STRUCTURAL ANALYSIS OF TRISACCHARIDES AS PERMETHYLATED TRISACCHARIDE ALDITOLS BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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(Received June 18th, 1970; accepted for publication, July 24th, 1970)

ABSTRACT

The separation and structural analysis of 21 trisaccharides as their permethylated trisaccharide alditols by gas–liquid chromatography (g.l.c.) and gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) is described. Most of the alditol derivatives could be separated from one another by g.l.c. with SE-30 or OV-22 stationary phases.

The molecular weights of the monosaccharide units and their sequence if the masses are different, as well as the presence of chain branching, are disclosed by analysis with g.l.c.–m.s. The position of the glycosidic linkage next to the alditol unit in straight-chain trisaccharide alditol derivatives can be established by m.s. if the reduction is carried out with borodeuteride; (1→3)- or (1→2)-linkages next to the non-reducing end can also be readily identified, whereas differentiation of (1→6)- and (1→4)-linkages in this position may require previous knowledge of the stereochemistry of the sugar units.

Analysis as alditols instead of the parent sugars simplifies g.l.c. analyses of trisaccharide mixtures because of the absence of peaks due to different anomers. The interpretation of the mass spectra of methylated trisaccharide alditols is generally more straightforward, and therefore the structural conclusions are somewhat more reliable than when the methyl glycosides are employed.

INTRODUCTION

G.l.c. and combined g.l.c.–m.s. of permethylated methyl glycosides of trisaccharides have been shown recently to afford an efficient method for their separation and for their partial structural assignment[1]. The analysis can, however, be simplified if the trisaccharides are converted into the corresponding alditols before methylation. As a result, each trisaccharide forms only one peak in g.l.c. The structurally very similar alditol units, which are formed from saccharides containing (1→3)- and (1→4)-linkages at the reducing end, can be distinguished by m.s. if reduction is effected with borodeuteride[2,3].

In this work the g.l.c.–m.s. analysis of the 21 trisaccharides previously studied as permethylated methyl glycosides[1] is presented. Examples of straight-chain trisaccharides having all of the 9 possible combinations of (1→6)-, (1→4)-, and

(1→3)-linkages are included, in addition to a few miscellaneous trisaccharides containing (1→2)-linkages or pentoses, or possessing a branched-chain structure.

MATERIALS AND METHODS

The following trisaccharides were studied:

O-β-D-Glc*p*-(1→6)-*O*-β-D-Glc*p*-(1→6)-D-Glc, gentiotriose (**1**);
O-α-D-Gal*p*-(1→6)-*O*-α-D-Gal*p*-(1→6)-D-Glc, manninotriose (**2**);
O-β-D-Gal*p*-(1→6)-*O*-β-D-Gal*p*-(1→6)-D-Gal (**3**);
O-α-D-Glc*p*-(1→6)-*O*-α-D-Glc*p*-(1→4)-D-Glc, panose (**4**);
O-β-D-Gal*p*-(1→6)-*O*-β-D-Gal*p*-(1→4)-D-Glc (**5**);
O-β-D-Glc*p*-(1→6)-*O*-β-D-Glc*p*-(1→3)-D-Glc (**6**);
O-α-D-Glc*p*-(1→4)-*O*-α-D-Glc*p*-(1→6)-D-Glc (**7**);
O-α-D-Glc*p*-(1→4)-*O*-α-D-Glc*p*-(1→4)-D-Glc, maltotriose (**8**);
O-β-D-Gal*p*-(1→4)-*O*-β-D-Gal*p*-(1→4)-D-Glc (**9**);
O-β-D-Man*p*-(1→4)-*O*-β-D-Man*p*-(1→4)-D-Man (**10**);
O-β-D-Glc*p*-(1→4)-*O*-β-D-Glc*p*-(1→3)-D-Glc (**11**);
O-β-D-Glc*p*-(1→3)-*O*-β-D-Glc*p*-(1→6)-D-Glc (**12**);
O-β-D-Glc*p*-(1→3)-*O*-β-D-Glc*p*-(1→4)-D-Glc (**13**);
O-β-D-Gal*p*-(1→3)-*O*-β-D-Gal*p*-(1→4)-D-Glc (**14**);
O-β-D-Glc*p*-(1→3)-*O*-β-D-Glc*p*-(1→3)-D-Glc, laminaritriose (**15**);
O-β-D-Glc*p*-(1→2)-*O*-β-D-Glc*p*-(1→4)-D-Gal, lycotriose (**16**);
O-β-D-Glc*p*-(1→2)-*O*-β-D-Glc*p*-(1→2)-D-Glc (**17**);
O-α-D-Man*p*-(1→2)-*O*-α-D-Man*p*-(1→2)-D-Man (**18**);
O-β-D-Gal*p*-(1→6)-*O*-β-D-Gal*p*-(1→3)-L-Ara (**19**);
O-β-D-Xyl*p*-(1→3)-*O*-β-D-Xyl*p*-(1→4)-D-Xyl (**20**);
and *O*-β-D-Glc*p*-(1→3)-*O*-[β-D-Glc*p*-(1→6)]-D-Glc (**21**).

The trisaccharides or compounds containing these trisaccharides were obtained as gifts from private investigators or puchased from commercial sources[1]. The samples (*ca.* 100 μg) were reduced with borohydride or borodeuteride, methylated with methyl iodide in the presence of methylsulphinyl carbanion, and analysed by g.l.c. or combined g.l.c.–m.s. as previously described[2,3] (see preceding paper).

Symbols A–J, employed by Kochetkov *et al.*[4], supplied with lower-case letters *a*, *b*, and *c* to designate the monosaccharide units[5], are used in discussing mass-spectral fragmentations. Thus, $abcJ_1$ stands for the first ion of the J series, arising through fragmentation of ring *a* (non-reducing end of the original trisaccharide) and being substituted by ring *b* and the alditol unit *c*. The intensities of the peaks in m.s. are expressed as percentages of the highest peak (base peak).

RESULTS AND DISCUSSION

Analysis by g.l.c. — G.l.c. analysis of the permethylated trisaccharide alditols is shown in Table I. The permethylated alditols are generally eluted from the columns

slightly more rapidly than the corresponding methylated methyl glycosides[1]. Most of the alditols were well-resolved from one another, and essentially no additional peaks were present in the chromatograms. G.l.c. of permethylated alditols seems therefore very suitable for the resolution, preliminary identification, and quantitative analysis of trisaccharide mixtures. In the case of overlapping peaks, approximate quantitative ratios can be determined by m.s., if the mass spectra of the compounds are different.

TABLE I

RELATIVE RETENTION TIMES OF PERMETHYLATED TRISACCHARIDE ALDITOLS IN G.L.C.

Compound	*Stationary phase*	
	2.2% SE-30 (260°)	*1% OV-22 (265°)*
1	1.43	1.33
2	1.40	1.69
3	1.92	2.42
4	0.96	0.92
5	1.20	1.39
6	1.04	0.87
7	1.32	1.41
8	1.00[a]	1.00[b]
9	1.00	1.04
10	1.36	1.52
11	1.18	1.03
12	1.64	1.56
13	1.09	0.95
14	1.17	1.23
15	1.14	1.01
16	0.98	0.80
17	0.99	0.83
18	0.69	0.67
19	0.90	0.97
20	0.52	0.42
21	1.19	1.03

[a]Retention time, 15 min. [b]Retention time, 9 min.

General characteristics of the mass spectra. — Table II shows the partial mass spectra of the permethylated alditols. Included are the 10 highest peaks of each spectrum plus peaks otherwise pertinent to the structural interpretations. The spectra of both deuterated and nondeuterated derivatives were recorded. The stationary phase used in g.l.c.–m.s. was SE-30.

The ion at *m/e* 88 (mainly H_1^2 according to Kochetkov *et al.*[4]) forms the base peak in the spectra of all the methylated trisaccharide alditols having a (1→6)- or a (1→4)-linkage between the *a* and *b* units. In these compounds, H_1^2 ions can arise from both *a* and *b* units, in contrast to the (1→2)- or (1→3)-linked alditols where only the *a* unit can form this ion. Therefore, the ion at *m/e* 88 has a smaller intensity in the respective spectra, and in only one case (**18**) forms the base peak.

TABLE II

PARTIAL MASS SPECTRA OF PERMETHYLATED TRISACCHARIDE ALDITOLS

m/e	1	2	3	4	5	6	7	8	9	10	11
45	29	35	46	38	41	34	42	49	51	49	43
71	34	35	56	35	43	28	32	38	36	26	26
75	25	26	37	33	32	30	32	35	37	28	28
88	100	100	100	100	100	100	100	100	100	100	100
89	26	36	32	28	32	24	28	34	33	26	32
101	51	62	74	58	67	49	52	64	59	42	50
103	8	6	7	5	5	4	5	4	3	2	2
111	18	23	26	30	31	19	27	41	39	19	33
115	13	14	32[a]	20	25	17[a]	19	31	28	29	34[a]
133	8[a]	7[a]	16	7[a]	8[a]	4	8[a]	9[a]	9[a]	6[a]	5
143	3	5	7	5	6	4	5	5	8	4	4
145	14[a]	14[a]	15[a]	6	6	5	16[a]	7	6	5	6
155	10	9	8	13	11	10	15	22	14	15	9
159	2	3	3	2	5	3	2	2	6	2	2
171	10[a]	10[a]	18[a]	10[a]	12[a]	9[a]	10[a]	14[a]	15[a]	17[a]	16[a]
175	1	1	1	1	1	1	1	1	1	1	1
177	4[a]	4[a]	2[a]	—	—	—	6[a]	—	—	—	—
187	34	32	35	63	40	37	57	35	49	38	61
191	—	—	—	—	—	—	—	—	—	—	—
201	1	1	1	1	1	—	1	—	2	1	1
203	3[a]	4[a]	4[a]	2[a]	2[a]	2[a]	4[a]	2[a]	3[a]	1[a]	2[a]
219	5	12	13	10	15	8	8	9	21	27	7
235	21[a]	22[a]	47[a]	27[a]	27[a]	17[a]	21[a]	40[a]	47[a]	35[a]	29[a]
251	—	—	—	—	—	—	—	—	—	—	—
295	5[a]	7[a]	2[a]	2[a]	1[a]	3[a]	4[a]	1[a]	3[a]	5[a]	6[a]
303	—	—	—	—	0.5	—	0.1	—	0.6	—	—
305	0.4	0.5	0.3	0.6	0.4	0.4	0.8	0.8	0.6	0.6	0.7
319	—	0.2	0.3	0.2[a]	0.2	—	—	—	0.3	0.5	0.6[a]
335	—	—	—	—	1.0	0.3[a]	0.1	0.7	0.4	0.4	0.5[a]
347	—	—	—	—	0.1	—	0.3	—	0.2	—	—
351	0.2	—	—	—	0.2	0.2[a]	—	—	—	—	—
359	0.2	—	0.1	0.2	0.2	0.3	0.3	0.3	0.1	0.6	0.3
363	—	—	—	—	—	—	—	—	0.1	—	0.2
365	—	0.2[a]	—	—	—	—	—	0.3[a]	—	0.2[a]	—
375	1.2[a]	0.2[a]	—	0.7	—	0.4[a]	0.3[a]	0.2[a]	0.8[a]	0.4[a]	0.2[a]
391	0.4	0.3	0.2	0.6	0.3	0.7	0.3	0.4	0.1	0.4	0.3
395	—	—	0.2	—	—	—	—	—	—	—	—
407	3.0[a]	0.3[a]	0.2[a]	3.6[a]	0.2[a]	2.1[a]	1.6[a]	0.6[a]	2.0[a]	0.7[a]	1.0[a]
411	—	—	—	—	—	—	—	—	—	—	—
423	—	0.2	0.2	0.2	0.5	0.3	0.6	1.0	0.3	0.3	0.4
439	0.8[a]	12[a]	14[a]	1.1[a]	13[a]	0.9[a]	2.1[a]	1.9[a]	0.4[a]	0.9[a]	1.0[a]
455	—	—	—	—	—	—	—	—	—	—	—
499	2.4[a]	0.2[a]	0.2[a]	2.0[a]	0.1[a]	0.9[a]	0.8[a]	0.4[a]	1.5[a]	0.5[a]	0.2[a]
541	0.5	0.5	0.5	—	—	—	0.2	—	—	—	—
585	—	—	—	0.1	0.2	0.4[a]	—	—	0.3	—	0.2[a]

[a]In the deuterated derivative, >50% of the ion is converted into the next higher *m/e*. [b]The symbol refers to and **20**, [f]For compound **19**.

12	13	14	15	16	17	18	19	20	21	*Symbol*
58	92	75	58	89	87	80	46	100	27	
69	50	76	48	48	71	64	45	92	25	
39	35	53	39	39	46	44	21	57	26	J_1
67	50	33	46	83	88	100	100	33	100	H_1
37	47	40	36	52	61	56	37	35[a]	22	
101	89	100	75	99	100	96	67	88	51	F_1
9	5	4	4	6	8	7	5	39	—	
50	60	67	54	58	57	35	16	18	26	
19	31	35	27[a]	28	30[a]	19	11	41	7[a]	
9[a]	9[a]	9[a]	6	12[a]	16	14	—	12[a]	—	
5	9	10	7	10	8	9	5	55	4	aA_2[b]
18[a]	8	8	9	13	27	26	4	9	5	
28	35	14	29	23	22	16	6	—	11	aA_3[c]
17	16	35	20	3	3	4	8[a]	12[a]	3	cA_2[d]
9[a]	9[a]	17[a]	11[a]	20[a]	18[a]	20[a]	—	—	5[a]	cA_3[e]
1	2	1	5	2	1	2	1	29	—	aA_1[b]
5[a]	—	—	—	—	2	3	—	—	—	
93	100	90	100	100	97	83	16	—	56	aA_2[c]
—	—	—	—	2	—	—	3[a]	66[a]	—	cA_1[d]
1	—	—	—	8	7	4	1	—	—	
2[a]	1[a]	3[a]	3[a]	3[a]	2[a]	2[a]	2	—	1[a]	cA_2[e]
12	14	38	13	13	10	54	5	—	8	aA_1[c]
18[a]	25[a]	40[a]	14[a]	32[a]	93[a]	79[a]	—	—	3[a]	cA_1[e]
—	—	—	—	—	—	—	16[a]	—	—	bcJ_1[d]
—	—	—	—	6[a]	1[a]	—	—	—	—	bcJ_1[e]
—	—	—	—	0.7	—	0.2	0.2[a]	0.6	0.2	baA_2[b]
—	—	0.2	0.2	1.0[a]	0.8	0.8	0.4	—	0.4	
—	—	—	0.3[a]	0.4[a]	0.2	—	0.2	0.9[a]	—	bcA_2[b]
—	0.3	0.5	0.3[a]	1.1	—	—	0.2[a]	1.1	—	baA_1[b]
0.8[a]	0.5[a]	0.5[a]	0.4[a]	0.5[a]	—	—	—	0.2	—	
—	—	—	—	—	—	—	0.2	0.2[a]	—	bcA_1[b]
1.3	2.5	1.1	1.8	0.9	0.7	0.4	0.1	—	—	baA_3[c]
—	—	—	—	—	—	—	0.3[a]	—	—	bcA_2[f]
1.0[a]	1.3[a]	0.2[a]	1.8[a]	0.3	0.3	0.5	—	0.4[a]	—	
0.7[a]	0.2[a]	—	0.2[a]	—	0.2[a]	0.3[a]	—	—	0.2[a]	bcA_3[e]
0.6	1.0	1.2	1.2	40	29	10	—	—	0.2	baA_2[c]
—	—	—	—	—	—	—	13[a]	—	—	bcA_1[f]
1.3[a]	0.9[a]	0.7[a]	0.9[a]	5.7[a]	1.4[a]	8.2[a]	—	—	0.6[a]	bcA_2[e]
—	—	—	—	—	—	—	—	1.0[a]	—	$abcJ_1$[b]
0.9	2.3	5.7	2.2	4.6	3.3	3.4	0.3	—	0.2	baA_1[c]
0.7[a]	0.6[a]	0.9[a]	0.8[a]	1.9[a]	1.8[a]	0.5[a]	—	—	3.0[a]	bcA_1[e]
—	—	—	—	—	—	—	0.1[a]	—	—	$abcJ_1$[f]
0.8[a]	0.3[a]	0.4[a]	0.4[a]	2.1[a]	1.3[a]	3.5[a]	—	—	2.2[a]	$abcJ_1$[e]
1.2	—	0.5[a]	0.1[a]	—	0.1[a]	0.1[a]	—	—	—	M−133[e]
—	0.6	0.5	0.4[a]	0.3	1.9[a]	0.6[a]	—	—	—	M−89[e]

compound **20**, [c]For all compounds except **20**, [d]For compounds **19** and **20**, [e]For all compounds except **19**

The major ions between *m/e* 40 and *m/e* 300 are the same as in the spectra of permethylated disaccharide alditols[3]. The ions baA_{1-3}, bcA_{1-3}, and $abcJ_1$ are the most prominent in the mass range from *m/e* 300 to *m/e* 500, the intensities being of the same order as those of the corresponding ions in the spectra of permethylated trisaccharides[1]. The relatively few ions above *m/e* 500 are those formed through the loss of 31, 89, or 133 mass units[3] from the molecular ion, sometimes coupled with a secondary loss of methanol. The molecular ion itself is not visible. The molecular weights of the monosaccharide units and the order of units of different molecular weight can easily be determined on the basis of the above ions.

The assignment of the position of the b→c *linkage.* — The position of the *b→c* linkage of straight-chain trisaccharide alditols can be determined in the same manner as with disaccharide alditols[3]. The ion *m/e* 177 (containing four carbon atoms of the alditol chain) and the increase of the intensity of the ion at *m/e* 145 (177−MeOH) are found only in the spectra of trisaccharide alditols having (1→6)- and (1→2)-linkages in this position. The shift of the ion at *m/e* 177 to 178 and of the ion at *m/e* 145 to 146 in the spectra of deuterated **1**, **2**, **3**, **7**, and **12** distinguish these (1→6)-linked compounds from the (1→2)-linked compounds **17** and **18**. The (1→6)- and (1→2)-linked trisaccharide alditols can also be differentiated on the basis of ions in the mass range above *m/e* 500 (Table II). Since compounds having (1→5)- and (1→2)-linkages next to the alditol are expected, by analogy with disaccharide alditols[6], to undergo similar fragmentations (but exhibiting different deuterium effects), all of these linkages when present in the *b→c* position can probably be distinguished from one another by m.s.

The ion at *m/e* 133 (containing three carbon atoms of the alditol chain) can be used to differentiate (1→4)(deuterium effect)- and (1→3)(no deuterium effect)-linkages in the *b→c* position. If, in the latter case, the alditol unit is a pentitol (**19**), the ion at *m/e* 133 is totally absent. This is also the case when the alditol unit carries two glycosyl substituents separated by the C-3–C-4 linkage (compound **21**).

The changes induced by deuteration on the position of M−89 (*m/e* 585) and M−133 (*m/e* 541) peaks are analogous to those observed with disaccharide alditols[2,3]. A shift of the ion at *m/e* 585 to 586 is noted only in the spectra of the (1→3)- and (1→2)-linked trisaccharide alditols, whereas the change of the ion at *m/e* 541 to 542 is characteristic for (1→4)-, (1→3)-, and (1→2)-linkages.

The assignment of the position of the a→b *linkage.* — The (1→3)- and (1→2)-linkages in an *a→b* position can, as mentioned above, be distinguished from (1→6)- and (1→4)-linkages by the lower intensity of the peak at *m/e* 88. Another feature common to the two former types is the predominance of *ba*A ions over *bc*A ions, whereas the fragmentation of the two latter types proceeds mainly through the *bc*A pathway.

The ion at *m/e* 295 (bcJ_1) was found to be present in variable intensities in the spectra of (1→6)- and (1→4)-linked trisaccharide alditols but absent from the spectra of (1→3)-linked compounds. Its absence in the latter case can be explained by the lack of a free methoxyl group attached to C-3 of the *b* unit, which prevents fragmentation through pathway J. However, the absence of *m/e* 295 from the spectrum of

compound **18** and the variations of its intensity in the other spectra indicate that other structural factors also influence the generation of this ion (see also Ref. 1).

Characteristic for a (1→3)-linkage in an *a*→*b* position is the increased intensity of ions at *m/e* 159 (composed probably of fragments of ring *b*, see Ref. 1), and at *m/e* 347 (331+16), and *m/e* 365 (349+16). A distinguishing feature for (1→2)-linked compounds is the greatly increased intensity of the ions of the *ab*A series[1] (especially of baA_2, *m/e* 391), and the presence of an ion at *m/e* 201. These ions allow the identification of (1→3)- and (1→2)-linkages in an *a*→*b* position.

Compounds having (1→6)- and (1→4)-linkages in *a*→*b* positions produce very similar mass spectra. Although differences in the intensities of certain ions of low intensity were observed, *e.g.*, the ratio of *m/e* 306 to *m/e* 305 was in general greater in the spectra of the deuterated derivatives having (1→6)-linkages than in those having (1→4)-linkages, it seems that unambiguous differentiation of these two linkages in an *a*→*b* position is not easy in the absence of previous knowledge of the molecule.

Fragmentation of trisaccharide alditols containing pentoses, and of a branched-chain trisaccharide alditol. — The presence of pentose units and their location in the trisaccharide molecule can be established by the characteristic shifts of 44 or 2×44 mass units in the position of the ions of the A and J series (compounds **19** and **20**). The spectrum of **20** shows, as was to be expected, an ion at *m/e* 133 (*m/e* 134 in the deuterated derivative) due to a (1→4)-linkage between *b* and *c* units, and an increased intensity of the ion at *m/e* 115 (159−44), consistent with a (1→3)-linkage between *a* and *b* units. The (1→3)-linkage in the *b*→*c* position in compound **19** is reflected by the absence of *m/e* 133 from its mass spectrum. The (1→6)-linkage between *a* and *b* and the presence of galactose as the middle sugar unit of compound **19** explain the very high bcA_1 peak (see below) at *m/e* 395.

The presence of chain branching in compound **21** is reflected by the low intensity of the *c*A ions in the spectrum. The absence of the ion at *m/e* 133 is in accordance with a structure in which the two glycosyl substituents of the alditol unit *a* reside on different sides of the C-3–C-4 linkage.

Influence of monosaccharide stereochemistry on m.s. fragmentation. — As the mass-spectral analysis of permethylated trisaccharides has shown[1], configurational differences in the middle monosaccharide unit greatly influence the relative intensities of the disaccharide ions of the A series. Empirical rules analogous to those proposed for the differentiation of (1→4)- and (1→6)-linkages in an *a*→*b* position in a trisaccharide can be formulated for trisaccharide alditols. In this case, the mass difference of 16 units between *bc*A and *ba*A ions considerably facilititates the interpretation of these effects.

Consequently, an intense ion bcA_1 at *m/e* 439 (*m/e* 395 in the spectrum of **19**) in the spectra of trisaccharide alditol derivatives containing galactose as the *b* unit and an intensity ratio $\ll 1$ of bcA_2 to bcA_1 indicate a (1→6)-linkage between *a* and *b* units. A ratio clearly greater than unity and a moderate intensity of these ions indicate a (1→4)-linkage between these units. When the middle sugar is glucose, the approximate ratios are >2 for a (1→6)-linkage and $\leqq 1$ for a (1→4)-linkage. The observed

differences in intensity are more prominent in the spectra of alditol derivatives than in the spectra of methylated trisaccharides and they seem to be in this case uninfluenced by the $b \rightarrow c$ linkage.

ACKNOWLEDGMENTS

I am grateful to Drs. K. Nisizawa, G. Chihara, G. O. Aspinall, S. Russi, J. R. Turvey, P. A. J. Gorin, A. Gauhe, D. Löw, B. H. Howard, and D. J. Manners for their generous gifts of reference materials and to Drs. H. Adlercreutz and T. Luukkainen for permission to use the g.l.c.–m.s. instrument at the Department of Clinical Chemistry, University of Helsinki. This work was supported by the National Research Council for Medical Sciences, Finland, and by the Finnish Medical Foundation.

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