the major parts of the fragmentation took place in the same way. The fragmentation pathways could be classified into four classes common to all of the compounds examined.

The first class of fragmentation was of the aA type. Compounds **1**, **3**, and **4** respectively showed base peaks at *m/e* 317, 331, and 331. Compound **2** showed an intense peak (69.2%) at *m/e* 326. These peaks correspond to aA_1 fragment ions The series of fragment ions (*m/e* 257, 215, 197, and 155 for **1**; *m/e* 263, 219, 200, 156, and 155 for **2**; and *m/e* 271, 229, 211, 169, and 109 for **3** and **4**) formed by the successive release of acetic acid and ketene from aA_1 ions had relatively high intensities. Therefore, the main pathways of fragmentation of the compounds were of the aA type. It has been reported that, for oligosaccharides, the fragmentation of the sugar residues follows principles similar to those for monosaccharides[4], but these results were markedly different from those obtained for the mass spectra of methyl aldohexopyranoside peracetates[3,5,6]. In the spectra of permethylated disaccharides having a (1→2)-linkage, the intensity of the peak for aA_1 ions was relatively low[4,7], showing



1 R = Ac, R' = CO_2Me
2 R = Ac-d_3 R' = CO_2Me
3 R = Ac, R' = CH_2OAc



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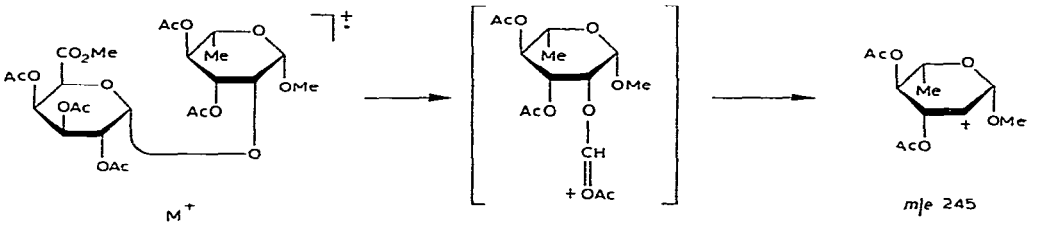


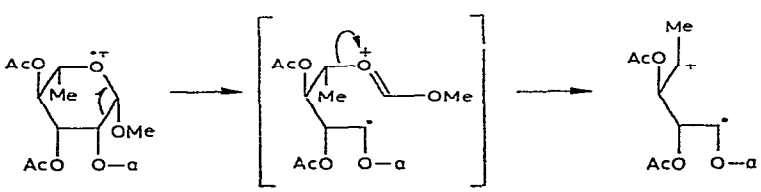
Scheme 1

that there are large differences between the fragmentation pathways of peracetylated and permethylated disaccharides.

The second class of fragmentation was initiated by the formation of the abJ_1 ion from the molecular ion. The C-1–C-2 bond of the a ring of the molecular ion of **1** was split radically, and then the acetoxyl group on C-2 was transferred to C-1. The resulting, abJ_1 ion (*m/e* 333) was converted into the *m/e* 245 ion by the loss of AcO–CHO, as shown in Scheme 1. The peaks corresponding to these ions were found at *m/e* 342 and 251 in the spectrum of **2**, showing that three and two acetoxyl groups were present in the abJ_1 ion and the fragment ion of *m/e* 245, respectively. These facts showed that this scheme was reasonable. The formation of AbJ_1 ions having high intensity was reported for a permethylated aldobiouronic acid[8] and a permethylated methyl pseudoaldobiouronate[9]. Therefore, this type of fragmentation is universal to disaccharides. The release of acetic acid and ketene gave a series of fragment ions of *m/e* 185, 143, 125, and 83. A similar fragmentation pathway was found for **3**. The fragment ions (*m/e* 245, 185, 143, 125, and 83) had the same structure as those from **1**, because the b ring of **1** and **3** had the same structure. This fragmentation pathway was presumed to be present in the spectrum of **4**, but the abJ_1 ion (*m/e* 331) and the ions included in this class had the same values of *m/e* as the aA_1 ion and a series of aA ions; moreover, the aA fragment-ions had relatively high intensities. Therefore, the ions belonging to this class of fragmentation pathway were not distinguished.

The third class of fragmentation was initiated by the release of a methoxyl or acetoxyl radical from C-1 of the b ring of the molecular ion, to give baA_1, which was further degraded by the loss of acetic acid and ketene molecules. In the mass spectra of **1** and **3**, the ions included in this class (*m/e* 547, 445, and 385 for **1**, and *m/e* 561, 399, and 357 for **3**) had relatively high intensities in the regions of high mass units. In the spectrum of **4**, the intensity of the baA_1 ion (*m/e* 619) was low, and other fragment-ions included in this class were not observed, showing that release of the acetoxyl group on C-1 of the b ring was difficult for **4**.

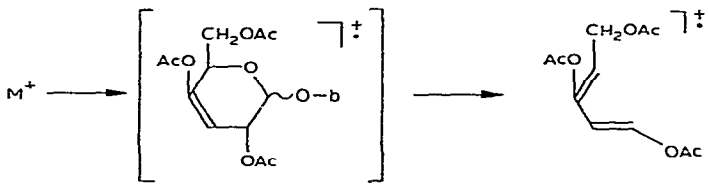
The fourth class of fragmentation pathway was of the C type. The molecular ion of **1** was degraded by the loss of 60 mass units, to give the ion of *m/e* 518, having a high intensity. The peak corresponding to this ion was observed at *m/e* 535 in the spectrum of **2**, showing that the fragment released from the molecular ion was not acetic acid, but $OHC–OCH_3$, released by the C type of fragmentation, as shown in Scheme 2. Therefore, the ion of *m/e* 518 was the baC_1. This baC_1 ion generated various



Scheme 2

kinds of baC_2 ions having *m/e* 459 by the loss of acetoxyl radical. The loss of acetic acid from baC_2 ions gave the ions of *m/e* 399, and this is supported by the presence of ions of *m/e* 408 in the spectrum of **2**. This process was also found in the spectra of **3** and **4**. In the spectrum of **3**, baC_1 and baC_2 ions (*m/e* 472) gave intense peaks in the high-mass region. In the spectrum of **4**, the peak at *m/e* 590 corresponded to the baC_1 ion, because the substituent at C-1 was an acetoxyl group, and the fragment released was OHC–OAc. The baC_2 and further-degraded ions were not detected. The ratios of the intensities of the ions of *m/e* 519 and 518 in the spectrum of **1**, and of *m/e* 535 and 534 in that of **2**, were not settled. The ions of *m/e* 519 and 535 were presumed to correspond to the processes of release of the carboxymethyl radical, but the reason for the irregularity was unclear.

The retro-Diels–Alder reaction on the fragment ion generated by the loss of one molecule of acetic acid from the molecular ions of **3** and **4** gave the fragment ion

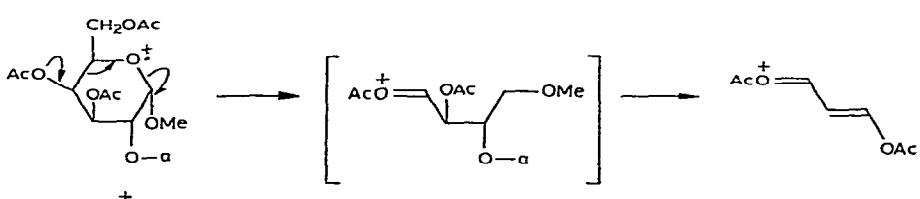


Scheme 3

of *m/e* 242, as shown in Scheme 3. Successive release of acetic acid and ketene gave the ions of *m/e* 200, 140, and 98. The intensities of these ions were ~10% in the spectra of **3** and **4**, but the ions corresponding to this fragmentation pathway were not found in the spectrum of **1**. This observation showed that a retro-Diels–Alder reaction on the b ring of the peracetylated methyl (methyl aldobiosid)uronate was rare.

Chizhov *et al.*[10] reported that the fragment ion of *m/e* 259 formed from the b ring was characteristic for the peracetate of a (1→2)-linked disaccharide (sophorose). This fragment ion was found in the spectra of **1**, **3**, and **4** (*m/e* 245).

It was reported[4] that permethylated disaccharides or aldobiouronic acids having a (1→2)-linkage generate baA_1 and baF_1 fragments as characteristic fragment-ions. Compounds **3** (see Scheme 4) and **4** generated baF_1 fragment (*m/e* 445), but neither baB_1 nor baF_1 fragment ions were observed in the spectrum of **1**.



Scheme 4

From these results, the structures of (1→2)-linked disaccharides could be determined by measurement of the mass spectra of their peracetylated derivatives; the structures of the b and a rings could be determined from the aA, baA, and abJ ions, and the kind of linkage, from the ions of baB, baC, and baF.

EXPERIMENTAL

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 are expressed in intensities relative to that of the base peak. The symbols used to denote fragment ions are those employed by Kochetkov and Chizhov[2].

*Methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-*D*-galactopyranosyluronate)-β-*L*-rhamnopyranoside* (**1**). — Compound **1** was prepared from 2-*O*-(α-D-galactopyranosyluronic acid)-β-L-rhamnopyranose by methyl glycosidation–methyl esterification with methanolic hydrogen chloride followed by acetylation. Details of the preparation and n.m.r. data have been given[11].

*Methyl 3,4-di-*O*-acetyl-*d_3*-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-*d_3*-α-D-galactopyranosyluronate)-β-L-rhamnopyranoside* (**2**). — Compound **1** (100 mg) in absolute methanol (10 mL) was stirred with 0.1M sodium methoxide (0.05 mL) for 30 min at room temperature. The solution was passed through a column of Amberlite IR-120 (H^+) resin and then evaporated. The residual syrup was acetylated with 1:1 $(CD_3CO)_2O$–pyridine (1 mL) for 24 h at room temperature, and the product extracted with chloroform. Crystallization, and recrystallization, from 1:1 ethanol–ether gave pure **2** (82 mg). The acetoxyl signal at ~2 p.p.m. was completely absent from the n.m.r. spectrum of **2**.

*Methyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-L-rhamnopyranoside* (**3**). — A solution of compound **1** (100 mg) dissolved in oxolane (5 mL) containing sodium borohydride (50 mg) was stirred for 1 h at room temperature The solution was made neutral with acetic acid, passed through a column of Amberlite IR-120 (H^+) resin and then evaporated The residual syrup was acetylated with 1:1 acetic anhydride–pyridine as usual. As crystallization could not be achieved, pure compound **3** was obtained by preparative t.l.c. (78 mg); R_F 0.66 (4:1 benzene–acetone); n.m.r. data ($CDCl_3$): δ 1.41–1.22 (3 H, Rha-CH_3, $J_{5,6}$ 6.4 Hz), 1.99–2.16 (18 H, 6 OAc), 3.36 (s, 3 H, OCH_3), 3.73 (o, 1 H, Rha-H-5, $J_{4,5}$ 7.3 Hz), 3.94–4.04 (3 H, Gal-H-6, Gal-H-5), 4.32 (d, 1 H, J 6.5 Hz), 4.44 (d, 1 H, Rha-H-1, $J_{1,2}$ 1.9 Hz), 4.82–5 39 (4 H), and 5.46 (d, 1 H, Gal-H-4, $J_{3,4}$ 3.6 Hz).

*1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose* (**4**). — Compound **4** was synthesized by the method of Helferich and Zirner[12] with slight modifications. 1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose (1 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.12 g) were dissolved in absolute acetonitrile (10 mL) in a flask covered with metal foil. Mercuric cyanide (0.36 g) and mercuric bromide (0.514 g) were added and the mixture was stirred for

30 min at room temperature, and evaporated. The residue was twice extracted with chloroform (100 mL), and the extract was dried (sodium sulfate), and evaporated. Crystallization from ether gave a powder (530 mg). The same treatment of the mother liquor gave a second crop (421 mg). The combined powder gave two spots in t.l.c. on silica, R_F 0.34 and 0.47 (4:1 benzene–acetone) in the ratio of ~2:3. The material of R_F 0.47 was isolated (451 mg) by using a silica column. This material gave pure **4** (382.5 mg) by two recrystallizations from ether; R_F 0.46 (4:1 benzene–acetone); n.m.r. data ($CDCl_3$): δ 1.80–2.01 (15 H, 3 *eq*-OAc + 2 CH_2OAc), 2.08–2.14 (9 H, 3 *ax*-OAc), 3.8 (6 H), 4.53 (d, 1 H, H-1 of nonreducing end, J 7.5 Hz), 4.9–5.08 (2 H), 5.2–5.5 (3 H), and 6.32 (d, 1 H, H-1 of reducing end, J 4.0 Hz).

Anal. Calc for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.52; H, 5.60.

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