PHYSICAL STUDIES ON OLIGOSACCHARIDES RELATED TO SUCROSE

PART II. MASS-SPECTRAL IDENTIFICATION OF D-FRUCTOFURANOSYL RESIDUES*

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ABSTRACT

Sucrose (**1**), melibiose (**2**), 1-kestose (**3**), planteose (**4**), raffinose (**5**), nystose (**6**), and stachyose (**7**) have been converted into their respective peracetates (**1a**, **2a**, **3a**, **4a**, **5a**, **6a**, and **7a**) and per(trimethylsilyl) ethers (**1b**, **2b**, **3b**, **4b**, **5b**, **6b**, and **7b**), and the two series of derivatives have been subjected to electron-impact ionization in a high-resolution mass spectrometer. The ethers show detectable molecular-ion peaks (up to *m/e* 1674 for **7b**) together with $M^+ - \cdot CH_3$ peaks, and the acetates show $M^+ - \cdot OAc$ peaks. From the fragmentations observed, and the relative intensities of the fragments, it is possible to recognize in the oligosaccharides (*a*) the presence or absence of ketohexofuranosyl residues, (*b*) the terminal or nonterminal location of ketohexofuranosyl residues, and (*c*) the presence of ketohexofuranosyl residues (and their number) attached to adjacent residues through "methylene bridges". A substantial temperature-dependence was observed in the intensities of certain ions.

INTRODUCTION

Electron-impact ionization mass spectrometry of suitable derivatives of sugars can be used to differentiate between aldopyranoses, aldofuranoses, and ketopyranoses[1,2], and to indicate linkage patterns in disaccharides[3–6]. Studies on disaccharides were initiated[3] with permethyl ethers, but the use of per(trimethylsilyl) ethers[4,5,7] and peracetates[6] offers some advantages because of their ease of preparation. Characteristic ions can be recognized[2,3,5,8] that are diagnostic of linkage position (although not of stereochemistry) in the disaccharide derivatives.

Sugars containing D-fructofuranosyl residues are widespread in Nature. No comparative mass-spectral study of sugars containing ketofuranose residues has, however, been reported. The previous paper in this series[9] described the structural characterization, by n.m.r. spectroscopy, of a series of acetylated oligosaccharides

*Preliminary report, W. W. Binkley, R. C. Dougherty, and D. Horton, *Abstracts Papers Amer. Chem. Soc. Meeting*, 155 (1968) c 69.

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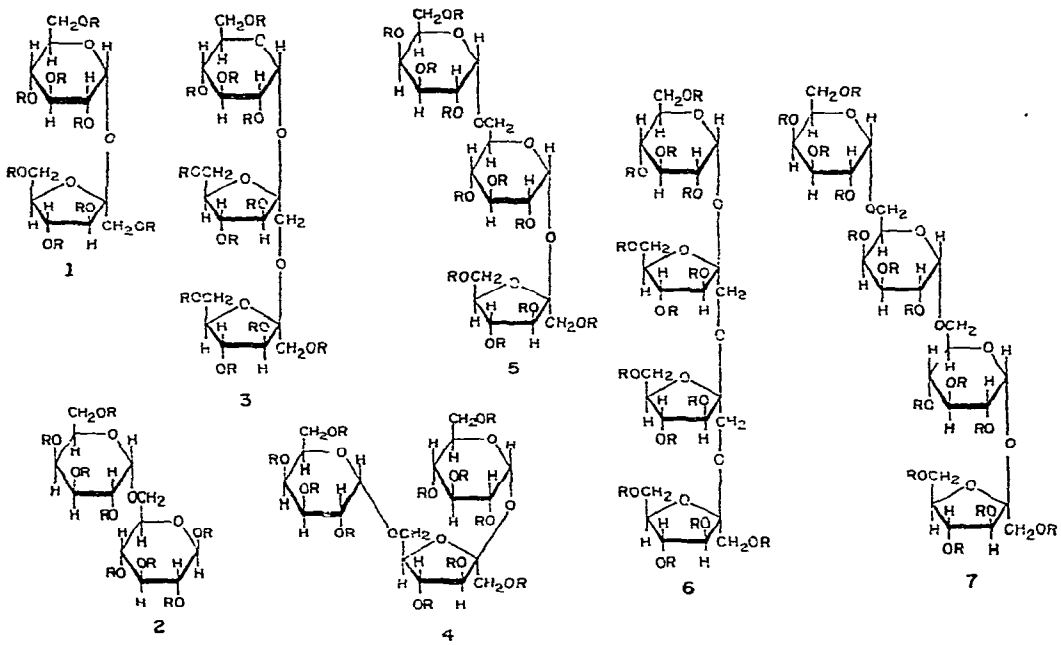
[from sucrose (**1**), 1-kestose (**3**), and nystose (**6**)] that contain, respectively, one, two, and three-D-fructofuranosyl residues together with one D-glucopyranosyl group; 1-kestose and nystose can be regarded as being derived from sucrose by homologous extension at O-1 of the D-fructofuranosyl group. Extension of sucrose by attachment of a D-galactopyranosyl group at O-6 of the D-glucopyranosyl group gives the trisaccharide raffinose (**5**), and repetition of this step gives the tetrasaccharide stachyose **7**). Detachment of the D-fructofuranosyl group from raffinose gives melibiose (**2**).



Attachment of a D-galactopyranosyl group at O-6 of the D-fructofuranosyl group of sucrose gives planteose (**4**). These seven oligosaccharides, as their peracetates (**1a**, **2a**, **3a**, **4a**, **5a**, **6a**, and **7a**) and per(trimethylsilyl) ethers (**1b**, **2b**, **3b**, **4b**, **5b**, **6b**, and **7b**) have now been examined by mass spectrometry.

Decomposition patterns previously determined for simpler carbohydrates[2] were, for the most part, found adequate for interpreting the fragmentation processes of these oligosaccharide derivatives.

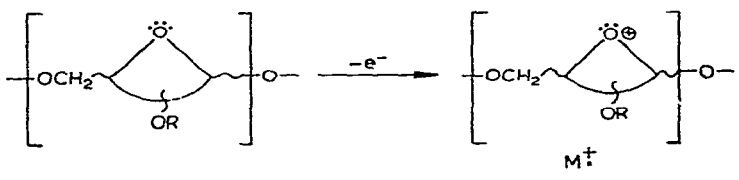
DISCUSSION

The mass spectra of these oligosaccharide derivatives have relatively few ions, considering that the molecules contain up to 250 atoms. It has been proposed[5] that the mass-spectral fragmentation of oligosaccharides proceeds by localized ionization at a single residue and subsequent fragmentation almost exclusively within that residue. The results that follow appear to be in essential agreement with this hypothesis; however, extensive labeling experiments would be required to confirm the hypothesis

in these cases. Table I lists mass numbers and relative intensities of peaks in the seven peracetates (**1a–7a**), and Table II lists similar data for the per(trimethylsilyl) ethers (**1b–7b**). Tables I and II list all observable peaks of high mass number (exclusive of isotope clusters), and peaks below *m/e* ~300 that have intensities greater than 1% of the base (most intense) peak are also listed. For convenience, most of the data are grouped into homologous series of ions. Each series can be considered to represent one particular type of ion in the fragmentation of a component monosaccharide residue. As this fragmentation can (in principle) occur in any one of the residues in the oligosaccharide, there are observed, in each series, the monomer fragment, the dimer fragment and, for the higher oligosaccharides, the appropriate trimer and tetramer fragments. Ions within a given series are separated by the mass number of a fully substituted monosaccharide residue, that is, 288 daltons [$C_6H_7O_2(OAc)_3$] for the acetates and 378 daltons [$C_6H_7O_2(OSiMe_3)_3$] for the per(trimethylsilyl) ethers.

The assignment given for each series represents the probable mode of fragmentation responsible for the ions listed, consistent with principles delineated with simpler sugars[2]; it is, however, recognized that an individual peak, especially in the lower *m/e* range, may result from more than one species of contributing ion. The term $M^{+\cdot}$ refers to the radical cation of the intact oligosaccharide molecule, and Gl^+ denotes an oligosaccharidyl or monosaccharidyl cation formed by cleavage of one of the interglycosidic linkages to give a positively charged, even-electron, glycosyl cation and a neutral, radical species.

Molecular ions and fragmentations not involving glycosyl cleavage. — General. In line with established principles[2], the primary ionization process can be expected to involve removal of one electron from an oxygen atom (most probably a ring-oxygen atom) of the oligosaccharide derivative to give the molecular radical-ion $M^{+\cdot}$. For the acetates of the di-, tri-, and tetra-saccharide derivatives, the mass number of



this fragment will be 678, 966, and 1254, respectively; for the trimethysilyl ethers, the corresponding *m/e* values are 918, 1296, and 1674.

Per(trimethylsilyl) ethers. — For five of the trimethylsilyl ethers (**1b**, **2b**, **4b**, **5b**, and **7b**), small, but distinct, molecular-ion peaks are observed. Loss of a methyl radical from the molecular ion, an established pattern[10] for trimethylsilyl ethers, gives detectable $M^{+\cdot} - \cdot CH_3$ peaks at *m/e* 1659 for both of the tetrasaccharide derivatives **6b** and **7b**, and also (at *m/e* 1281) for the trisaccharide derivative **3b** not showing a detectable molecular-ion peak. These observations suggest that mass spectrometry of per(trimethylsilyl) ethers can be a useful technique for measuring the molecular weight of oligosaccharides, for molecular weights of the derivative up to

TABLE I

MASS-SPECTRAL DATA FOR OLIGOSACCHARIDE ACETATES[a]

m/e	*Relative intensities (% of base peak) for peracetylated*							
	Sucrose (1a)	*Melibiose* (2a)	*1-Kestose* (3a)	*Planteose* (4a)	*Raffinose* (5a)	*Nystose* (6a)	*Stachyose* (7a)	*Assignment*[b,c]
1195						0.001	0.008	$M^+ - \cdot OR$
907			0.02	0.02	0.21	0.46	3.4	
619	0.004	0.02	1.0	3.2	2.4	4.8	2.5	
331	44.0	9.9	20.3	25.0	100	83.5	57.0	
43	100	100	100	100	88	95	100	CH_3CO^+
1135						0.02	0.05	$M^+ - \cdot OR - AcOH$
847			0.01	0.15	0.31	0.02	0.47	
559	0.002	0.18	0.04	0.13	0.10	0.19	0.04	
271	1.27	0.06	1.0	1.1	0.8	2.5	0.7	
1093						—	—	$M^+ - \cdot OR - Ac_2O$ (or
805			—	—	—	0.03	0.01	$M^+ - \cdot OR - AcOH - CH_2CO$)
517	0.001	0.04	0.06	0.05	0.04	0.07	0.28	
229	1.6	0.5	1.1	0.85	4.0	2.3	1.8	
1075						0.01	0.008	$M^+ - \cdot OR - 2AcOH$
787			0.004	0.02	0.14	0.03	0.03	
499	0.001	0.17	0.09	0.07	0.05	0.19	0.02	
211	47	0.8	16	12	41	34.5	25.7	
1033						—	0.001	$M^+ - \cdot OR - Ac_2O - AcOH$
745			—	—	—	0.03	0.02	(or $M^+ - \cdot OR - 2AcOH - CH_2CO$)
457	0.002	0.12	0.13	2.1	0.11	0.40	0.33	
169	77	8.4	39.3	39	43.8	100	31.7	
1153						—	—	$M^+ - \cdot OR - CH_2CO$
865			—	—	—	0.02	—	
577	—	0.01	0.02	—	—	0.14	0.03	
289	0.3	0.1	0.09	0.3	0.7	3.0	1.2	
415	0.002	0.3	0.05	0.08	0.05	0.18	0.09	$M^+ - \cdot OGl - 2Ac_2O$
127	9.4	3.6	4.6	6.4	10.2	11.7	8.4	
187	1.8	0.5	1.5	0.8	0.8	2.6	1.5	$M^+ - \cdot OR - Ac_2O - CH_2CO$
397	0.002	0.02	0.06	0.19	—	0.29	0.06	$M^+ - \cdot OR - 2AcOH - Ac_2O$

TABLE I (*continued*)

MASS-SPECTRAL DATA FOR OLIGOSACCHARIDE ACETATES[a]

m/e	*Relative intensities (% of base peak) for peracetylated*							
	Sucrose (1a)	*Melibiose* (2a)	*1-Kestose* (3a)	*Planteose* (4a)	*Raffinose* (5a)	*Nystose* (6a)	*Stachyose* (7a)	*Assignment*[b,c]
109	52.7	11.5	19.9	26	33	44.2	28.7	
966						—	0.02	$[GlOAc]^+$
678			—	0.02	0.08	0.37	0.01	
390	0.002	0.01	0.37	—	—	0.70	0.01	
1181						0.60	0.003	$M^+ - \cdot CH_2OR$
893			0.94	0.006	0.04	7.2	0.008	
605	0.001	0.01	0.01	0.08	0.04	0.11	0.05	
317	0.02	3.2	0.01	5.7	4.8	0.05	5.5	
1121						0.0001	0.02	$M^+ - \cdot CH_2OR - AcOH$
833			0.01	0.02	0.22	0.05	0.007	
545	—	—	0.05	—	—	0.26	0.01	
257	0.01	0.6	0.01	0.07	1.3	0.05	0.65	
1061						—	—	$M^+ - \cdot CH_2OR - 2AcOH$
773			0.02	0.11	0.13	0.02	0.14	
485	0.006	0.06	—	—	—	0.03	0.01	
197	0.04	1.5	0.04	0.2	1.4	0.08	1.6	
1139						0.02	0.001	$M^+ - \cdot CH_2OR - CH_2CO$
851			0.03	—	—	0.17	—	
139	3.7	1.9	2.0	6.4	6.2	4.7	4.2	$M^+ - \cdot CH_2OGl - 2AcOH - CH_2CO_2$
1152						—	—	$M^+ - OHCCH_2OR$
864			—	—	—	—	0.02	
576	0.01	0.01	—	0.04	0.10	—	0.01	
288	0.2	0.02	0.02	0.07	0.07	0.6	0.2	
1092						—	—	$M^+ - OHCCH_2OR - AcOH$
804			—	—	0.02	—	0.005	
516	0.001	0.03	—	0.05	0.04	0.03	0.02	
228	0.6	0.5	0.03	0.25	0.4	1.1	0.8	
1050						—	—	$M^+ - OHCCH_2OR - Ac_2O$
762			—	0.001	0.02	—	0.004	
474	0.001	—	0.05	0.04	—	0.04	—	
186	1.0	0.2	0.1	0.3	0.3	1.3	0.8	

TABLE I (*continued*)

MASS-SPECTRAL DATA FOR OLIGOSACCHARIDE ACETATES[a]

m/e	*Relative intensities (% of base peak) for peracetylated*							
	Sucrose (1a)	*Melibiose* (2a)	*1-Kestose* (3a)	*Planteose* (4a)	*Raffinose* (5a)	*Nystose* (6a)	*Stachyose* (7a)	*Assignment*[b,c]
1079						0.02	—	$M^{+}-OHCCH_2OR-\cdot CH_2OAc$
791			0.02	—	—	0.07	—	
503	0.001	0.003	0.19	0.02	0.02	0.43	0.02	
215	0.09	2.0	0.05	0.2	0.7	1.0	2.1	
1019						—	0.001	$M^{+}-OHCCH_2OR-\cdot CH_2OAc-AcOH$
731			0.01	0.09	0.12	0.04	0.08	
443	0.006	0.06	0.18	0.02	0.03	0.60	0.03	
155	0.5	6.4	1.5	1.1	7.0	1.5	6.3	
243	0.01	2.2	0.04	2.1	8.0	0.1	1.4	$M^{+}-HCO_2Gl-\cdot OAc$
183	0.05	0.3	0.2	0.1	0.1	0.6	0.2	$M^{+}-HCO_2Gl-\cdot OAc-AcOH$
144	0.4	1.1	1.0	0.6	3.0	1.0	0.7	$[AcO\overset{+}{C}HCHOAc]^{+}$
389	0.005	0.05	2.2	—	0.1	4.3	0.08	$OHC\overset{+}{C}HOGl$
101	5.4	0.6	4.3	3.4	4.8	6.7	2.6	$OHC\overset{+}{C}HOAc$
200	0.2	1.7	—	0.9	1.8	—	0.8	
140	0.5	2.8	—	2.1	5.0	—	1.6	
498	0.002	0.01	—	0.09	0.05	—	0.01	
171	1.0	0.4	3.0	0.8	0.4	3.0	0.7	
141	0.5	3.4	—	2.8	5.2	—	3.0	
126	1.4	0.6	0.6	0.5	0.6	5	1.8	
97	6.3	3.6	5.6	5.8	8.0	9.8	5.3	hydroxypyrylium^{+}
81	2.6	10.9	2.9	8.0	10.5	4.2	9.8	pyrylium^{+}
73	3.6	1.7	4.3	2.5	—	3.6	1.7	$^{+}CH_2OAc$

[a]Partial data, including all detectable peaks above *m/e* ~300, and, below *m/e* 300, those >1% of the base peak. [b]These designations represent probable identities of the corresponding fragments, consistent with established principles[2]. Data not discussed in the text are not essential to arguments at hand, and are included as reference material. [c]*R* indicates the acetyl group (CH_3CO) in fragments above the stepped line transsecting sections of the data, and a glycosyl residue (Gl) in fragments below the line.

TABLE II

MASS-SPECTRAL DATA FOR TRIMETHYLSILYL ETHERS[a] OF OLIGOSACCHARIDES

m/e	*Relative intensities (% of base peak) for the per(trimethylsilyl) ethers of*							
	Sucrose (1b)	*Melibiose* (2b)	*1-Kestose* (3b)	*Planteose* (4b)	*Raffinose* (5b)	*Nystose* (6b)	*Stachyose* (7b)	*Assignment*[b,c]
1674						—	0.0009	M^+
1296			—	0.001	0.002	—	0.01	$[GlOSiMe_3]^+$
918	0.001	0.002	0.005	0.003	0.0001	0.0001	0.009	
540	0.09	0.01	0.1	0.15	0.06	0.03	0.04	
1659						0.0005	0.0005	$M^+ - \cdot CH_3$
1281			0.002	—	—	—	—	$[GlOSiMe_3]^+ - \cdot CH_3$
903	0.01	0.003	0.001	0.004	0.001	0.002	0.0025	
525	0.18	0.01	0.05	0.04	0.09	0.2	0.06	
1569						0.002	0.007	$M^+ - \cdot CH_3 - Me_3SiOH$
1191			0.002	0.04	0.01	0.001	0.06	$[GlOSiMe_3]^+ - \cdot CH_3 - Me_3SiOH$
813	0.02	0.05	0.5	0.09	0.008	0.01	0.01	
435	1.35	0.34	0.55	0.29	0.44	0.28	0.31	
1479						—	0.001	$M^+ - \cdot CH_3 - 2Me_3SiOH$
1101			0.003	0.003	0.005	0.002	0.006	$[GlOSiMe_3]^+ - \cdot CH_3 - 2Me_3SiOH$
723	0.11	0.02	0.25	0.05	0.005	0.11	0.03	
345	1.8	0.4	0.6	0.5	0.54	0.33	0.39	
1585						—	0.0001	$M^+ - \cdot OR$
1207			0.001	—	—	0.0007	0.02	
829	0.001	0.001	0.36	0.001	0.001	0.16	0.09	
451	10.8	1.4	23.8	4.4	11.0	16.4	21.8	
73	100	66.0	87	100	93.5	74.5	40.1	Me_3Si^+
1495						—	—	$M^+ - \cdot OR - Me_3SiOH$
1117			0.002	0.001	0.001	0.0005	0.007	
739	0.01	0.002	0.3	1.15	0.001	0.3	0.07	
361	70.0	7.7	94.0	93.5	100	39.0	73.8	
1405						—	—	$M^+ - \cdot OR - 2Me_3SiOH$
1027			—	—	—	0.004	0.012	
649	0.01	0.01	0.36	0.12	0.002	0.85	0.07	
271	11.0	1.5	11.9	12.7	14.7	10.7	9.9	
1584						—	0.001	$M^+ - ROH$
1206			0.002	0.002	—	—	—	
828	0.03	0.003	0.01	0.2	0.01	—	0.02	
440	0.5	0.001	0.8	0.02	0.9	0.4	0.5	

TABLE II (*continued*)

MASS-SPECTRAL DATA FOR TRIMETHYLSILYL ETHERS[a] OF OLIGOSACCHARIDES

m/e	*Relative intensities (% of base peak) for the per(trimethylsilyl) ethers of*							
	Sucrose (1b)	*Melibiose* (2b)	*1-Kestose* (3b)	*Planteose* (4b)	*Raffinose* (5b)	*Nystose* (6b)	*Stachyose* (7b)	*Assignment*[b,c]
1494						—	—	$M^{+}-ROH-Me_3SiOH$
1116			0.007	0.001	—	—	—	
738	0.02	0.002	0.01	—	0.001	0.05	0.006	
350	0.04	0.05	0.08	0.05	0.01	0.2	0.1	
1571						—	0.009	$M^{+}-\cdot CH_2OR$
1193			0.003	0.04	0.005	0.003	0.006	
815	0.01	0.03	0.13	6.1	0.004	0.04	0.03	
437	9.6	0.3	16.0	0.15	13.0	8.0	9.6	
1481						—	0.003	$M^{+}-\cdot CH_2OR-Me_3SiOH$
1103			0.002	0.003	0.004	0.001	0.004	
725	0.07	0.01	0.11	0.06	0.008	0.17	0.01	
347	0.8	0.3	0.5	0.4	0.5	0.5	0.4	
1391						0.003	—	$M^{+}-\cdot CH_2OR-2Me_3SiOH$
1013			0.004	0.001	—	0.002	0.002	
635	0.03	0.005	0.03	0.04	0.002	0.03	0.009	
257	2.4	1.9	2.3	1.7	1.2	2.0	1.0	
1303						—	—	$M^{+}-\cdot CH_2OR-2Me_3SiOH-CH_2Me_2SiO$
925			—	0.002	—	—	—	
547	0.003	0.001	0.02	0.06	0.01	0.03	0.01	
169	6.0	2.5	10.9	12.2	11.8	11.4	7.7	
1338						—	0.01	$ROCHCHOSiMe_3^{+}$[d]
960			0.003	0.02	0.009	0.003	0.01	
582	0.01	0.04	0.02	0.006	0.0003	0.05	0.005	
204	5.8	100	4.8	80.0	80.0	3.6	100	
1265						—	—	$ROCHCHO^{+}$[d]
887			0.004	—	—	0.03	0.003	
509	0.04	0.004	0.57	0.02	0.04	0.87	0.02	
131	3.8	2.1	2.7	3.1	3.0	1.7	1.7	
1325						—	0.008	$ROCHOS\ Me_3^{+}$[d]
947			0.005	0.02	0.0003	0.01	0.005	
569	—	0.6	—	0.4	0.04	—	0.04	
191	7.2	16.0	8.0	11.0	9.0	6.3	9.7	

TABLE II (*continued*)

MASS-SPECTRAL DATA FOR TRIMETHYLSILYL ETHERS[a] OF OLIGOSACCHARIDE

m/e	*Relative intensities (% of base peak) for the per(trimethylsilyl) ethers of*							
	Sucrose (1b)	*Melibiose* (2b)	*1-Kestose* (3b)	*Planteose* (4b)	*Raffinose* (5b)	*Nystose* (6b)	*Stachyose* (7b)	*Assignment*[b,c]
1351						—	0.0001	$ROCHCH{=}CHOSiMe_3$
973			0.02	0.001	—	0.002	0.02	
595	0.002	0.004	0.1	0.09	0.002	0.07	0.01	
217	32.5	18.0	100	50.8	51.0	100	41.7	
786	0.002	—	0.004	0.001	0.0005	—	0.006	$M^{\ddagger}-OHCCH_2OR$
408	—	0.3	0.04	0.2	0.2	0.05	0.2	
1527						—	0.001	$M^{\ddagger}-OHCCH_2OR-\cdot CH_3$
1149			0.002	0.006	0.001	—	0.01	
771	0.004	0.02	0.005	0.01	0.001	0.01	0.006	
393	0.2	0.46	0.12	0.53	0.20	0.1	0.17	
1452						—	0.001	$M^{\ddagger}-OHCCH_2OR-Me_3SiOH$
1074			—	0.005	0.005	—	0.005	
696	0.001	0.02	0.02	0.004	—	—	0.004	
318	0.5	0.7	0.3	0.8	0.6	0.2	0.9	
1453						—	0.002	$M^{\ddagger}-OHCCH_2OR-Me_3SiO\cdot$
1075			—	0.006	0.001	—	0.007	
697	0.001	0.01	0.01	0.006	0.001	—	0.008	
319	5.7	1.5	7.7	6.9	8.2	5.3	6.0	
1439						—	0.001	$M^{\ddagger}-OHCCH_2OR-\cdot CH_2OSiMe_3$
1061			0.002	0.005	—	0.001	0.006	
683	0.03	0.03	0.02	0.03	0.001	0.05	0.008	
305	2.2	3.0	2.6	3.0	3.0	1.8	3.0	
81	1.0	0.5	1.2	1.1	1.3	0.9	1.0	pyrylium$^+$
711	0.005	0.002	0.009	0.005	0.001	0.05	0.01	
333	1.3	0.6	0.8	1.3	1.0	0.4	1.2	
379	0.4	0.05	3.6	0.2	0.2	3.2	0.1	
317	0.7	1.0	0.4	1.0	1.1	0.2	2.9	
289	2.5	0.2	4.1	0.6	1.2	5.3	1.6	
219	5.0	2.2	10.1	4.6	4.9	12.2	4.6	
205	6.2	20.0	4.3	20.4	13.6	4.0	20.5	

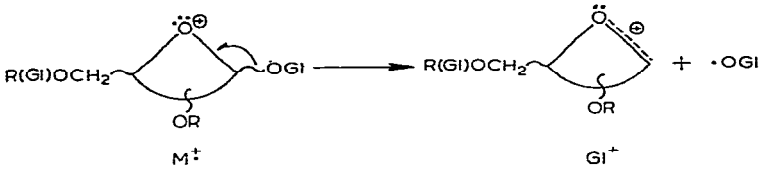
[a,b]See footnotes *a* and *b* to Table I. [c]*R* indicates the trimethylsilyl group (Me_3Si) in fragments above the stepped line transsecting sections of the data, and a glycosyl residue (Gl) in fragments below the line. [d]The convention described in footnote *c* is reversed for these ions.

at least 2000 daltons. With convenient micro methods for trimethylsilylation available, and with the very small sample requirements of the mass spectrometer, it should be feasible to obtain molecular weights for microgram samples of oligosaccharides recovered from paper chromatograms.

For each of the ethers, additional peaks can be observed corresponding to loss from the $M^{+\cdot} - \cdot CH_3$ ion of one, or of two, molecules of Me_3SiOH ($M^{+\cdot} - 105$ and $M^{+\cdot} - 195$, respectively) that further serve to indicate the *m/e* value of the molecular ion. Related ions can also be observed, derived from glycosyl fragments (Gl^+) of the molecular ion, having *m/e* values corresponding to $Gl^+ - Me_3SiOH$, and $Gl^+ - 2\,Me_3SiOH$.

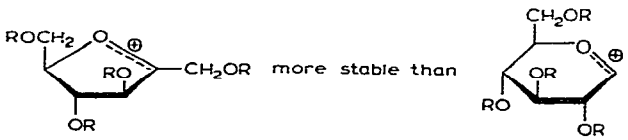
Acetates. — The acetates do not show appreciable molecular-ion peaks, but all seven examples showed weak (moderate with **5a**) peaks at M − 59 corresponding to loss of an acetate radical from the molecular ion ($M^{+\cdot} - \cdot OAc$). Also present in each example are weak peaks at M − 119 and M − 179 corresponding to subsequent loss of one, or two, acetic acid fragments (*m/e* 60) from the $M^{+\cdot} - \cdot OAc$ fragment; the formation of these further fragmentation products of the molecular ion serves to confirm assignment of the M − 59 fragment, and hence corroborates the molecular weight assigned to the parent derivative.

Fragmentations involving cleavage of glycosyl groups. — General. Various families of ions can be recognized in both of the series of derivatives that result from initial rupture of the molecule at one of the interglycosidic linkages to give a glycosyl cation, followed by a sequence of fragmentations within that glycosyl residue by patterns already established for simpler sugar derivatives. Such a fission converts the odd-electron, molecular ion into an even-electron, glycosyl cation (Gl^+), with elimination of a glycosyloxy radical, by the general process shown.



Acetates. For the acetates, this cleavage gives rise to a tetra-*O*-acetyl-glycosyl cation of *m/e* 331 from all examples studied, and the trisaccharides examined show, also, the homologous hepta-*O*-acetyldisaccharidyl cation at *m/e* 619. The spectra of the tetrasaccharides show, additionally, the deca-*O*-acetyltrisaccharidyl cation at *m/e* 907. All seven examples (**1a–7a**) show appropriate Gl^+ peaks of this type. From these Gl^+ cations, there is observed the sequential splitting out of neutral, even-electron species by successive loss of the elements of acetic acid to give the ions $Gl^+ - 60$, of the elements of acetic acid and ketene to give the ions $Gl^+ - 102$, of two molecules of acetic acid to give the ions $Gl^+ - 120$, or of the elements of acetic acid and acetic anhydride (or 2 AcOH and ketene) to give the ions $Gl^+ - 162$. Again, all of the peaks expected are observed in the spectra.

The intensities of the peaks in this series show significant correlation with molecular structure. Observation of the intensities of the *m/e* 169 fragment (monosaccharide $Gl^+ - 162$) or, better, the sums of the intensities of the monosaccharide Gl^+ ions and their sequence of degradation products, for each of the seven acetates, reveals that the Gl^+ series of ions for melibiose acetate (**2a**) is much weaker than for all of the other six examples. Melibiose is the only example that does not contain a ketohexofuranosyl residue. The interpretation may be advanced that high intensities in the Gl^+ series of ions are indicative of the presence of one or more ketohexofuranosyl residues in the molecule, as a result of the greater stability of the ketohexofuranosyl cation over the corresponding hexopyranosyl cation. This observation accords with the report of Biemann *et al.*[1] that 2-tetrahydrofurylium ions derived



from aldofuranoses far exceed the corresponding 2-tetrahydropyrylium ions in stability.

In the planteose derivative **4a**, the stability of the *m/e* 457 ion (disaccharide $Gl^+ - 162$) is noteworthy, as it is in accord with the aldohexopyranosyl→ketohexofuranosyl→aldohexopyranose structure, and shows favored cleavage at the ketohexofuranosyl linkage. The monosaccharide series of Gl^+ ions for **4a** presumably arises, in part, by cleavage of a monosaccharide fragment from the disaccharide Gl^+ ions.

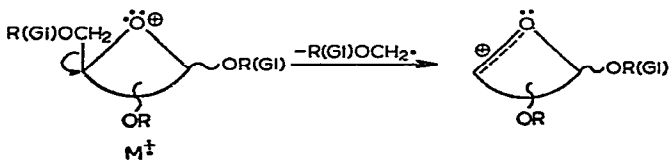
Per(trimethylsilyl) ethers. For all of the ethers **1b–7b**, interglycosidic cleavage from M^+ to give $Gl^+ + \cdot OGl$ affords monosaccharide Gl^+ cations (*m/e* 451) from the disaccharide derivatives **1b** and **2b**; additional peaks for the disaccharide Gl^+ cations (*m/e* 829) are observed with the trisaccharide derivatives **3b**, **4b**, and **5b**. For the tetrasaccharide derivatives **6b** and **7b**, very weak peaks at *m/e* 1207 (trisaccharide Gl^+ cations) are also observed. In addition, for each type of Gl^+ ion, peaks are observed for lower homologs at $Gl^+ - 90$ and $Gl^+ - 180$, corresponding to loss of one, or two, Me_3SiOH fragments, respectively. As with the acetates, the presence of one or more ketohexofuranosyl residues in the molecule greatly increases the intensity of these Gl^+ ions and their fragmentation products; inspection of the relative intensities of the monosaccharide Gl^+ series of ions at *m/e* 451, 361, and 271 reveals that, in each case, the intensity of these ions for the melibiose derivative **2b** (the only one not containing one or more ketohexofuranosyl residues) is much lower than for the other six examples. As with the acetate series, the presence of an intense monosaccharide Gl^+ ion (and its fragmentation products) is strongly indicative of the presence of one or more ketohexofuranosyl residues.

The high intensity of the *m/e* 739 (disaccharide $Gl^+ - Me_3SiOH$) ion in the planteose derivative **4b** is analogous to the behavior observed in the acetate series (where the disaccharide $Gl^+ - AcOH - Ac_2O$ ion is prominent), and can be correlated

with the location of the ketohexofuranosyl residue in the middle of the trisaccharide molecule.

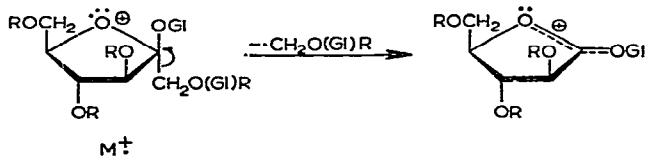
The loss of an $\cdot OSiMe_3$ radical, and subsequent loss of Me_3SiOH, from the parent ion $M^{+\cdot}$, is not an important fragmentation pathway for the ethers **1b**—**7b**.

Fragmentations involving cleavage of a $\cdot CH_2OR$ radical. — General. Fragmentations involving scission of an exocyclic, substituted hydroxymethyl group are well established[2] in the cleavage of hexopyranose and pentofuranose derivatives. As in the



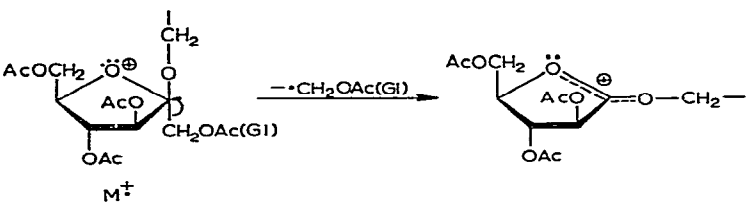
fragmentation mode already discussed, the ions formed from five-membered rings are more stable than those from six-membered ones.

In the ketohexofuranosyl derivatives, another mode for loss of a $\cdot CH_2OR$ radical is possible, namely, by excision of the C-1 residue. This mode of fragmentation



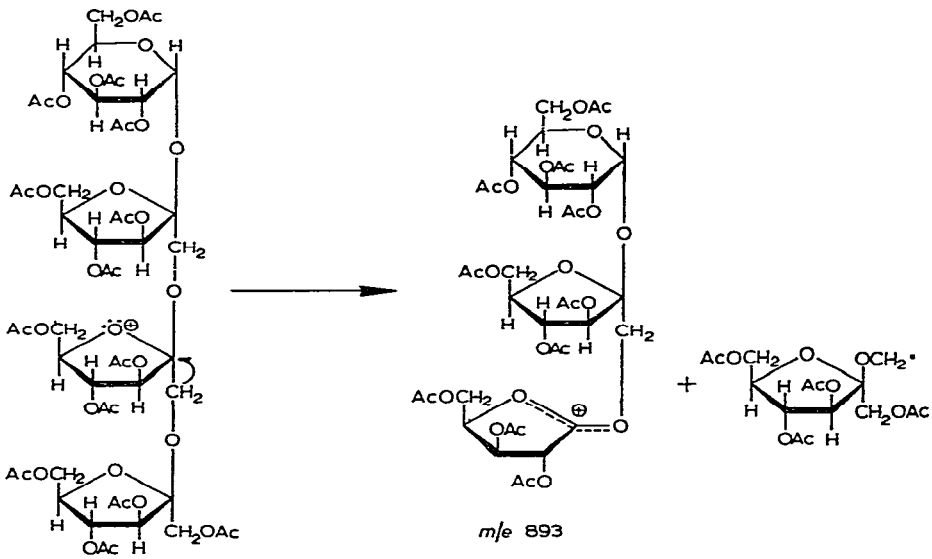
will predictably be favored over the mode involving cleavage of the C-6 group, because the positive charge in the ion formed by loss of C-1 can be delocalized over three atoms (O-5, C-2, and O-2) instead of the two (C-5 and O-5) available by loss of a C-6 fragment. It will be shown that the importance of the ion formed by loss of the C-1 fragment is markedly influenced by the nature of the group attached to O-2.

Acetates. For the acetates, a striking difference is observed, as a function of structure, in the M−73 fragment (loss of a $\cdot CH_2OAc$ radical from the molecular ion). For the sucrose (**1a**), melibiose (**2a**), planteose (**4a**), raffinose (**5a**), and stachyose (**7a**) derivatives, this fragment is relatively insignificant, but, for the 1-kestose (**3a**) and nystose (**6a**) derivatives, the fragment is relatively enormous for an ion of such a high mass number (*m/e* 893 and 1181, respectively). Such an ion of high intensity may be diagnostic of the structural feature of a ketohexofuranosyl residue attached by a "methylene bridge"[9] to another residue, and it indicates that the process shown leads to an ion of particularly high stability. For the compounds studied, such a "methylene



bridge"-linked structure is present only in **3a** and **6a**; in the other examples having D-fructofuranosyl residues, the glycosidic oxygen atom is attached not to a methylene group but directly to the electron-withdrawing glycosidic (anomeric) atom of another sugar residue. Because of the relative inductive effects of the methylene group as compared with the glycosyl residue, the electron density at C-2 of the D-fructofuranosyl residue in the "methylene bridge"-linked structure will be higher than in the glycosyl-linked structures, a situation favoring formation of the $M^{+} - \cdot CH_2OAc$ ion.

It may be noted that 1-kestose possesses one "methylene bridge", whereas nystose has two such groups. It is not surprising, therefore, to find in the spectrum of the nystose derivative **6a** an extremely intense peak at *m/e* 893 that evidently results from cleavage of the terminal D-fructofuranosyl group and the methylene bridge from the penultimate residue by the process shown. Predictably, the process is not a

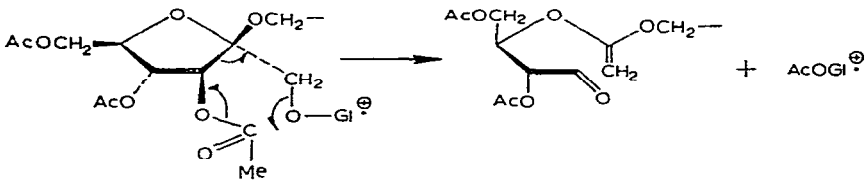


major pathway at the stage of a lower homolog, nor is a major ion for loss of a (D-fructosyloxy)methylene radical observed with 1-kestose acetate (**3a**), because, in each instance, a methylene-bridge group would not assist in formation of the resultant, 5-membered ion.

For each of the $M^{+} - \cdot CH_2OAc$ or $M^{+} - \cdot CH_2OGl$ types of ion observed for the acetates there can be recognized a family of peaks that can be rationalized as degradation products formed by successive loss of one, or two, acetic acid molecules, or of ketene, or of a combination of these.

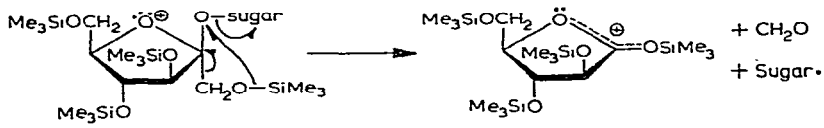
A similar phenomenon related to the mode of linkage is observed for the acetates with respect to formation of ions having *m/e* 390 and 678. Neither of these ions is significant in the spectra of **1a**, **2a**, **4a**, **5a**, and **7a**, but a notable peak at *m/e* 390 occurs in the spectra of acetylated 1-kestose (**3a**) and nystose (**6a**); the latter also

shows a substantial peak at *m/e* 678. The peak at *m/e* 390 appears characteristic of a terminal, acetylated ketohexofuranosyl group linked by a "methylene bridge" to the rest of the oligosaccharide. A possible route for forming this ion would involve detachment of the terminal D-fructofuranosyl group, with accompanying acetyl-group migration from the penultimate residue, to give the D-fructofuranose pentaacetate radical-ion. This process could be assisted sterically by the six-membered-ring transi-



tion-state, and electronically by the presence of the methylene bridge and the keto-hexofuranosyl residue. In the nystose derivative **6a**, a similar situation is also possible for cleavage at the penultimate D-fructofuranosyl moiety, to release a radical-ion of *m/e* 678 (a radical-ion of 1-*O*-*β*-D-fructofuranosyl-D-fructofuranose octaacetate). These observations complement those on the $M^{+\cdot} - \cdot CH_2OAc(Gl)$ fragment for diagnosing the structural element of one or more acetylated ketohexofuranosyl residues attached through a "methylene bridge".

Per(trimethylsilyl) ethers. In the series of ethers **1b–7b**, the $M^{+\cdot} - \cdot CH_2OSiMe_3$ fragmentation is not an important pathway of decomposition. However, each of the oligosaccharides having a terminal D-fructofuranosyl group (**1b**, **3b**, **5b**, **6b**, and **7b**) shows a major peak at *m/e* 437, whereas the melibiose and planteose derivatives, which have no terminal D-fructofuranosyl group, show a relatively insignificant peak at *m/e* 437. Formation of an ion having *m/e* 437 from a terminal D-fructofuranosyl group can be rationalized in one step from the molecular ion by the process shown.



m/e 437

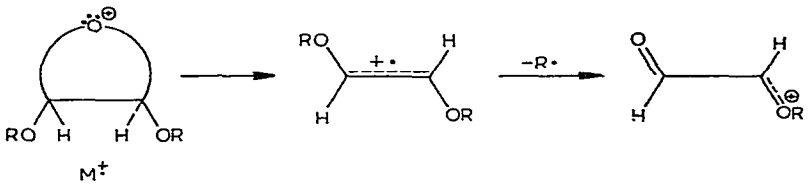
Such an ion, formed by simultaneous loss of formaldehyde and a radical from the rest of the sugar chain, will predictably be a stable one, having charge delocalization over three atoms in a five-membered ring-structure.

Although the *m/e* 437 fragment in the planteose derivative (**4b**) is insignificant, a relatively enormous peak is observed at *m/e* 815. Based on the rationalization for excision of the *m/e* 437 ion from the derivatives having terminal D-fructofuranosyl groups, the occurrence of an important ion at *m/e* 815 for the planteose derivative **4b** is entirely predictable; a similar cleavage to split off the sugar residue at O-2 of the D-fructofuranosyl group would leave an ion identical with the structure formulated for the *m/e* 437 ion, except that a glycosyl instead of a trimethylsilyl group would be

attached at O-6. The intense ion at *m/e* 815 may be diagnostic of a non-terminal ketohexofuranosyl residue in such a derivative as **4b**; recognition of an ion of this type may be useful in locating the position of a ketohexofuranosyl residue within an oligosaccharide chain.

Various series of ions are recognizable that can be rationalized as being derived from the ($M^{+\cdot} - CH_2O - \cdot$sugar) ion by successive loss of one, or two, Me_3SiOH units. An ion at *m/e* 169 appears indicative of ketohexofuranosyl residues, since it is of high intensity for **1b**, **3b**, **4b**, **5b**, **6b**, and **7b**, but is weak for **2b**. Several plausible routes may be formulated for formation from a ketohexofuranosyl residue of an ion having *m/e* 169.

Excision of a disubstituted, vicinal-diol group. — An established[2] degradative pathway from the molecular ion of a substituted sugar involves formation of a 1,2-disubstituted ethylene radical-ion, which then loses a substituent homolytically to generate an even-electron ion.



In the present series of acetates, the first (odd-electron) ion (*m/e* 144) is virtually absent. The second (even-electron) ion (*m/e* 101) is present for all seven examples, but its intensity is much lower for **2a** than for the others, suggesting that the pathway to the ion of *m/e* 101 is favored by the presence of ketohexofuranosyl residues.

The per(trimethylsilyl) ethers show the odd-electron fragment (*m/e* 204) as a major ion, but, in this series, its intensity is particularly high in those examples rich in aldohexopyranosyl residues, whereas it is considerably less intense in those (**3b** and **6b**) that are rich in ketohexofuranosyl residues. The even-electron ion is observed (*m/e* 131), but its intensity varies little throughout the series.

Other correlations. — It is noteworthy that an ion having *m/e* 126, a regular feature of the mass spectra of peracetylated ketohexopyranoses[1], is virtually absent in all seven examples, thus indicating a clear distinction in the behavior of ketohexopyranoses and ketohexofuranoses.

The ratio of intensities of the ions having *m/e* 81 and 97 (probably pyrylium and hydroxypyrylium ions, respectively) is greater than unity when aldohexopyranosyl residues preponderate in the acetylated oligosaccharide, and is less than unity when the number of ketohexofuranosyl residues equals or exceeds the number of aldohexopyranosyl residues. Ions of low intensity at *m/e* 200 and 140 are present in those examples (**2a**, **4a**, **5a**, and **7a**) in which aldohexopyranosyl residues preponderate; these ions are weak for **1a** and absent for **3a** and **6a**.

All seven ethers (**1b**–**7b**) show peaks, unidentified, at *m/e* 205, 289, 317, and 379. The peaks at *m/e* 205 and 317 are smaller for **1b**, **3b**, and **6b** than for **2b**, **4b**, **5b**, and **7b**; for the peaks at *m/e* 289 and 379, the reverse is true.

Other signals, mostly corresponding to established fragmentation patterns, abound in the spectra, but these were not found useful in the elucidation of the composition and sequence of attachment of subunits of these oligosaccharides.

Temperature effects. — The presence of certain characteristic ions in the mass spectra of per(trimethylsilyl) ethers of oligosaccharides has been rationalized[5] in terms of linkage-positions of the component monosaccharide residues. Accordingly, it was anticipated that substantial ion-currents would be observed at *m/e* 569 in the mass spectrum of **2b**, and at *m/e* 583 in those of **2b**, **4b**, and **7b**, as an indication of the (1→6) linkages present. However, fragmentation of the melibiose derivative **2b** in two different A.E.I MS-9 spectrometers, and reexamination with a freshly prepared sample of **2b**, failed to disclose the presence of either of these ions to a noteworthy extent. Accordingly, the inlet part of the mass spectrometer was cooled below 150°, and the system was allowed to return gradually to its normal operating temperature (250°), whilst the fragmentation pattern of **2b** was recorded at temperature intervals of 25°. From the variation in intensity with temperature of several ions produced by **2b** (see Table III), a striking effect of temperature is immediately recognizable; at

TABLE III

INTENSITIES OF SELECTED IONS IN THE MASS SPECTRA OF OCTA-*O*-(TRIMETHYLSILYL)MELIBIOSE (**2b**) MEASURED AT DIFFERENT TEMPERATURES

m/e	*Relative intensities (% of base peak) found at temperature (degrees)*				
	250	*225*	*200*	*175*	*150*
73	65.0	69.0	85.0	65.0	100.0
147	16.0	16.6	24.0	20.0	22.0
204	100.0	100.0	100.0	100.0	33.0
361	7.7	8.1	9.6	10.0	2.8
451	1.3	2.0	2.4	3.3	1.6
569	0.55	1.3	2.3	4.8	1.2
583	0.32	0.6	1.3	2.9	0.5

175°, the ions having *m/e* 569 and 583 are ~10 times as intense as at 250°. This phenomenon probably arises as a consequence of alternative fragmentation pathways' becoming available with increasing thermal energy of the molecule. Minor quantitative variations between results of different authors[5] are to be expected, because of differences in spectrometer characteristics, but the existence of this strong thermal dependence dictates that discretion be exercised in the extrapolation of conclusions from data measured at one inlet temperature to those measured at another.

EXPERIMENTAL

Mass-spectral measurements. — Spectra were recorded with an AEI MS-902 high-resolution, double-focusing, mass spectrometer at an accelerating potential of 8 kV (for **1a–5a** and **1b–5b**) or 6 kV (for **6a**, **6b**, **7a**, and **7b**), and a source temperature

of 250° where not otherwise specified. Spectra were calibrated by direct counting of spectra recorded at a very high recorder-speed and a recorder gain high enough to provide a signal at almost every mass number. Mass numbers thus obtained were verified by comparison with signals at established *m/e* values in the spectrum of an added standard (perfluorokerosene or heptacosafluorotributylamine). Relative intensities were measured with a Gerber Variable Scale, Model TP 007100 B (The Gerber Scientific Instrument Co., Hartford, Connecticut).

The experiments in which the temperature was varied were conducted by cooling the source to ~100°, inserting a sample, allowing the source temperature to rise gradually, and recording mass spectra as the source temperature increased. Temperatures recorded are considered accurate to within ±10°.

Acetylated oligosaccharides. — Acetylation of **2**, **3**, **4**, **5**, **6**, and **7** was achieved by the action, at 80–90°, of acetic anhydride (5 ml) and powdered, fused sodium acetate (500 mg) on the respective sugar (500 mg, recrystallized 2 to 5 times) until the dissolution of the reactants was complete (~2 h), plus an additional 2 h of heating at the same temperature. Solvents were evaporated under diminished pressure at the same temperature, chloroform (15 ml) was added to the residue, and the mixture was washed with saturated, aqueous sodium hydrogen carbonate at 10° until all of the acetic anhydride had been removed. The chloroform solution was dried (sodium sulfate) and evaporated; crystalline acetates were recrystallized twice from 95% ethanol. Noncrystalline acetates were obtained as chromatographically homogeneous syrups[9].

Sucrose octaacetate (**1a**). The commercial material (Matheson Coleman & Bell, Norwood, Ohio) was recrystallized twice from 95% ethanol; m.p. 85–86°, $[\alpha]_D^{20}$ +60° (*c* 4, chloroform) [lit.[11] m.p. 89°, $[\alpha]_D^{20}$ +59.6° (chloroform)].

β-Melibiose octaacetate (**2a**). The acetate was prepared crystalline; m.p. 177–178°, $[\alpha]_D^{20}$ +103.5° (*c* 3, chloroform) [lit.[12] m.p. 177–178°, $[\alpha]_D^{20}$ +104° (*c* 0.8, chloroform)].

1-Kestose hendecaacetate (**3a**). The acetate prepared was a syrup; $[\alpha]_D^{20}$ +31.8° (*c* 3.7, chloroform)[9].

Planteose hendecaacetate (**4a**). The acetate was prepared crystalline; m.p. 134–135°, $[\alpha]_D^{20}$ +97.2° (*c* 4.0, chloroform) [lit.[13] m.p. 135°, $[\alpha]_D^{26}$ +97° (*c* 1, chloroform)].

Raffinose hendecaacetate (**5a**). The acetate was prepared crystalline; m.p. 99–100°, $[\alpha]_D^{20}$ +92.3° (*c* 4.0, ethanol) [lit.[14] m.p. 99–101°, $[\alpha]_D^{20}$ +92° (*c* 8, ethanol)].

Nystose tetradecaacetate (**6a**). The acetate prepared was a syrup; $[\alpha]_D^{20}$ +19.1° (*c* 2.0, chloroform)[9].

Stachyose tetradecaacetate (**7a**). The acetate prepared was a syrup; $[\alpha]_D^{20}$ +120.0° (*c* 3.8, ethanol) [lit.[15] $[\alpha]_D^{22.5}$ +120.2° (ethanol)].

(Trimethylsilyl)ated oligosaccharides. — Per(trimethylsilyl)ation of **1**, **2**, **3**, **4**, **5**, **6**, and **7** was performed essentially by the method of Sweeley *et al.*[7]. A mixture of the respective sugar (25 mg, recrystallized 2 to 5 times) and the (trimethylsilyl)ating reagent [3 ml of 9:3:1 (v/v/v) pyridine–hexamethyldisilazane–chlorotrimethylsilane] was heated for 2 min at 80° to ensure complete dissolution of the sugar, and heating

was continued for an additional 12 h at 40°. The pyridine and unreacted (trimethylsilyl)ating reagents were removed under diminished pressure at 50–60°. Any residual pyridine was removed by repeated addition and evaporation of 2-ml portions of fresh benzene. The residue was extracted with ether, the extracts were filtered, and the solvent was evaporated at 25° to yield colorless syrups, chromatographically homogeneous by t.l.c. T.l.c. was performed with 250-μm layers of Silica Gel G (Merck) on glass plates, with 2:1 (v/v) benzene–hexane as developer and 20% sulfuric acid as indicator.

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