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AN ELECTRON-IMPACT, MASS-SPECTROMETRIC FRAGMENT-ION THAT IDENTIFIES 3-LINKED D-GLUCOPYRANOSYL RESIDUES IN PER-*O*-ALKYLATED, LINEAR β-D-GLUCOPYRANO-OLIGOSACCHARIDE-ALDITOLS*,**

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ABSTRACT

A previously unreported fragment-ion, designated J_0, was observed in the electron-impact, mass spectra of per-*O*-alkylated, linear di- and tri-β-D-glucopyranosylalditols containing 3-linked D-glucopyranosyl residues. The J_0 fragment-ion was absent from, or present in very low abundance in, the spectra of per-*O*-alkylated, linear di- and tri-β-D-glucopyranosylalditols composed of only 2-, 4-, or 6-linked residues. The presence of the J_0 fragment-ion and the absence of the J_1 fragment-ion were found indicative of the presence of 3-linked D-glucopyranosyl residues, and may be indicative of the presence of all 3-linked-glycosyl residues in per-*O*-alkylated oligosaccharide-alditols. A possible mechanism for the formation of the J_0 fragment-ion is proposed.

INTRODUCTION

Mass-spectrometric analysis has become one of the most commonly used methods for determining glycosyl sequences of per-*O*-alkylated oligosaccharide-alditols of known glycosyl-linkage compositions[1–7]. Three types of electron-impact mass spectrometry (e.i.-m.s.) fragment-ions, namely, the A, J, and alditol-cleavage (ald) series (see Fig. 1), provide most of the information necessary to determine the sequence of the glycosyl residues in per-*O*-alkylated oligosaccharide-alditols[1–7]. With these methods, 3-linked glycosyl residues are distinguished from 2-, 4-, and 6-linked glycosyl residues by the absence of the J_1 fragment-ion in the e.i.-mass spectrum of per-*O*-alkylated oligosaccharides[5,6] (see Fig. 1). However, the presence of a 3-linked glycosyl residue is sometimes difficult to establish, because

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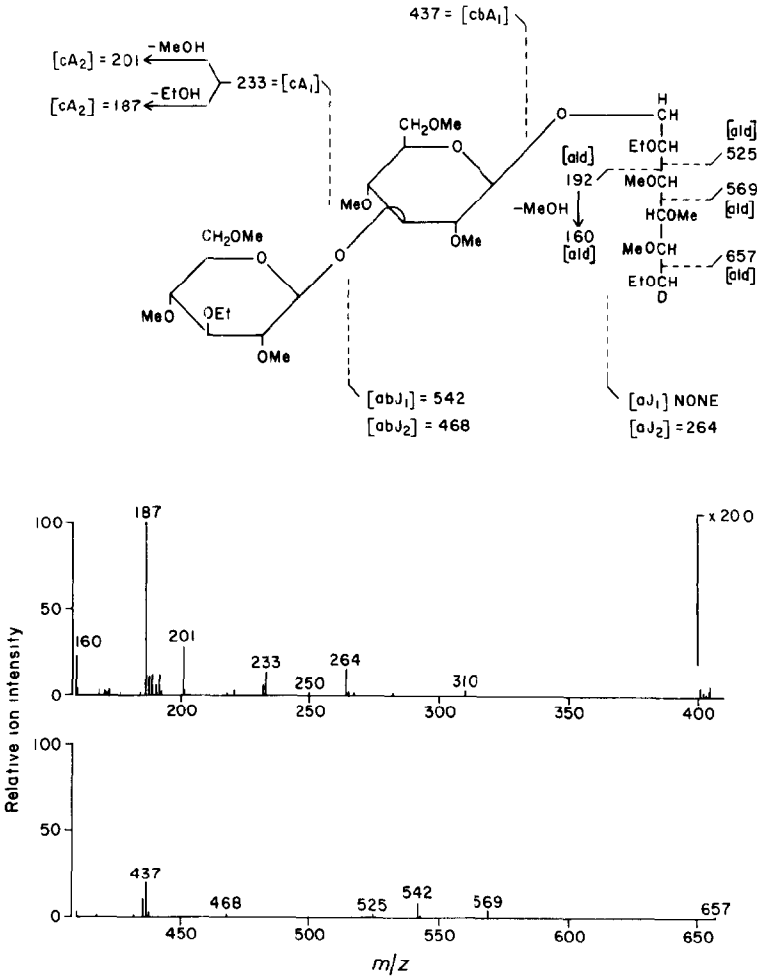


Fig. 1. E.i.-mass spectrum of a per-*O*-alkylated β-glucopyrano-trisaccharide-alditol in which residue b is 3-linked (compound 2). The origins of the fragment-ions in the mass spectrum are illustrated in the top half of the Figure. The nomenclature is that of Kochetkov and Chizhov[4], as modified by Åman *et al.*[16]. Glycosyl residues are identified by the letters a, b, c, *etc.*, from the alditol end of the oligosaccharide-alditol. The type of fragment-ion is designated by a capital letter or "ald" (for alditol fragment), and the specific fragment-ion is designated by including the letters of the intact glycosyl residues of which the fragment ion is composed. Residue b is 3-linked, because no aJ_1 is observed[6], but another fragment-ion at *m/z* 310 is observed (see text).

small amounts of a sample may produce an e.i.-mass spectrum in which a J_1 fragment-ion of low abundance might be overlooked and be interpreted as absent. We now describe a newly observed, e.i.-m.s. fragment-ion that provides positive evidence for the presence of 3-linked glucopyranosyl, and probably most (or all) other 3-linked glycosyl. residues in per-*O*-alkylated oligosaccharide-alditols.

MATERIALS AND METHODS

Chemicals. — Pustulan, a polymer of β-D-(1→6)-linked D-glucopyranosyl residues, was obtained from Calbiochem. Laminaran, a polymer of β-D-(1→3)-linked D-glucopyranosyl residues, was purchased from Sigma Chemical. The 4-linked hexa-β-glucopyranosylalditol was purified as described[8]. The 2-linked β-glucopyrano-oligosaccharide was a gift of W. York[9]. Per-*O*-alkylated di- and tri-β-glucopyranosylalditols, generated by the sequence analysis of lichenan (Sigma Chemical)[7,10], were gifts of M. McNeil. The S-glucan, from *Rhizobium japonicum* strain 3l1b71a[11], was a gift of W. Dudman.

Generation of per-O-alkylated di- and tri-β-glucopyranosylalditols. — A mixture of per-*O*-alkylated β-glucopyrano-oligosaccharide-alditols was generated from each polysaccharide, as described[7,10]. The per-*O*-alkylated β-glucopyrano-oligosaccharide-alditols generated from pustulan, laminaran, and S-glucan were fractionated by 3.5-MPa, reversed-phase, liquid chromatography on a column (4.6 mm × 25 cm) of Supelco Spherisorb-5 ODS equilibrated in 52% acetonitrile in water at a flow rate of 0.5 mL/min. The fractions containing the more-abundant per-*O*-alkylated di- and tri-β-glucopyranosylalditols were separately pooled for g.l.c.–m.s. analysis. The per-*O*-alkylated β-glucopyrano-oligosaccharide-alditols generated from the β-2- and β-4-linked glucopyrano-oligosaccharides were not fractionated by 3.5-MPa l.c. before g.l.c.–m.s. analysis.

G.l.c.–m.s. analysis of the per-O-alkylated β-glucopyrano-oligosaccharide-alditols. — A Hewlett–Packard 5840A Gas Chromatograph and 5985 GC/MS System were used for g.l.c.–m.s. analysis. Samples of per-*O*-alkylated β-glucopyrano-oligosaccharide-alditols (1 μg/μL) were dissolved in 1:1 decane–acetone. Samples (0.5 μL) were directly injected into a DB-1 g.l.c. column (0.25-μm film thickness, 30 m × 329 μm i.d., J and W Scientific)[12]. The effluent end of the column was placed in the ion source of the mass spectrometer[13]. The flow rate of the carrier gas, grade Z-1 helium, was 40 cm/s. The gas chromatograph was programmed at 150° for 3 min, followed by a rate of 30°/min to 190°, followed by a rate of 6°/min to 340°. The mass spectrometer was set at an ionization energy of 70 eV, an ionization current of 300 μA, a source temperature of 200°, and an electron-multiplier voltage of 2.200 kV. Scans were made at 800 amu/s over a range of 160 to 1000 *m/z*. Data were collected with a Hewlett–Packard 1000 Series E computer with the Hewlett–Packard AQUIRE program.

RESULTS AND DISCUSSION

The e.i.-mass spectra of the per-*O*-alkylated di-β-glucopyranosylalditols **1**, **2**, and **6**, in which residue c was linked to O-3 of residue b, contained a previously unreported fragment-ion of *m/z* 310 (*m/z* 309 for **6**) (see Table I and Fig. 1). As expected for 3-linked glucopyranosyl residues, these spectra lacked aJ_1 fragment-ions of significant abundance. The fragment-ions of *m/z* 310 or 309 corresponded to an

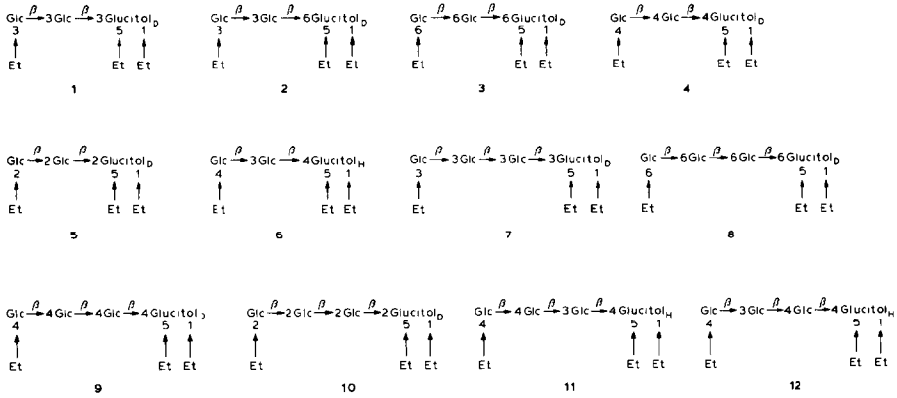
TABLE I

DIAGNOSTIC E.I.-M.S. FRAGMENT-IONS FOR SOME PER-*O*-ALKYLATED GLUCOPYRANOTRISACCHARIDE-ALDITOLS

Compound[a]	cA_1	cA_2		cbA_1	aJ_1	aJ_0	aJ_2	abJ_1	abJ_2	*Ald*		
1	233	201	187	437	324	310	264	542	468	672	614	
	(14.2)	(27.9)	(100)	(1.6)	(0.2)	(1.1)	(11.3)	(0.2)	(0.2)	(0.1)	(0.2)	
2	233	201	187	437	—	310	264	542	468	569	192	160
	(13.0)	(27.4)	(100)	(1.0)		(3.4)	(15.4)	(0.5)	(0.1)	(0.2)	(10.6)	(24.7)
3	233	201	187	437	324	310	264	528	468	569	192	160
	(10.4)	(100)	(10.4)	(0.7)	(11.2)	(0.2)	(43.6)	(3.9)	(0.5)	(0.3)	(10.7)	(33.8)
4	233	201	187	437	324	310	264	528	468	672	614	613
	(17.3)	(100)	(72.6)	(1.0)	(13.0)	(0.3)	(97.1)	(0.6)	(0.6)	(0.1)	(0.1)	(0.3)
5	233	201	187	437	324	310	264	528	468	614	570	191
	(9.9)	(100)	(20.4)	(3.4)	(1.0)	(0.2)	(97.6)	(2.2)	(1.6)	(1.0)	(0.1)	(2.2)
6	233	201	187	437	323	309	263	527	467	613		
	(14.0)	(100)	(63.1)	(2.3)	(0.2)	(1.9)	(33.6)	(0.2)	(0.3)	(0.1)		

[a]All compounds have *O*-ethyl (Et) groups where indicated, and *O*-methyl groups in place of all other hydroxyl groups. The D designates the presence of a deuterium label on C-1 in those compounds reduced with $NaBD_4$; the H label designates those compounds reduced with $NaBH_4$. The authenticity of alditol fragments of low abundance was assessed by selected-ion profiling of the chromatogram. The dash indicates that no fragment-ion was observed.

m/z of 46 amu more than the aJ_2, and will be referred to as aJ_0 fragment-ions. The e.i.-mass spectra of di-β-glucopyranosylalditols **3**, **4**, and **5**, which had glucopyranosyl residue c respectively linked at O-6, O-4, and O-2 of residue b, contained, as expected, relatively abundant aJ_1 fragment-ions and only very low abundances of the aJ_0 fragment-ion.



The e.i.-mass spectra of tri-β-glucopyranosylalditols **7** and **11** contained relatively abundant aJ_0 fragment-ions (see Table II). Compounds **7** and **11** both had residue c attached to O-3 of residue b, and, as expected, their spectra lacked an aJ_1

TABLE II

DIAGNOSTIC E.I.-M S. FRAGMENT-IONS FOR SOME PER-*O*-ALKYLATED GLUCOPYRANOTETRASACCHARIDE-ALDITOLS

Compound[a]	cA_1	cA_2		cbA_1	$dcbA_1$	aJ_1	aJ_0	aJ_2	abJ_1	abJ_0	abJ_2	$abcJ_1$	$abcJ_2$	*Ald*	
7	233	201	187	437	641	—	310	264	—	514	468	746	672	818	
	(18.3)	(32.2)	(100)	(3.2)	(0.6)		(0.5)	(27.6)		(0.3)	(0.6)	(0.1)	trace[b]	(0.1)	
8	233	201	187	437	—	324	310	264	528	—	468	732	—	192	160
	(13.2)	(100)	(20.7)	(0.9)		(9.8)	(0.2)	(39.8)	(2.4)		(0.3)	(0.9)		(5.8)	(17.1)
9	233	201	187	437	641	324	310	264	528	514	468	732	—	—	
	(11.2)	(100)	(88.8)	(1.6)	(0.1)	(4.9)	(0.2)	(61.4)	(0.5)	(0.1)	(0.4)	(0.1)			
10	233	201	187	437	641	324	310	264	528	514	468	732	672	818	
	(11.1)	(100)	(21.2)	(2.2)	(0.1)	(0.9)	(0.2)	(98.8)	(0.6)	trace[b]	(0.9)	(0.6)	trace[b]	(0.2)	
11	233	201	187	437	641	—	309	263	527	—	467	731	671	—	
	(9.2)	(100)	(55.2)	(0.5)	(0.5)		(1.1)	(46.6)	(0.8)		(0.4)	(0.1)	(0.1)		
12	233	201	187	437	641	323	309	263	—	513	467	731	671	—	
	(10.9)	(61.7)	(67.7)	(1.8)	(0.1)	(6.2)	(0.2)	(100)		(0.6)	(0.5)	(0.1)	(0.0)		

[a]See footnote *a*, Table I. [b]The *m/z* detected was <0.1 in relative abundance.

fragment-ion. The spectra of tri-β-glucopyranosylalditols **8**, **9**, and **10** (which had glucopyranosyl residue c attached to O-6, O-4, and O-2, respectively, of residue b) contained aJ_1 fragment-ions of relatively high abundance and aJ_0 fragment-ions of very low abundance.

The e.i.-mass spectra of tri-β-glucopyranosylalditols **7** and **12**, which had glucopyranosyl residue d attached to O-3 of residue c, contained relatively abundant abJ_0 fragment-ions of *m/z* 514 and 513, respectively (see Table II). No abJ_1 fragment-ion was observed in the spectra of compounds **7** and **12**. Comparable abJ_0 fragment-ions of very low abundance were seen in the spectra of compounds **9** and **10**, in which residue d was attached to O-4, and O-2, respectively, of residue c. The e.i.-mass spectra of compounds **8** and **11**, in which residue d was attached to O-6

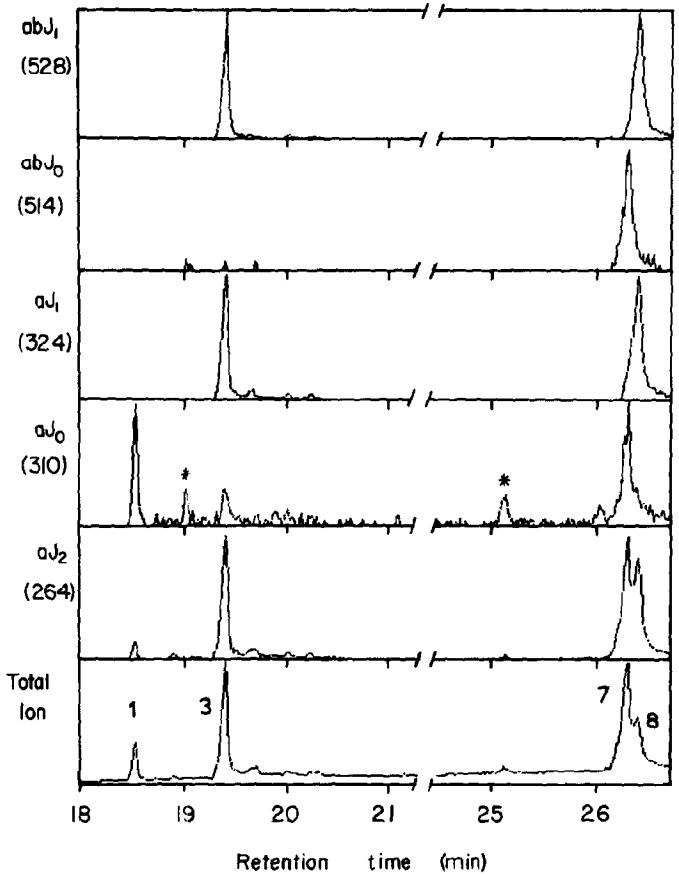


Fig. 2. Selected-ion profiles of a g.l.c.–mass spectrogram of compounds **1**, **3**, **7**, and **8**. The fragment-ions profiled are shown on the *y* axis and their *m/z* values are in parentheses. Asterisks indicate peaks due to weak per-*O*-alkylated oligosaccharide-alditols of undetermined structure. The selected-ion profile of the aJ_2 fragment-ion is added for comparison.

TABLE III

RATIOS OF THE J_0/J_1 FOR THE PER-*O*-ALKYLATED β-GLUCOPYRANO-TRI- AND -TETRASACCHARIDE-ALDITOLS

Compound	aJ_0/aJ_1	abJ_0/abJ_1
1	5.5	
2	∞	
3	0.02	
4	0 02	
5	0.2	
6	9.5	
7	∞	∞
8	0.02	0
9	0.04	0.2
10	0.2	0
11	∞	0
12	0.03	∞

and O-4 of residue c, respectively, had no detectable abJ_0 fragment-ion. The e.i.-mass spectra of compounds **8**, **9**, **10**, and **11** possessed, again as expected, relatively abundant abJ_1 fragment-ions.

Selected-ion profiling of a chromatogram can be used to determine whether the observed abundance of a J_0 fragment-ion is indicative of the presence of a 3-linked glucopyranosyl residue. For instance, selected-ion profiling of per-*O*-alkylated di-β-glucopyranosylalditol **1** (see Fig. 2), which contained residue c linked to O-3 of residue 6, showed a strong aJ_0 at *m/z* 310 and no detectable aJ_1 fragment-ion. This suggested that the *m/z* value corresponding to an aJ_1 fragment-ion in its spectrum was due to the background. A similar conclusion can be drawn from the selected-ion profiling of per-*O*-alkylated tri-β-glucopyranosylalditol **7**, which has both residues d and c linked to O-3 of c and b, respectively. Both the abJ_0 and aJ_0 fragment-ions of *m/z* 514 and *m/z* 310, respectively, are clearly present, but the abJ_1 or aJ_1 fragment-ions, at *m/z* 528 and *m/z* 324, respectively, cannot be detected above the background. Weak aJ_0 fragment-ions of *m/z* 310 were observed in the selected-ion profiles of per-*O*-alkylated di- and tri-β-glucopyranosylalditols **3** and **8**, both of which had residue c linked to O-6 of residue b, suggesting that the aJ_0 ion may be a weak component of these spectra. However, the strong abJ_1 or aJ_1 fragment-ion, or both, of *m/z* 528 and *m/z* 324, respectively, clearly show(s) that these are per-*O*-alkylated oligoglucosides having no 3-linked glucopyranosyl residues. Two other peaks in the profile of *m/z* 310 (see Fig. 2, asterisks) were due to small amounts of per-*O*-alkylated oligosaccharides of undetermined structure, although some evidence indicated that they, too, contained 3-linked glucosyl residues (data not shown). In conclusion, selected-ion profiling can be used to compare the J_0 and J_1 fragment-ions in determining the presence of 3-linked glucopyranosyl residues in per-*O*-alkylated gluco-oligosaccharide-alditols.

The ratio of the abundances of the *m/z* value of a J_0 *vs.* a J_1 fragment-ion in the e.i.-mass spectra of the per-*O*-alkylated glucopyrano-oligosaccharide-alditols is

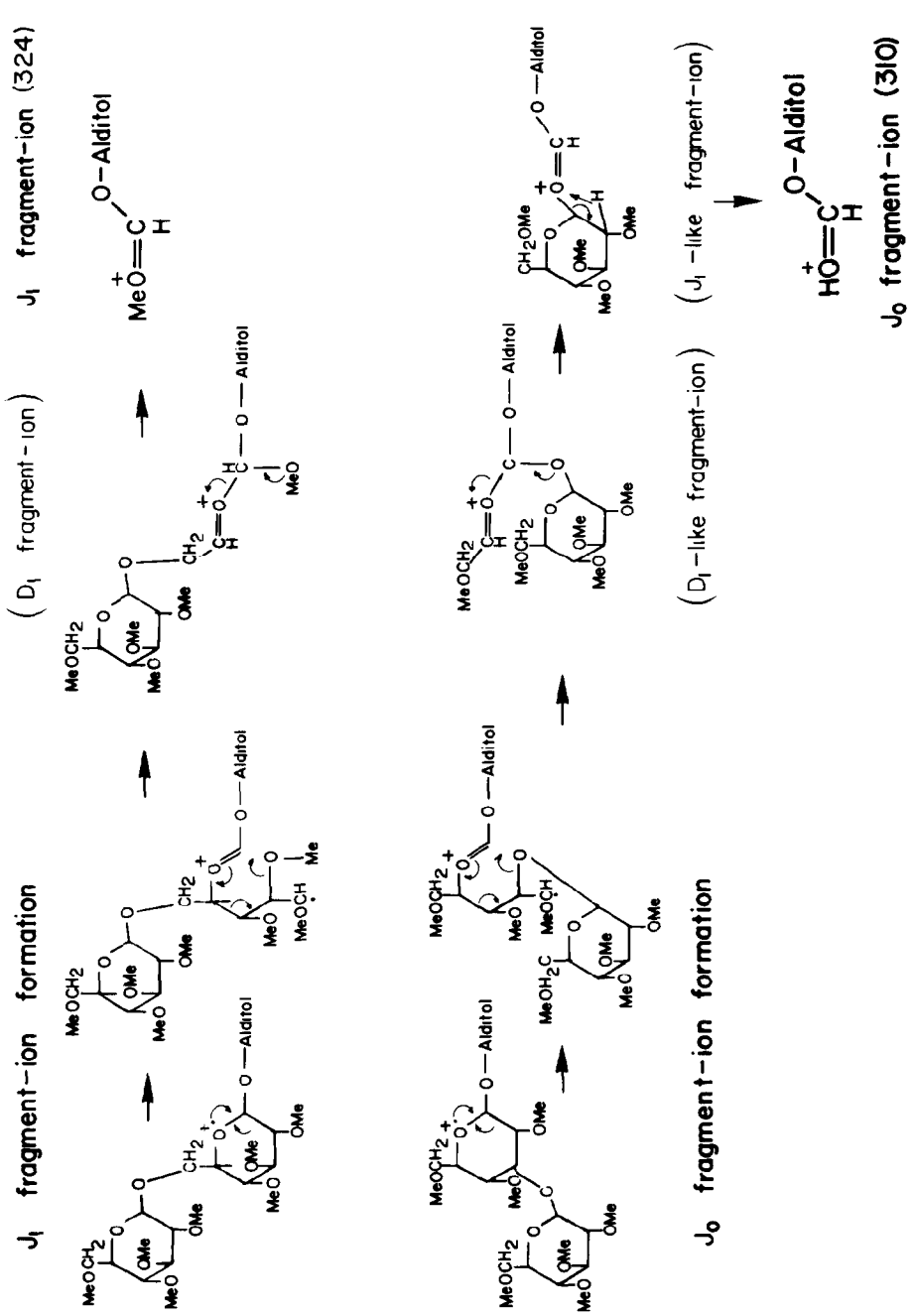


Fig. 3. A possible mechanism for the formation of the J_0 fragment-ion. The alditol is the same as that represented in Fig. 1, with a molecular weight of 264. The first scheme is the formation of the J_1 fragment-ion in a per-*O*-alkylated glucopyrano-trisaccharide-alditol in which residue c is linked to O-6 of residue b (modified from refs. 1 and 4). The second scheme is a possible mechanism for the formation of the J_0 in a per-*O*-alkylated glucopyrano-trisaccharide-alditol in which residue c is attached to O-3 of residue b (see text).

the best available indication of the presence of a 3-linked glucopyranosyl residue (see Table III). The aJ_0/aJ_1 ratio is greater than unity (actually, ≥ 5.5) in the e.i.-mass spectra of the per-*O*-alkylated glucopyrano-oligosaccharide-alditols having residue c linked to O-3 of residue b (**1**, **2**, **6**, **7**, and **11**, see Table III). Similarly, the abJ_0/abJ_1 ratio of per-*O*-alkylated glucopyrano-oligosaccharide-alditols **7** and **12**, in which residue d is linked to O-3 at residue c, is also greater than unity (actually, ∞). In contrast, the aJ_0/aJ_1 and abJ_0/abJ_1 ratios are <1 for all of the glucopyranosyl residues that are not 3-linked (actually, <0.2).

The formation of the J_0 fragment-ion may occur in a manner similar to that of the J_1 fragment-ion[1,4] (see Fig. 3). First, a D_1-like fragment-ion might be formed, followed by the transfer to C-1 of the glycosyl residue on C-3 in the same way that an alkyl ether is transferred in the formation of a J_1 fragment-ion (see Fig. 3)[1,4]. The J_1-like fragment-ion may rearrange[14] to form the J_0 fragment-ion. The formation of the J_0 fragment-ion may be facilitated by the alditol, because no comparable fragment-ion was reported in e.i.-mass spectra of per-*O*-alkylated oligosaccharide methyl glycosides having 3-linked glycopyranosyl residues[15].

In summary, a previously unreported, e.i.-m.s. fragment-ion (the J_0 fragment-ion) with an *m/z* equivalent to 46 amu greater than the *m/z* of the J_2 fragment-ion was observed for di- and tri-β-glucopyranosylalditols containing 3-linked glucopyranosyl residues. J_0 fragment-ions have also been observed in the spectra of di- and tri-glycosylalditols having α-linked hexosyl residues as well as for 3-linked deoxyhexosyl and pentosyl residues[17]. Therefore, in the e.i.-mass spectrum of a per-*O*-alkylated oligosaccharide-alditol, the presence of a J_0 fragment-ion, combined with the absence of a corresponding J_1 fragment-ion, provides positive evidence for the presence of a 3-linked glycopyranosyl residue.

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