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CHEMICAL IONIZATION–MASS SPECTRA OF ALDOBIOURONIC ACIDS AND DIALDOSE DIANHYDRIDES*

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ABSTRACT

Chemical ionization (c.i.) mass spectra with isobutane as the reagent gas are reported for the peracetates of aldobiouronic acids and related compounds, and for peracetates and permethylated derivatives of dialdose dianhydrides. Ions ($M^+ + 43$) having relatively high intensities were detected in the spectra of disaccharides lacking the dianhydride structure. Peracetylated dialdose dianhydrides showed very weak ($M^+ + 43$) ions, and permethylated dianhydrides did not show them. The ($M^+ + 43$) ion consisted of molecular ion and acetoxyl radical (but not of the reagent gas). In the c.i. mass spectra of the usual disaccharide peracetates, ($M^+ - 31$) and ($M^+ - 60$) ions had large intensities. In contrast, c.i. mass spectra extremely similar to the corresponding e.i. mass spectra were obtained for dialdose dianhydrides.

INTRODUCTION

Analyses of fragmentation pathways in the electron impact (e.i.) mass spectra of some peracetylated disaccharides, and peracetylated and permethylated dialdose dianhydrides, have previously been reported[1–3]. In these spectra, the intensities of the fragment ions in the high-mass regions were very small, molecular ions were not found in many cases, and the abundant breakdowns of the molecules, caused by the impact of high-energy electrons, increased the complexity of the spectra.

Chemical ionization (c.i.) mass spectra with ammonia, methane, or isobutane as the reagent gas give molecular ions and several fragment-ions with high intensities in the high-mass regions[4,5]. As the mass spectra of sugar derivatives generally show only small peaks in the high-mass regions of the e.i. mass spectra, c.i. mass spectra are useful for analysis of the structures of sugar derivatives. Therefore, many studies on the c.i. mass spectra of sugars have been made[4–6], but very few reports on aldobiouronic acids and dialdose dianhydrides are at present available.

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*Mass Spectrometry of Dialdose Dianhydrides, Part IV. For Part III, see ref. 1.

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TABLE I

C.I. (ISOBUTANE) MASS SPECTRA OF PERACETATES OF ALDOBIOURONIC ACIDS, AND RELATED COMPOUNDS[a]

Compound	m/z *(relative intensity in parentheses)*									
1	621	578	547	519	427	399	385	331	317	257
	(1.7)	(0.4)	(5.7)	(0.9)	(1.2)	(0.6)	(0.6)	(1.9)	(100)	(11.1)
2	639	563	562	534	531	459	436	408	392	326
	(2.4)	(7.2)	(25.6)	(1.6)	(1.6)	(1.2)	(3.8)	(1.4)	(1.2)	(87.2)
3	635	561	533	501	473	459	441	413	389	331
	(2.5)	(4.3)	(2.6)	(0.3)	(0.3)	(1.1)	(0.7)	(1.1)	(0.8)	(100)
4	619	457	415	410	401	378	341	331		
	(7.5)	(0.7)	(0.4)	(1.3)	(0.7)	(2.1)	(3.1)	(100)		
5	679	636	605	577	545	517	505	503	473	457
	(3.4)	(0.5)	(15.1)	(6.8)	(12.5)	(7.5)	(1.5)	(1.5)	(1.5)	(1.8)
	443	431	429	425	373	331	317			
	(3.2)	(2.6)	(6.1)	(2.5)	(12.5)	(10.5)	(51.5)			

[a] Only the ions having higher masses than aA_1 ions are listed, because the ions smaller than aA_1 ions showed no significant differences from those in the corresponding e.i. mass spectra.

We here deal with the c.i. mass spectra of peracetylated aldobiouronic acids and their derivatives, and peracetylated and permethylated dialdose dianhydrides.

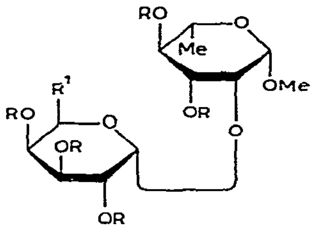
RESULTS AND DISCUSSION

1. C.i. mass spectra of the peracetates of aldobiouronic acids and related compounds. — The compounds examined in this study (see Table I) were methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 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**), 1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**4**), and methyl 2,3,6-tri-*O*-acetyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-β-D-galactopyranoside (**5**).

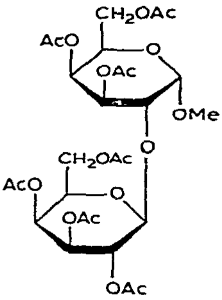
In this experiment, the c.i. (isobutane) mass spectra of these disaccharide peracetates were essentially the same as the corresponding e.i. mass spectra, although small differences were present. The increase of intensities of fragment-ions having high masses was observed. The number of these fragment ions was very small and, therefore, the spectra were quite simple.

In all cases, the aA_1 fragment-ions, which were formed by splitting of the glycosidic linkage at the nonreducing-end sugar group, were the base peaks in the spectra (*m/z* 317 for **1** and **5**, 326 for **2**, and 331 for **3** and **4**). The aA_1 fragments were very effective for determination of the structures of nonreducing-end sugars.

All spectra contained no [or weak (for **1**)] molecular-ion peak, but the spectra of **1**, **2**, **3**, and **5** contained relatively clear peaks having higher masses than those of the



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



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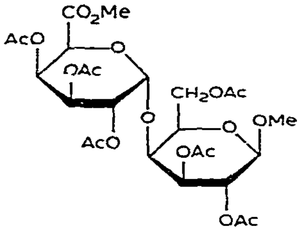
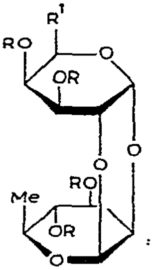




6 R = Ac, R^1 = CO_2Me
7 R = Ac-d_3, R^1 = CO_2Me
8 R = Ac, R^1 = CH_2OAc
9 R = Me, R^1 = CH_2OMe
10 R = Me-d_3, R^1 = CH_2OMe-d_3

molecular ions (*m*/*z* 621 for **1**, 639 for **2**, 635 for **3**, and 679 for **5**). Dougherty *et al.*[5] reported that the ions with masses higher than that of the molecular ion are reagent gas–molecular ion complexes in c.i. mass spectra with a reagent gas consisting of a mixture of ammonia and isobutane. In this experiment, the excess of mass was 43 for **1**, **3**, and **5**, but 46 for **2**; this result showed that these ions were not reagent gas–molecular ion complex, because the reagent gas (isobutane) gives a fragment ion with *m*/*z* 43 (and not *m*/*z* 46). Consequently, the complexes consisted of molecular ion and acetoxyl radical ($\dot{O}COCH_3$). It was also reported[5] that all disaccharides used in their experiments gave these ions, but **4** gave neither a molecular ion nor an acetoxyl radical–molecular ion complex.

In the spectra of **1**, **2**, **3**, and **5**, two intense peaks were observed in the high-mass regions. The peaks with higher masses (*m*/*z* 547, 562, 561, and 605 for **1**, **2**, **3**, and **5**, respectively) were the ions formed by the release of methoxyl group. The *m*/*z* value of **2** (*m*/*z* 562, namely, $M^+ - 31$) supported the release of the OCH_3 group linked to the glycosidic center of the reducing-end sugar. The peaks with lower masses (*m*/*z* 519, 533, and 577 for **1**, **3**, and **5**, respectively) were smaller by 59 mass units than those of the molecular ions. There are three possible explanations for this observation. The first is the release of acetoxyl radical from the molecular ion, the second is the release of acetic acid followed by the addition of one hydrogen atom, and the third is the release of OHC · OCH_3 (baC-type fragmentation) followed by addition of a hydrogen atom. The decrease of mass from the molecular ion was 62 and 59 in the spectrum of **2**, showing that the second and third pathways were operative. In **4**, the release of acetoxyl radical from the glycosidic center gave *m/z* 561 ($M^+ - 59$), and the release of acetic acid gave the fragment-ion having the same mass (*m/z* 561, $M^+ - 60 + 1$). The fragment ions due to the third process were not observed in the spectra of **4** and **5**. It had already been reported[3] that baC-type fragmentations predominate in the e.i. mass spectra of **1** and **4**, but the intensities of baC-type fragment-ions were low in the c.i. mass spectra. It was presumed that the baC-type fragmentations (which require high energy) could readily occur in e.i. mass spectrometry, because of the high energy of electron impact. In the step involving the release of the methoxyl group from the glycosidic center of the potentially reducing end, the addition of hydrogen atoms to the newly formed fragment-ions was not observed. The difference was considered due to the difference in the stability of the fragment ion. By the release of the methoxyl radical, a double bond is formed between the ring-oxygen atom and C-1, stabilizing the ion. In contrast, release of acetic acid does not stabilize the positive charge on the ring-oxygen atom. Therefore, stabilization of the fragment ion was achieved by the addition of a hydrogen atom, and the fragment ions had the methoxyl group on C-1.

In the spectra of **1**, **2**, and **3**, some differences were observed in the region lying between *m/z* 350 and 500. The baA_3 ions (*m/z* 445 for **1**, and 455 for **2**), clearly observable in the e.i. mass spectra, were not detected. In the spectrum of **3**, the *m/z* 459 ion was observed, but this ion was presumed to be the baA_3 fragment-ion (not observed in the e.i. mass spectrum of **3**). The baC fragments (*m/z* 459 for **1**, 464 for **2**, and 473 for **3**) were not observed in the e.i. mass spectrum. The intensity of the *m/z* 426 ion for **1** (*m/z* 436 for **2**, and 441 for **3**) increased markedly, to give characteristic patterns.

The c.i. mass spectrum of **4** was very simple, and the molecular ion and a series of baA fragment-ions were not observed. The fragment ions appeared at *m/z* 457, 410, 401, and 378, and had intensities of 0.5–2%, but attempts at assignments were unsuccessful.

The c.i. mass spectrum of **5** showed a pattern similar to that in the e.i. mass spectrum, except that the fragment-ions of *m/z* 516, 372, 288, and 228 in the e.i. mass spectrum appeared at *m/z* 517, 373, 289, and 229 (by addition of a hydrogen atom). The c.i. mass spectrum of **5** was also similar to those of **1**, **2**, and **3**. The intensities of the fragment-ions of *m/z* 605 ($M^+ - 31$), 577 ($M^+ - 60 + 1$), 545 ($M^+ - 31 - 60$), and 517 ($M^+ - 60 - 60 + 1$) were very high in comparison with those in other spectra, although the intensities of both the molecular ion (*m/z* 636) and the ($M^+ + 43$) ions were small. These characteristics indicated that differentiation of the kinds of linkage, and of the component sugars, was possible.

2. C.i. mass spectra of the peracetylated and permethylated dialdose dianhydrides.

TABLE II

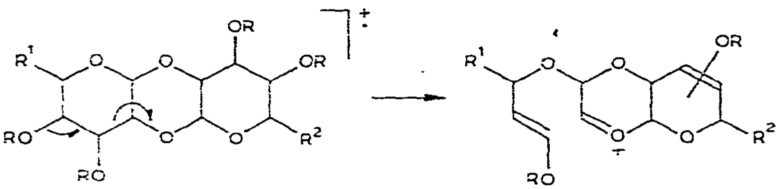
C.I. (ISOBUTANE) MASS SPECTRA OF PERACETATES AND PERMETHYLATED DERIVATIVES OF DIALDOSE DIANHYDRIDES[a]

Compound	m/z *(relative intensity in parentheses)*									
6	547	505	444	402	385	358	343	330	325	316
	(0.1)	(1.1)	(1.2)	(3.0)	(11.6)	(2.4)	(2.3)	(1.4)	(2.2)	(12.0)
	303	288	275	266	256					
	(7.5)	(15.3)	(7.9)	(5.0)	(1.8)					
7	562	517	453	409	394	391	365	339	331	322
	(0.1)	(0.5)	(1.1)	(2.7)	(2.3)	(11.3)	(2.2)	(1.7)	(2.5)	(6.5)
	309	295	281	265	259					
	(6.8)	(7.4)	(6.5)	(7.1)	(2.4)					
8	561	519	458	416	399	385	372	356	343	330
	(0.4)	(0.7)	(5.7)	(1.0)	(5.9)	(3.0)	(2.1)	(1.4)	(0.9)	(9.7)
	317	303	301	289	279					
	(9.3)	(2.4)	(1.8)	(8.6)	(0.8)					
9	379	345	333	314	301	289	283	269	259	246
	(2.5)	(3.0)	(4.1)	(2.3)	(9.6)	(4.4)	(9.1)	(1.5)	(8.9)	(4.6)
	241	227	214	203	187					
	(6.5)	(4.5)	(12.5)	(24.5)	(44.8)					
10	394	358	345	323	315	310	301	289	268	255
	(1.3)	(1.1)	(4.1)	(2.5)	(4.5)	(13.5)	(3.1)	(14.8)	(11.3)	(5.8)
	247	220	212	196	187					
	(6.7)	(15.8)	(21.8)	(32.5)	(11.5)					

[a] Fifteen ions having high masses are listed.

— The compounds studied in this experiment (see Table II) were the peracetate (**6**) and per(deuteroacetate) (**7**) of the 1,2′:1′,2-dianhydride of β-L-rhamnopyranose and methyl α-D-galactopyranosyluronate, and the peracetate (**8**) and permethylated (**9**) and per(deuteromethyl)ated (**10**) derivatives of the 1,2′:1′,2-dianhydride of β-L-rhamnopyranose and α-D-galactopyranose.

The c.i. mass spectra of compounds **6**, **7**, and **8** were similar to the corresponding e.i. spectra, and the intensities of the fragment-ions with high masses were similar, except that several fragment-ions were smaller than those found in the case of **1**–**5**. These observations showed that all fragmentation pathways observed in the e.i. spectra occurred in the c.i. mass spectrometry. In contrast, the molecular ions were extremely weak (or not detected) in the e.i. spectra of **6**–**8**, but the molecular ions or ($M^+ + 1$) ions were observed. However, their intensities were very small, and the intensities of the complex ions with masses higher than those of the molecular ions were less than 0.4%, showing marked contrast to the results reported by Dougherty *et al.*[5]. A large increase in the intensity was observed at *m/z* 385 (**6**), 391 (**7**), and 399 and 385 (**8**). The fragment-ion of *m/z* 391 in the spectrum of **7** had two acetoxyl groups and, hence, it was produced from the fragment ion of *m/z* 516 by the loss of one acetoxyl radical and one acetic acid by the radical-transfer illustrated in Scheme 1.



in1, 504 $\longrightarrow$ 385, R = OAc, R^1 and R^2 = CO_2Me and Me
in2, 516 $\longrightarrow$ 391, R = OAc-d_3, R^1 and R^2 = CO_2Me and Me
in3, 518 $\longrightarrow$ 315, R = OAc, R^1 and R^2 = CH_2OAc and Me

Scheme 1

A small increase in the intensity of the *m/z* 394 ion (7) and the *m/z* 385 (8) ion, and the high intensity of the *m/z* 399 ion (8) showed that fragmentation by the radical-transfer depicted in Scheme 1 was dominant compared to the loss of the C-5 substituent in the c.i. spectra.

The c.i. mass spectra of the permethylated (9) and per(deuteromethyl)ated (10) dialdose dianhydrides were almost the same as the e.i. spectra of **2** and **10**. The molecular ion or (M^{+} + 1) ion had small intensities, but ions with masses higher than those of the (M^{+} + 1) ions were not detected in the spectra of **9** and **10**. A new type of fragment-ions with relatively high intensities was found at *m/z* 283 and 341 in the spectrum of **2**. The corresponding fragment-ions were also found at *m/z* 289 and 247 in that of **10**. Therefore, it was certain that both fragment-ions had two methoxyl groups (but the structures could not be determined).

In general, the c.i. mass spectrum gives a molecular ion–reagent gas complex having a high intensity. In this work, the disaccharides having the usual structures gave (M^{+} + 43) ions with relatively high intensities in the high-mass regions, although they were small in comparison with the data in the literature[5]. The c.i. mass spectra of the peracetylated and the permethylated dialdose dianhydride were slightly different from the e.i. mass spectra, and the intensities of the ions with masses higher than those of the molecular ions were extremely small. The intensities of the ions were small, in general agreement with those in the e.i. spectra, presumably because the dialdose dianhydrides have very rigid structures, caused by the presence of a third, fused ring, and show strong resistance to the impacts.

EXPERIMENTAL

C.i. mass spectrometry. — The c.i. mass spectra were recorded by direct introduction of the samples at an ionizing potential of 70 eV. The temperature in the ionizing chamber was in the range of 180–190°. In the mass spectrometry of the permethylated dialdose dianhydrides (**9** and **10**), the samples were introduced into a g.l.c. column (1 m) of 5% of OV-1. Isobutane was used as the reagent gas. All spectra were recorded with a Hitachi M-70 mass spectrometer. The intensities of the peaks are expressed relative to that base peak above the reagent-gas regions (m/z >60). The symbols used to denote fragment-ions are those employed by Kochetkov and Chizhov[7].

Materials. — Compounds **1–4** were prepared as described[3]. Compound **5** was prepared from the disaccharide fraction of the water-soluble polysaccharide of *P. amurense* as described[8]. N.m.r. and i.r. data for **5** are given in an accompanying paper[9]. The peracetates (**6–8**) and the permethylated derivatives (**9** and **10**) of the dialdose dianhydrides have been reported[1,2].

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REFERENCES

1 T. Fujiwara and K. Arai, *Carbohydr. Res.*, 87 (1980) 201–207.
2 T. Fujiwara and K. Arai, *Carbohydr. Res.*, 86 (1980) 17–26.
3 T. Fujiwara and K. Arai, *Carbohydr. Res.*, 87 (1980) 11–17.
4 A. M. Hogg and T. L. Nagabhushan, *Tetrahedron Lett.*, (1972) 4827–4830.
5 R. C. Dougherty, J. D. Roberts, W. W. Binkley, O. S. Chizhov, V. I. Kadentsev, and A. A. Solov'yov, *J. Org. Chem.*, 39 (1974) 451–455.
6 D. C. DeJongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dowson, and C. C. Sweeley, *J. Am. Chem. Soc.*, 91 (1969) 1728–1740.
7 N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
8 T. Fujiwara, *Mem. Nara Univ.*, 10 (1982) in press.
9 T. Fujiwara and K. Arai, *Carbohydr. Res.*, 101 (1982) 323–325.