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ELIMINATION OF INTERNAL GLYCOSYL RESIDUES DURING CHEMICAL IONIZATION-MASS SPECTROMETRY OF PER-*O*-ALKYLATED OLIGOSACCHARIDE-ALDITOLS*

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ABSTRACT

Glycosyl-residue elimination occurred in per-*O*-alkylated oligosaccharide-alditols during chemical ionization-mass spectrometry. This elimination produced fragment ions equivalent in mass to an (M + 1) ion of a per-*O*-alkylated oligosaccharide-alditol containing one less glycosyl residue than the parent per-*O*-alkylated oligosaccharide-alditol. Labeling experiments determined that the elimination fragment-ion was formed from protonated-parent, per-*O*-alkylated oligosaccharide-alditols by the loss of the internal glycosyl residue that was directly linked to the alditol. Some of the fragment ions produced by the glycosyl elimination underwent further glycosyl-residue eliminations.

INTRODUCTION

Chemical ionization-mass spectrometry (c.i.-m.s.) is a useful method for determining the molecular weight and other structural features of per-*O*-alkylated oligosaccharide-alditols[1–4]. Mixtures of per-*O*-alkylated oligosaccharide-alditols are often produced during the structural characterization of complex carbohydrates[2,3,5]. The per-*O*-alkylated oligosaccharide-alditols are frequently separated by liquid chromatography (l.c.), and detected and analyzed by c.i.-m.s.[2]. The per-*O*-alkylated oligosaccharide-alditols may also be separated by gas–liquid chromatography (g.l.c.), and analyzed by electron impact-mass spectrometry (e.i.-m.s.)[2,5], as well as by c.i.-m.s.[3]. Understanding the formation of the fragment ions in c.i. mass spectra of oligosaccharide-alditols provides information of value in characterizing the alditols. The formation is now described of diagnostic fragment-ions that, if incorrectly interpreted, could probably lead to incorrect structural assignments, but, when interpreted correctly, could provide valuable information regarding the sequence.

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EXPERIMENTAL

Preparation of per-O-alkylated oligosaccharide-alditols.— Compound **13** (ref. 6); compounds **7**, **8**, **14**, and **15** (ref. 7); compounds **4** and **5** (ref. 8); and compound **16** (ref. 9) were prepared as described. Compound **1** was prepared by the reduction ($NaBH_4$) of maltotriose (Sigma) and per-*O*-methylation of the resulting maltotriitol. Preparation of compound **2** (from starch, Merck), compounds **3** and **6** (from nigeran, Koch–Light), compounds **9** and **12** (from dextran, Pharmacia), compound **10** (from pustulan, Calbiochem), and compound **11** [from β-(1→2)-linked D-glucan[10]] was accomplished by methylation, partial hydrolysis with acid, reduction, ethylation, and purification by g.l.c. or l.c., as previously described[7]. The mixture of per-*O*-methylated methyl α- and β-maltotriosides was prepared by methylation of maltotriose.

Isobutane c.i.-m.s. — All spectra reported in Tables I–III were obtained by isobutane c.i.-m.s., using a Hewlett–Packard 5985 g.l.c.–m.s. system, unless otherwise noted. The compounds were introduced into the mass spectrometer by g.l.c., using a DB-1 durabond fused-silica capillary column (0.32 mm × 30 m, J and W Scientific). A solution (0.5 μL) of sample (200–700 ng) in 1:4 acetone–decane was injected by use of a Hewlett–Packard on-column injector. The g.l.c. was programmed at 150° for 2 min, followed by a rise of 30°/min to 220°, and finally a rise of 6°/min to 350°. Isobutane was introduced into the source at a pressure of ~93.3 Pa (~0.7 torr). The source temperature was 145°.

Acetonitrile–water l.c.–c.i.-m.s. — Some of the c.i.-mass spectra (of compounds **1**, **13**, **14**, and **15**) were obtained during l.c.–m.s. analysis, performed as described[2,6]. The source temperature was maintained at 165°. Water and acetonitrile were the ionization gases. (These conditions usually result in less fragmentation than during isobutane c.i., probably because of the cooling that occurs when the jet containing the acetonitrile–water, l.c.-column eluant is vaporized.)

RESULTS AND DISCUSSION

C.i.-mass spectra of oligosaccharide-alditols. — The formation of most of the major ions produced during c.i.-m.s. of per-*O*-alkylated oligosaccharide-alditols has been reported[1–4]. The formation of the major ions J_2 (OH_2), A_1, and (M + 1) for a typical per-*O*-alkylated oligosaccharide-alditol (compound **4**) is illustrated in Fig. 1. The process leading to the formation of the glycosyl-residue-elimination fragment-ion (*m/z* 516) is also shown in Fig. 1, and is substantiated by the data presented next.

Possible fragmentation theories that do not explain the observed glycosyl-residue-elimination fragment-ions. — The glycosyl-residue-elimination fragment-ions are not produced by loss of the nonreducing (nonalditol terminal) glycosyl group and migration of an alkyl or alkoxyl group, as had been proposed[1,4,6]. This loss–migration theory was shown to be incorrect by the *m/z* values of the glycosyl-elimi-

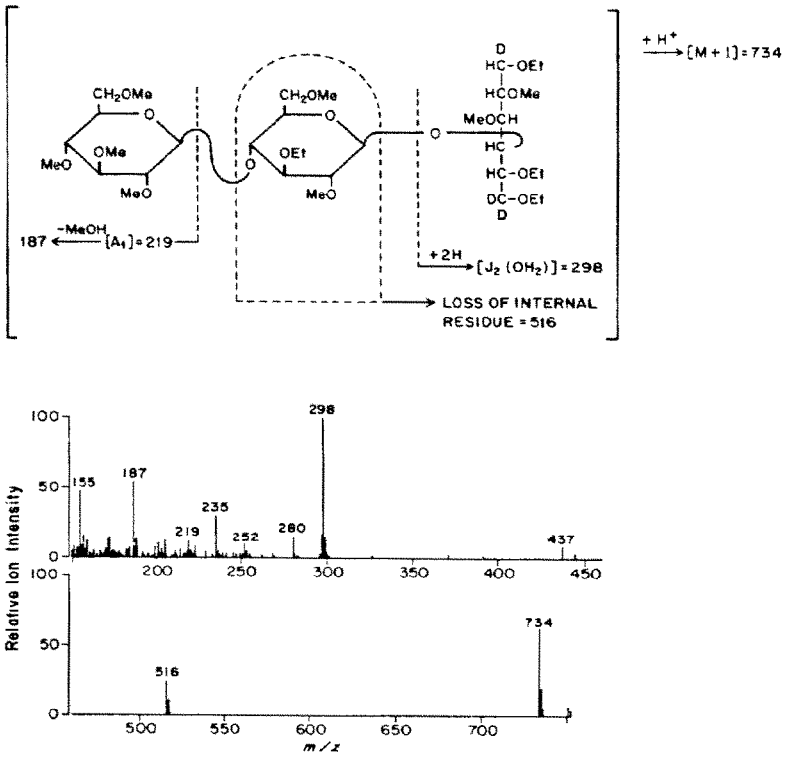

Fig. 1. The isobutane c.i. mass spectrum of per-*O*-alkylated trisaccharide-alditol **4** (see Table I). [The m.s. conditions are detailed in the Experimental section.]

nation fragment-ions of, variously, methyl, ethyl, and deuterium-substituted per-*O*-alkylated oligosaccharide-alditols (see Table I). If the elimination occurred by such a process, the fragment ions of both compounds **2** and **3** would involve an ethyl or an ethoxyl shift, because both compounds yielded a fragment ion at *m/z* 514, not at *m/z* 500 (*m/z* 500 is the result of a methyl or a methoxyl shift). However, it is very probable that, for either compound **2** or **3**, the fragment ion would correspond to a methyl or methoxyl migration, because the *O*-ethyl groups on the terminal (nonalditol) glycosyl group in compounds **2** or **3** were in different positions. Conclusive evidence against this theory is presented in the *m/z* value of the fragment ion of compound **4**. For this compound, loss of the nonreducing (nonalditol

TABLE I

THE *m/z* VALUES OF THE (M + 1) IONS AND THE GLYCOSYL-RESIDUE-ELIMINATION FRAGMENT-IONS FORMED DURING C.I.-M.S. OF SOME PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED TRISACCHARIDE-ALDITOLS

Compound		*(M+1)*[b] (m/z)	*Glycosyl-residue-elimination fragment-ion* (m/z)	*Difference between (M+1) and glycosyl-residue-elimination fragment-ion masses*	*Mass*[c] *of the internal glycosyl residue*
Number	*Formula*[a]				
1[d,e]	Glc$\xrightarrow{\alpha}$4Glc$\xrightarrow{\alpha}$4Glucitol	675 (100)	471 (30)	204	204
2[f]	*Et*→4Glc$\xrightarrow{\alpha}$4Glc$\xrightarrow{\alpha}$4Glucitol 5 1 ↑ ↑ Et Et	718 (60)	514 (40)	204	204
3[f]	*Et*→3Glc$\xrightarrow{\alpha}$4Glc$\xrightarrow{\alpha}$4Glucitol 5 1 ↑ ↑ Et Et	718 (20)	514 (17)	204	204
	Glc$\xrightarrow{\beta}$4Glc$\xrightarrow{\beta}$4Glucitol 3 6 5 1 ↑ ↑ ↑ ↑ Et Et Et Et	734 (75)	516 (30)	218	218
	Et→Glc$\xrightarrow{\beta}$4Glc$\xrightarrow{\beta}$4Fucitol 3 6 5 1 ↑ ↑ ↑ ↑ Et Et Et Et	718 (25)	498 (30)	220	220

[a]The positions of the glycosidic linkages and the *O*-ethyl groups are indicated. The remaining hydroxyl groups are methylated. [b]The relative abundance of the ions in % of base peak is in parentheses; when neither the (M + 1) ion nor the fragment ion is the base peak, the $J_2(OH_2)$ ion is the base peak (see Fig. 1). [c]The mass of the internal glycosyl residue, calculated as shown in Fig. 1. [d]The alditol was formed by reduction with $NaBH_4$, and contains only hydrogen atoms on C-1. [e]Chemical-ionization mass spectra obtained by l.c.–m.s. (see Experimental). [f]The alditol was formed by reduction with $NaBD_4$, and contains one deuterium atom on C-1. [g]The alditol has two deuterium atoms attached to C-6. [h]The internal glycosyl residue has two deuterium atoms attached to C-6.

terminal) glycosyl group would be accompanied by the migration of a methyl or methoxyl group, because this terminal glycosyl group had no *O*-ethyl substituents. Such loss and migration would yield an ion at *m/z* 530, instead of the observed ion at *m/z* 516.

Another plausible, but incorrect, explanation for the formation of the glycosyl-residue-elimination fragment-ion is that only part of the terminal glycosyl group was lost, and a fragment of it remained attached to the internal glycosyl residue.

TABLE II

THE *m/z* VALUES AND RELATIVE INTENSITIES OF THE (M + 1) IONS AND THE GLYCOSYL-RESIDUE-ELIMINATION FRAGMENT-IONS OF SOME DIFFERENTLY LINKED, PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED TRIGLUCOSIDE-ALDITOLS

Compound		*(M+1) ion*[b] (m/z)	*Glycosyl-residue-elimination fragment-ion* (m/z)	*Relative abundance of (M+1) ion divided by relative abundance of glycosyl-residue-elimination fragment-ion*
Number	*Formula*[a]			
6[c]	Et→4Glc→3Glc→4Glucitol (5 1; ↑ ↑; Et Et)	718 (30)	514 (1)	30
7[d]	Et→4Glc→3Glc→4Glucitol (5 1; ↑ ↑; Et Et)	717 (8)	513 (0.2)	40
3[c]	Et→3Glc→4Glc→4Glucitol (5 1; ↑ ↑; Et Et)	718 (20)	514 (17)	1.2
8[d]	Et→3Glc→4Glc→4Glucitol (5 1; ↑ ↑; Et Et)	717 (40)	513 (30)	1.3
9[c]	Et→6Glc→6Glc→6Glucitol (5 1; ↑ ↑; Et Et)	718 (80)	514 (90)	0.9
10[c]	Et→6Glc→6Glc→6Glucitol (5 1; ↑ ↑; Et Et)	718 (80)	514 (100)	0.8
11[c]	Et→2Glc→2Glc→2Glucitol (5 1; ↑ ↑; Et Et)	718 (3)	514 (2)	1.5

[a]The positions of the glycosidic linkages and the *O*-ethyl groups are indicated. The remaining hydroxyl groups are methylated. [b]The relative abundance of the ions in % of base peak is in parentheses; when neither of the peaks is listed at 100, the base peak is the $J_2(OH_2)$ ion (see Fig. 1). [c]The alditol was formed by reduction with $NaBD_4$, and contains one deuterium atom at C-1. [d]The alditol was formed by reduction with $NaBH_4$, and contains only hydrogen atoms at C-1.

This partial-elimination process could explain why the fragment ions of compounds **2** and **3** had the same *m/z* value. However, if this theory were correct, the *m/z* values of the glycosyl-residue-elimination fragment-ions of compounds **1** and **4** would be *m/z* 485 and *m/z* 530, respectively, not the *m/z* 471 and *m/z* 516 values seen (see Table I).

The fragmentation pathway resulting in the observed glycosyl-residue-elimination fragment-ion — The glycosyl-residue-elimination fragment-ion was formed by loss of an internal glycosyl residue. This was demonstrated by a comparison of the masses of the glycosyl-residue-elimination fragment-ions and the masses of the internal glycosyl residues. The mass of the internal glycosyl residue (calculated as shown in Fig. 1) was compared with the differences between the (M + 1) ion mass and the mass of the glycosyl-elimination fragment-ion for five differently labeled compounds (see Table I). The masses were identical in all cases. Most convincingly, compound **5** contained two deuterium atoms on C-6 of the internal glycosyl residue, and the masses of these deuterium atoms were not present in the fragment ion.

Effect of the linkage position and anomeric configuration of the internal glycosyl residue on the formation of the glycosyl-residue-elimination fragment-ion. — Appropriate, per-*O*-alkylated trisaccharide-alditols were prepared, in order to investigate the effect of the anomeric configuration and the linkage position of the internal glycosyl residue on the formation of the glycosyl-residue-elimination fragment-ion. The results (see Table II) showed that the anomeric linkage of the internal glycosyl residue appeared to have little effect on the formation of the glycosyl-residue-elimination fragment-ion (see compounds **6** *vs.* **7**, **3** *vs.* **8**, and **9** *vs.* **10**). However, the oxygen atom to which the internal glycosyl residues had a glycosyl group attached did affect the relative abundance of the fragment ion. When the internal glycosyl residue had a glycosyl group attached at O-3, the relative abundance of the glycosyl-residue-elimination fragment-ion decreased dramatically (compounds **6** and **7** *vs.* **3**, **8**, **9**, **10**, and **11**)*.

Glycosyl-residue-elimination during c.i.-m.s. of per-O-alkylated tetrasaccharide-alditols. — Several per-*O*-alkylated tetrasaccharide-alditols were found to produce glycosyl-residue-elimination fragment-ions. The glycosyl-residue elimination can occur twice in per-*O*-alkylated tetrasaccharide-alditols—first, yielding an ion corresponding in *m/z* value to the (M + 1) ion of a per-*O*-alkylated trisaccharide-alditol, and second, yielding an ion corresponding in *m/z* value to the (M

*Although it is useful to compare the ratio of the intensity of the (M + 1) ion to that of the glycosyl-residue-elimination fragment-ion, this comparison must be made with care. This ratio was very dependent on the source temperature, at higher source-temperatures, the ratio decreased. The intensities of both the rearrangement ion and the (M + 1) ion varied in comparison to the $I_2(OH_3)$ ion, depending on both the source temperature and the structure of the compound. The source temperature was maintained at 145° for the experiments whose results are shown in Table II. However, the effect of the linkage at O-3 of the internal glycosyl residue was so profound (see Table II) that it was noticeable at any source temperature; indeed, in weak spectra, the glycosyl-elimination fragment-ion was not detected.

TABLE III

THE m/z VALUES OF THE (M + 1) IONS AND THE GLYCOSYL-RESIDUE-ELIMINATION FRAGMENT-IONS FORMED DURING C.I.-M.S. OF SOME PARTIALLY *O*-METHYLATED, PARTIALLY *O*-ETHYLATED TETRASACCHARIDE-ALDITOLS

Compound		*(M + 1)* (m/z)	*Glycosyl-residue-elimination fragment-ions* (m/z)	
Number	*Formula*[a]			
12[c]	Et→6Glc$\xrightarrow{\alpha}$6Glc$\xrightarrow{\alpha}$6Glc$\xrightarrow{\alpha}$6Glucitol (5 ↑, 1 ↑; Et, Et)	922 (3)	718 (4)	514 (3)
13[c,d]	Et→3Glc$\xrightarrow{\beta}$6Glc$\xrightarrow{\beta}$6Glc$\xrightarrow{\beta}$4Glucitol (4 ↑, 5 ↑, 1 ↑; Et, Et, Et)	936 (100)	718 (25)	514 (20)
14[d,e]	Et→4Glc$\xrightarrow{\beta}$3Glc$\xrightarrow{\beta}$4Glc$\xrightarrow{\beta}$4Glucitol (5 ↑, 1 ↑; Et, Et)	921 (100)	717 (40)	513 (1)
15[d,e]	Et→4Glc$\xrightarrow{\beta}$4Glc$\xrightarrow{\beta}$3Glc$\xrightarrow{\beta}$4Glucitol (5 ↑, 1 ↑; Et, Et)	921 (100)	717 (3)	513 (1)
16[c]	Et→4Gal$\xrightarrow{\beta}$3Glc$\xrightarrow{\beta}$4Glc$\xrightarrow{\beta}$4Glucitol (6 ↑, 6 4 ↑ ↑, 5 ↑, 1 ↑; Et, Et Et, Et, Et)	964 (20)	760 (30)	528 (1)

[a]The positions of the glycosidic linkages and the *O*-ethyl groups are indicated. The remaining hydroxyl groups are methylated. [b]The relative abundance of the ions in % of base peak is in parentheses; when neither of the peaks is listed at 100, the base peak is the $J_2(OH_2)$ ion (see Fig. 1). [c]The alditol was formed by reduction with $NaBD_4$, and contains one deuterium atom at C-1. [d]Chemical-ionization mass spectra obtained by l.c.–m.s. (see Experimental). [e]The alditol was formed by reduction with $NaBH_4$, and contains only hydrogen atoms at C-1.

+ 1) ion of a per-*O*-alkylated disaccharide-alditol. Thus, compound **11** (see Table III) had an (M + 1) ion at m/z 922, and yielded glycosyl-residue-elimination fragment-ions of relative abundances similar to those of the (M + 1) ion at m/z 718 and m/z 514. (In compound **11**, the $J_2(OH_2)$ ion dominated the spectra; thus, the (M + 1) ion and the glycosyl-residue-elimination fragment-ions were found in smaller proportions.) The glycosyl-residue-elimination fragment-ions were also observed in the spectra of per-*O*-alkylated pentasaccharide-alditols and in those of even larger oligosaccharides containing as many as seven residues[11], although the formation of these glycosyl-residue-elimination fragment-ions was not then understood.

Order of elimination of internal glycosyl residues. — Only the internal glyco-

syl residue directly attached to the alditol was eliminated during c.i.-m.s. This was shown by the *m/z* values of the glycosyl-residue-elimination fragment-ions of compound **13**. In this compound, the two internal glycosyl residues have different masses. The 4-*O*-ethylated, 6-linked D-glucosyl residue, which is directly attached to the alditol, had a mass of 218 (calculated as in Fig. 1) and was eliminated first, yielding a fragment ion at *m/z* 718. The 6-linked D-glucosyl residue attached to the 4-*O*-ethylated, 6-linked D-glucosyl residue had a mass of 204 (calculated as in Fig. 1), and, had it been eliminated first, it would have yielded a fragment ion having an *m/z* value of 732, but this ion was not detected. Instead, the non-*O*-ethylated, 6-linked D-glucosyl residue became directly attached to the alditol in the first glycosyl-residue-elimination fragment-ion (*m/z* 718), and was subsequently eliminated, to yield a second glycosyl-elimination fragment-ion at *m/z* 514.

The results with compounds **14** and **15** confirmed this order of elimination. Each compound contains a 3-linked internal and a 4-linked internal glycosyl residue, but, in compound **14**, the 4-linked internal glycosyl residue is directly attached to the alditol, whereas, in compound **15**, it is the 3-linked internal glycosyl residue that is directly attached to the alditol. Three-linked glycosyl residues are rarely eliminated (see Table II). Thus, compound **14** should yield a more-abundant, glycosyl-residue-elimination fragment-ion at *m/z* 717 than compound **15** if only the glycosyl residue attached to the alditol were eliminated. Examination of the data presented in Table III showed this to be the case.

The order of glycosyl-residue-elimination was additionally confirmed by analysis of the spectrum of compound **16**. In this compound, the glycosyl residue attached to the alditol has a mass of 204 (calculated as in Fig. 1), whereas the remaining glycosyl residue has a mass of 232. The only glycosyl-residue-elimination fragment-ion corresponding to a protonated, per-*O*-alkylated trisaccharide-alditol was found at *m/z* 760 [loss of 204 from the (M + 1) ion]. No glycosyl-residue-elimination fragment-ion corresponding to the loss of 232 from the (M + 1) ion was found, although an ion corresponding to the loss of 232 from the glycosyl-residue-elimination fragment-ion at 760 (*m/z* 528) was detected. This ion was found at a low relative abundance, and agreed with the fact that the glycosyl residue that was eliminated to form this ion was 3-linked.

Glycosyl-residue-elimination fragment-ions produced during c.i.-m.s. of per-O-methylated methyl glycosides of oligosaccharides. — The glycosyl-residue-elimination process also occurred during c.i.-m.s. of per-*O*-methylated methyl maltotrioside (a mixture of α and β anomers). The ratios of the (M + 1) ion (*m/z* 659) to the first glycosyl-residue-elimination fragment-ion (*m/z* 455) to the second glycosyl-elimination fragment-ion (*m/z* 251) were 2:5:1, at a source temperature of 145°. Thus, a per-*O*-alkylated hexitol was not required for occurrence of the glycosyl-residue-elimination fragment-ion.

CONCLUSION

The glycosyl-residue-elimination process shown by these experiments is important for two reasons. First, it helps to determine the sequence of glycosyl residues of differing masses in per-*O*-alkylated oligosaccharide-alditols. Second, awareness of the existence of the glycosyl-residue-elimination fragment-ion is necessary in order to avoid misinterpreting c.i. mass spectra. Because the mass of the glycosyl-residue-elimination fragment-ion corresponds to the mass of (M + 1) ions of smaller per-*O*-alkylated oligosaccharide-alditols, the fragment ions are readily mistaken for the (M + 1) ions of oligosaccharide-alditols that do not actually exist in the sample being analyzed. This is especially true when mass spectrometers having limited mass ranges are used. Thus, with a mass spectrometer having a high-mass detection-limit of *m/z* 1000, the (M + 1) ion of a per-*O*-alkylated pentasaccharide-alditol cannot be detected. However, the glycosyl-residue-elimination fragment-ion corresponding to a per-*O*-alkylated tetrasaccharide-alditol could be detected, and it could readily be confused with the (M + 1) ion of a per-*O*-alkylated tetrasaccharide-alditol. Such confusion can be eliminated by use of retention times during g.l.c.–c.i.-m.s. analysis, because per-*O*-alkylated di-, tri-, tetra-, and penta-saccharide-alditols are eluted in separate groups. However, in l.c.–m.s., per-*O*-alkylated oligosaccharide-alditols are not separated into distinct size-groups[2]; thus, retention times therein give no useful data on the size of the per-*O*-alkylated oligosaccharide-alditol.

The results presented herein show clearly that elimination fragment-ions are formed during c.i.-m.s. of per-*O*-alkylated oligosaccharide-alditols by the loss of the internal glycosyl residue that is glycosidically linked directly to the alditol. This glycosyl-residue-elimination fragment-ion process was unexpected, and no detailed mechanism or chemical rationalization for it is here proposed.

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