STRUCTURAL ANALYSIS OF TRISACCHARIDES AS PERMETHYLATED METHYL GLYCOSIDES BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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ABSTRACT

The separation and structural analysis of 21 permethylated trisaccharides by gas–liquid chromatography (g.l.c.) and combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) is described. The compounds include examples of straight-chain trisaccharides having all the 9 possible combinations of (1→6)-, (1→4)-, and (1→3)-linkages between the monosaccharide units. Analysis of a few miscellaneous compounds having (1→2)-linkages, containing pentoses or having a branched-chain structure, is also presented.

The results show that g.l.c. (stationary phases SE-30 or OV-22) is well suited for resolving trisaccharide mixtures into their components and for their preliminary identification. Analysis by g.l.c.–m.s. discloses the molecular weights of the monosaccharide units, the sequence of monosaccharides if they have different masses, and the presence of chain branching. The position of the glycosidic linkage next to the reducing end of straight-chain trisaccharides can generally be established by m.s., whereas differentiation of (1→6)- and (1→4)-linkages next to the non-reducing end may require previous knowledge of the stereochemistry of the sugar units.

The use of g.l.c.–m.s. in studies of partial hydrolysates of polysaccharides is discussed.

INTRODUCTION

Small oligosaccharides can be analysed by g.l.c. as their methyl or trimethylsilyl (TMS) derivatives. These derivatives have been used with success for the g.l.c. separation of several disaccharides or disaccharide alditols[1–6]. The possibility of applying g.l.c. for the resolution of mixtures of tri- or tetra-saccharides has been little investigated, in spite of the adequate volatility and thermal stability of their methyl and TMS derivatives under ordinary g.l.c. conditions[1,2].

M.s. of oligosaccharides has also been largely restricted to structural studies of disaccharides or their alditols[5–10]. Although relatively few compounds have been studied so far, the results clearly demonstrate that m.s. of TMS or methyl derivatives is very useful for the determination of the molecular weights of the monosaccharide units and of the sequence of monosaccharides of different molecular weight, as well as for the assignment of the position of the glycosidic linkage. Examples of the

m.s. of trisaccharides have been reported[8,11], but no comparative studies with trisaccharides containing, for example, different types of glycosidic linkages have been published.

Combined g.l.c.–m.s. of oligosaccharides has been employed only for the analysis of disaccharide alditols[5,6]. The great advantage of this method is the possibility of obtaining structural information about compounds present in very small amounts without their previous fractionation. Its application to the analysis of partial hydrolysates of polysaccharides seems, therefore, particularly attractive.

In this work, g.l.c. and g.l.c.–m.s. are applied to the study of permethylated trisaccharides. Besides being eluted somewhat more rapidly in g.l.c. than TMS derivatives, the methyl ethers have significantly lower molecular weights, which greatly simplifies m.s. analysis. A total of 21 different trisaccharides were studied, including a few containing pentoses and one having a branched-chain structure.

MATERIAL 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-Galp-(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**).

Compounds **4** and **8** were purchased from Koch–Light Laboratories, Colnbrook, Bucks, England, and the following were gifts: **1** (Drs. K. Nisizawa and G. Chihara), **3**, **10**, and **19** (Dr. G. O. Aspinall), **5** (Dr. S. Russi), **6**, **11**, **12**, **13**, and **21** (Dr. J. R. Turvey), **9**, **14**, **17**, and **18** (Dr. P. A. J. Gorin), **16** (Drs. A. Gauhe

and D. Löw), and **20** (Dr. B. H. Howard). Compounds **2**, **7**, and **15** were prepared, respectively, by partial hydrolysis of stachyose (Mann Research Laboratories, New York), *O*-α-D-Glc*p*-(1→4)-*O*-α-D-Glc*p*-(1→6)-*O*-α-D-Glc*p*-(1→4)-*O*-α-D-Glc*p*-(1→4)-D-Glc (a gift from Dr. D. J. Manners), and laminarin (Fluka A. G., Buchs SG, Switzerland).

Each trisaccharide (*ca.* 100 μg) was equilibrated in water and permethylated with methyl iodide in the presence of methylsulphinyl carbanion[6,12]. G.l.c. was carried out as previously described[5].

G.l.c.–m.s. was performed with an LKB Model 9000 Gas Chromatograph–Mass Spectrometer with helium as carrier gas. The energy of the bombarding electrons was 70 eV; the ionizing current was 60 μamp. The total mass range covered was from *m/e* 40–700. Mass numbers of the peaks in the high mass range were determined by using as a reference the spectrum of perfluorokerasene taken under identical conditions. Peaks due to background (mainly caused by column bleeding) were subtracted from each spectrum, and the intensities were expressed (Table II) as percentages of the highest peak (base peak).

Symbols A–J, employed by Kochetkov *et al.*[7], with lower-case letters *a*, *b*, and *c* to designate the monosaccharide units[10] (from the non-reducing end, in this order) are used to denote the fragment ions. The first lower-case letter indicates the part of the molecule from which the fragment originated. The second and third lower-case letters indicate the uncleaved part of the molecule substituting this fragment. Thus, for example, $abcJ_1$ stands for the first ion in the series formed through cleavage of ring *a* following pathway J being substituted by units *b* and *c*.

RESULTS

Analysis by g.l.c. — The relative retention times of the methyl ether methyl glycosides of the trisaccharides in g.l.c. are presented in Table I. The methylated trisaccharides are eluted from the non-polar SE-30 column considerably earlier than the corresponding TMS derivatives, the retention times being comparable to those of TMS derivatives of disaccharides. However, when the more-polar OV-22 column is employed, the retention times of the methyl and TMS derivatives of trisaccharides are approximately of the same order. The anomeric methyl glycosides are, in most cases, separated from each other, which facilitates the identification of the parent trisaccharide but also increases the possibility of overlapping with the other trisaccharide peaks. The quantitative ratio of the anomers of a given sugar was very constant. As seen from Table I, each trisaccharide forms a characteristic peak or a set of peaks that do not completely overlap with those of any other trisaccharides. Thus, in principle, qualitative and quantitative analysis is possible, provided the mixture is not very complicated.

General characteristics of the mass spectra. — Partial mass spectra of the permethylated trisaccharides are shown in Table II. The corresponding g.l.c. peaks are indicated in Table I. Only peaks relevant to the present discussion, in addition

to the 10 peaks of highest intensity in each mass spectrum, are included in the list.

The ions in the low-mass range (*m/e* 40–300) are largely the same as are found in the spectra of methylated disaccharides[7,9]. The ion at *m/e* 88 (H_1) usually forms

TABLE I

RELATIVE RETENTION TIMES* OF PERMETHYLATED TRISACCHARIDES IN G.L.C.

Compound	*Stationary phase*					
	2.2% SE-30 (260°)			1% OV-22 (265°)		
1	1.32[a]	(m)	1.47	1.22	(m)	1.52
2	1.26[a]	(m)	1.39	1.54	(m)	1.90
3	1.83		1.95[a] (m)	2.50		2.77 (m)
4	1.12[a]	(m)	1.19	1.15	(m)	1.40
5	1.28[a]	(m)	1.40	1.59	(m)	1.93
6	1.02[a]	(m)	1.13	0.89	(m)	1.09
7	1.27[a]			—		
8	1.23[a]			1.43	(m)	1.69
9	1.22[a]	(m)	1.27	1.36	(m)	1.60
10	1.50		1.66[a] (m)	2.02		2.38 (m)
11	1.36[a]			1.31	(m)	1.43
12	1.55[a]	(m)	1.70	1.48	(m)	1.63
13	1.30[a]			1.22	(m)	1.42
14	1.43[a]	(m)	1.48	1.63	(m)	1.89
15	1.31[a]	(m)	1.38	1.26	(m)	1.42
16	0.84[a]	(m)	0.90	0.74	(m)	0.84
17	1.18		1.24[a] (m)	1.15		
18	0.64[a]	(m)	0.67	0.68	(m)	0.82
19	1.02		1.16[a] (m)	1.27		1.50 (m)
20	0.68[a]			0.57		
21	1.58[a]	(m)	1.72	1.46	(m)	1.86

*Retention times are relative to the permethylated alditol of compound 8 (maltotriitol); absolute retention time of maltotriitol: SE-30 15 min, OV-22 9 min. [a]Mass spectrum shown in Table II. (m), major peak.

the base peak. Since the greatest contribution to its intensity is provided by the ion H_1^2, which contains C-2 and C-3 of the sugar ring, the intensity of the ion at *m/e* 88 is generally lower in the spectra of trisaccharides containing (1→2)- or (1→3)-linkages, especially if two linkages of this kind are present in the same molecule. Surprisingly, the ion at *m/e* 279 (usually thought to arise through pathway J) is also present, though in only moderate intensity, in the spectra of all trisaccharides having a (1→3)-linkage in an *a*→*b* position (compounds **12**, **13**, **14**, and **15**). Since fragmentation through pathway J requires the migration of a methoxyl group from C-3 (in this case of unit *b*)[7] and no such methoxyl group is present in the compounds in question, the ion at *m/e* 279 may, in this case, have a different origin.

The ions between *m/e* 300 and *m/e* 560 contain, as a rule, one or two intact monosaccharide units plus fragments of other sugar units. The most prominent ions in this area are those of the A (*ba*A_1 or *bc*A_1, *m/e* 423; *ba*A_2 or *bc*A_2, *m/e* 391; and

baA_3 or *bc*A_3, *m/e* 359) or of the J (*abc*J_1, *m/e* 483) series. The intensities of the A and J ions in this range are generally of the order of 10% of the intensities of the corresponding ions containing one sugar unit less.

The mass range above *m/e* 560 contained very few ions, and their intensities were always less than 1% of the base peak. The molecular ion was not visible, but peaks at M−31, M−(31+32), and M−45 could almost always be detected. These ions, together with the A and J ions discussed above, allow for the determination of the molecular weight of the trisaccharide and its sub-units, as well as of the order of the sub-units differing in molecular weight.

Identification of (1→3)-linkages. — The mass spectra of the methylated, hexose-containing trisaccharides having one or two (1→3)-linkages are characterized by an intense peak at *m/e* 159, regardless of the place of the linkage in the molecule. The ion at *m/e* 159 has also been found to be specific for a (1→3)-linkage in disaccharides[16], and for a (1→3)-linkage in an *a*→*b* position in trisaccharide alditols[13]. Trisaccharides having a (1→3)-linkage between the *a* and *b* units (compounds **12–15**) show, in addition, peaks at *m/e* 331 and *m/e* 349 in their spectra. These are absent or very weak in the spectra of trisaccharides having a (1→3)-linkage in the *b*→*c* position only (compounds **6** and **11**), but, in this case, a notable increase in the intensity of the ion at *m/e* 145 is observed. In the spectrum of **15**, a compound having two (1→3)-linkages, both of these effects are present.

The shift of the ion at *m/e* 159 to *m/e* 115 in the spectra of the pentose-containing compounds **19** and **20**, as well as the absence of high *m/e* 145 from the spectrum of **19** (probably shifted to *m/e* 101), indicate that these ions contain C-5 and C-6 of that hexose moiety to which the (1→3)-linkage is attached.

Identification of (1→2)-linkages. — The spectra of the trisaccharides containing (1→2)-linkages (compounds **16–18**) are characterised by the general increase of the intensities of the ions at *m/e* 423, 391, and 359 (*ba*A_{1-3} or *bc*A_{1-3}), an effect which is not seen in comparable magnitude in any other trisaccharide spectra, and by the presence of an ion at *m/e* 201. Compounds **17** and **18**, having two (1→2)-linkages, are readily distinguished from compound **16**, which contains one (1→2)- and one (1→4)-linkage, by the lower intensity of the peak at *m/e* 88 (see above).

Identification of (1→4)- and (1→6)-linkages. — The ion at *m/e* 557 (*cba*D_1) was found to be present exclusively in the spectra of methylated trisaccharides having a (1→6)-linkage in a *b*→*c* position (**1–3**, **7**, and **12**). In contrast, no ions specific for (1→6)-linkages in an *a*→*b* position were detected, and it seems that distinguishing this linkage from a (1→4)-linkage, without prior knowledge of the sugar composition (see below), may be difficult. However, it may be possible to identify this linkage in some special cases, *e.g.*, if the *b*→*c* linkage is also (1→6) (compounds **1–3**). The low intensity of the ion at *m/e* 305 in these spectra as compared to the spectrum of **7** might, in this case, serve to distinguish the (1→6)- and (1→4)-linkages.

Fragmentation of trisaccharides containing pentoses and of a branched-chain trisaccharide. — The two pentose-containing trisaccharides (**19**, **20**) were degraded essentially according to the same fragmentation mechanisms as the hexose-containing

TABLE II

PARTIAL MASS SPECTRA OF PERMETHYLATED TRISACCHARIDES

m/e	1	2	3	4	5	6	7	8	9	10
45	15	18	27	23	17	28	22	29	34	39
71	25	24	27	10	27	40	22	23	20	21
75	46	42	37	32	28	37	47	38	31	31
88	100	100	100	100	100	100	100	100	100	100
89	11	9	12	12	11	13	11	12	11	11
99	4	3	8	6	5	7	5	7	7	7
101	34	45	62	38	42	50	35	36	37	31
111	9	10	17	17	16	19	15	24	25	21
115	4	5	8	6	8	7	7	10	7	11
127	6	7	12	10	14	13	8	12	12	13
143	3	3	4	3	3	2	2	3	6	6
145	4	4	5	5	6	20	5	7	7	7
155	9	5	8	11	8	12	9	7	13	17
159	2	3	2	2	3	12	2	2	6	2
175	1	—	1	1	—	—	—	—	—	—
187	15	13	18	34	22	32	32	49	37	42
201	2	—	1	2	1	1	—	—	2	1
219	12	11	24	15	17	13	14	18	31	37
235	—	—	—	—	—	—	—	—	—	—
271	—	—	—	—	—	—	—	—	—	—
279	17	9	9	6	6	6	4	7	10	14
303	—	0.1	0.1	0.2	—	—	0.1	—	0.3	0.2
305	—	0.1	0.1	5.1	1.4	0.3	0.8	1.7	0.9	0.6
315	—	—	0.1	—	—	—	—	—	—	—
331	—	0.2	0.1	—	—	—	0.1	—	—	0.3
335	0.2	0.1	—	0.3	—	—	0.2	—	—	0.2
347	—	0.1	—	—	—	—	—	—	—	—
349	—	—	0.1	—	0.3	0.2	—	0.3	—	0.3
359	3.0	0.4	0.5	1.1	0.2	1.9	0.7	0.5	2.2	1.1
379	—	0.1	—	—	—	—	—	—	—	—
391	0.9	0.2	0.1	4.0	0.2	5.0	0.7	1.6	4.3	3.5
395	—	—	—	—	—	—	—	—	—	—
423	2.6	9.4	8.7	1.6	14	2.8	1.7	3.2	1.1	1.5
439	—	—	—	—	—	—	—	—	—	—
483	2.9	0.5	0.1	4.8	0.2	4.5	1.4	0.7	2.4	0.2
557	1.6	1.1	0.2	—	—	—	1.0	—	—	—

[a]The symbol refers only to compounds **19** and **20**. [b]For all compounds except **20**. [c]For all compounds

trisaccharides. The bcJ_1 ion (for its presence in the spectrum of **20**, see discussion above) is shifted to *m/e* 235 (279−44) in both of these spectra, and the $abcJ_1$ ion to *m/e* 439 (483−44) and to *m/e* 395 (483−2×44) in the spectra of **19** and **20**, respectively. The position of all the ions of the A series are shifted by 44 or 2×44 mass units in the spectrum of **20**, whereas two series of A ions (*a*A or *ba*A and *c*A or *bc*A), with a difference of 44 mass units, are observed in the spectrum of **19**. The above ions are in accordance with a structure of hexose–hexose–pentose for **19** and pentose–pentose–pentose for compound **20**.

Although the present results indicate that the position of the glycosidic linkages

12	13	14	15	16	17	18	19	20	21	*Symbol*
30	56	46	55	43	65	54	30	43	27	
57	54	72	73	26	59	51	25	46	28	
60	56	66	53	-32	51	73	24	68	34	
100	100	71	66	100	73	100	100	65	100	H_1
19	20	14	16	17	30	24	10	8	14	
7	12	12	8	6	14	11	6	27	5	
67	81	100	79	44	84	73	54	100	49	F_1
28	53	62	52	25	60	32	19	25	14	
7	16	13	10	7	13	9	24	73	5	
16	27	30	27	11	18	16	10	1	15	
3	5	4	5	5	12	15	12	63	3	aA_2[d] or cA_2[a]
11	11	12	34	4	10	8	3	15	8	
11	30	18	26	14	24	14	6	—	12	aA_3[b] or cA_3[c]
17	22	34	30	4	6	7	2	—	2	
—	1	—	1	—	1	2	6	43	—	aA_1[d] or cA_1[a]
54	97	88	100	53	100	70	17	—	46	aA_2[b] or cA_2[c]
—	—	—	—	5	7	5	—	—	—	
15	17	44	22	9	40	45	4	—	9	aA_1[b] or cA_1[c]
—	—	1	—	—	—	—	16	4	—	bcJ_1[a]
—	—	—	—	—	—	—	—	1	—	baA_3[d] or bcA_3[d]
3	3	3	4	7	8	13	—	—	—	bcJ_1[c]
—	—	—	—	0.8	0.2	0.2	—	2.6	—	baA_2[d] or bcA_2[d]
—	—	—	—	0.3	1.2	0.8	0.4	0.4	0.7	
—	—	—	0.2	0.1	0.2	0.2	0.4	—	—	bcA_3[e]
0.8	1.8	2.5	1.1	—	0.8	0.7	—	0.1	0.4	
—	—	—	—	2.2	—	—	—	1.6	—	baA_1[d] or bcA_1[d]
—	—	—	—	0.2	—	0.2	0.2	0.2	—	bcA_2[c]
0.5	1.8	0.6	3.4	—	—	0.2	0.2	—	—	
0.4	2.2	1.1	1.6	10	11	8.6	—	—	0.5	baA_3[b] or bcA_3[c]
—	—	—	—	—	—	—	15	—	—	bcA_1[e]
0.5	2.1	2.2	1.9	25	43	16	—	—	2.3	baA_2[b] or bcA_2[c]
—	—	—	—	—	—	—	—	2.8	—	$abcJ_1$[d]
1.6	5.2	4.5	5.5	8.3	7.0	4.4	0.1	—	1.1	baA_1[b] or bcA_1[c]
—	—	—	—	—	—	—	0.1	—	—	$abcJ_1$[c]
0.6	0.8	0.8	1.0	3.2	1.6	6.9	—	—	2.8	$abcJ_1$[c]
1.0	—	—	—	—	—	—	—	—	—	$cabD_1$[b]

19 and 20. [d]For compound 20. [e]For compound 19.

of the pentose-containing trisaccharides can most probably be assigned in a manner analogous to that for the hexose-containing compounds, this should be confirmed by the analyses of additional trisaccharides of this type.

The mass spectrum of the branched-chain trisaccharide **21** is very similar to the spectra of the other hexose-containing trisaccharides. However, the absence of the bcJ_1 ion at *m/e* 279 (in combination with low *m/e* 159) is not compatible with any possible straight-chain structure. Assignment of the position of the glycosidic linkages of the branched-chain trisaccharides undoubtedly requires more m.s. information of compounds of similar basic structure.

Other structural aspects influencing fragmentation. — The increase of the intensity of the ion at *m/e* 423 and the low intensity of the other ions of the A series in this mass range are prominent features of the spectra of compounds **2**, **3**, and **5**. An analogous increase in the intensity of the ion at *m/e* 379 at the expense of *m/e* 347 and *m/e* 315 is observed in the spectrum of **19**. All of these compounds contain galactose as *a* and *b* units and possess a (1→6)-linkage in an *a*→*b* position. The m.s. analysis of trisaccharide alditols[13] has shown that these effects are due to the increase of the intensity of the bcA_1 ion, where the configuration of the free methoxyl groups of the galactose unit *b* presumably makes the further fragmentation of this ion difficult[7,10]. Compound **9**, where the substituent at C-4 of the middle galactose unit has been eliminated in the generation of bcA_1, does not show this effect. The spectrum of **14**, a compound having a (1→3)-linkage attached to the middle galactose unit, is also very similar in this respect to the spectra of the (1→3)-linked glucose-containing compounds.

With regard to linkage analysis, it is tempting to make the following generalisations about the intensities of the ions *m/e* 391 and 423. When the middle sugar unit is galactose, an intense ion at *m/e* 423 and a ratio $\ll 1$ of *m/e* 391 to *m/e* 423 indicate a (1→6)-linkage between the *a* and *b* units. A ratio > 1 and a moderate intensity of the ion at *m/e* 423 indicate a (1→4)-linkage. If the middle sugar is glucose [and the *b*→*c* linkage (1→4) or (1→3)], a ratio clearly greater than unity indicates a (1→6)-linkage, and a ratio $\leqq 1$, a (1→4)-linkage in an *a* →*b* position. These differences in relative intensities may therefore afford a possibility of differentiating (1→6)- and (1→4)-linkages in an *a*→*b* position, if previous knowledge about the stereochemistry of the monosaccharide units is available. Configurational differences in the sugar unit *a* probably assume lesser importance in this respect.

Although the configuration of the glycosidic linkages between the monosaccharide units probably has little influence on the mass-spectral fragmentation, the orientation of the methoxyl group at the anomeric carbon of unit *c* seems to have a constant influence on the intensities of, for example, the ions at *m/e* 557 and 305. As a rule, the intensity of these ions in the spectrum of the anomer having the greater g.l.c. retention time (β-D anomer?) was approximately half of the intensity of the peaks in the spectrum of the faster moving anomer. Analogous differences in the relative intensities of corresponding ions have been detected in the mass spectra of anomeric methylated disaccharides[16].

DISCUSSION

Most biochemical applications of oligosaccharide analysis have been concerned with the isolation and characterisation of products liberated by partial hydrolysis of polysaccharides. Liquid chromatography (column, paper, or thin-layer) has usually been employed for the separation. Compared to g.l.c., liquid chromatography still generally lacks the sensitivity, accuracy of quantitative determination, as well as the speed and simplicity of operation offered by standard g.l.c. techniques.

The identification of the separated compounds on the basis of the chromatographic retention times is tentative and requires a large stock of reference materials. Direct analysis of the g.l.c. effluents with m.s. offers a convenient way of obtaining reliable structural information about an unknown oligosaccharide, although this technique alone is not sufficient for complete structural assignment.

The general approach presented here for the analysis of partial hydrolysates is as follows. The oligosaccharide mixture is permethylated and analysed by temperature-programmed g.l.c. Thereby, mono-, di-, tri-, tetra-, and penta-saccharides[16] can be eluted from the column at different temperature ranges. The oligosaccharides within each class are usually resolved. This makes possible the quantitative analysis of the oligosaccharides from microgram amounts of material. Mass spectrometry of the g.l.c. peaks can then be used (*a*) to determine the purity of the g.l.c. fractions and (*b*) to assign the molecular weights of the monosaccharide units, and, if their masses are different, their sequence in the molecule, as well as the position of the glycosidic linkages. For further structural analysis, microgram quantities of the individual oligosaccharide peaks can be collected by preparative g.l.c., and the monosaccharide units identified, after hydrolysis, by g.l.c.–m.s.[6].

The purpose of the present study is to provide a basis for the analysis of the compounds present in the trisaccharide range in the gas chromatograms. Since complete mutual overlapping of the g.l.c. peaks of the methylated trisaccharides was not observed, the individual sugars can be tentatively identified and quantitatively determined if the mixture is not very complicated. Thereby, advantage can be taken of the constancy of the ratio of the anomers and of the possibility of using several different g.l.c. columns. The position of the glycosidic linkages of trisaccharides can, in most cases, be assigned with m.s. Compounds containing (1→2)-linkages, as well as those containing pentoses or possessing a branched-chain structure, still need further investigation.

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