Tetrahedron Vol. 24, pp. 5587 to 5593. Pergamon Press 1968. Printed in Great Britain

MASS SPECTROMETRY OF OLIGOSACCHARIDES

N. K. KOCHETKOV, O. S. CHIZHOV and N. V. MOLODTSOV
Zelinsky Institute of Organic Chemistry, Academy of Science of U.S.S.R., Moscow

(*Received* 24 *January* 1968; *accepted for publication* 29 *March* 1968)

Abstract—The mass spectra of some trimethylsilyl ethers of di- and trisaccharides show that the sequence of monosaccharide units, the position of glycosidic bonds and other details of structure can be elucidated by this method. Mass spectrometry is insensitive to the stereochemistry of oligosaccharides. The fragmentation patterns of the compounds investigated have been discussed.

PROGRESS in the study of polysaccharides and other carbohydrate containing biopolymers depends on developing rapid, precise and sensitive methods for the elucidation of oligosaccharide structures. Application of mass spectrometry to monosaccharide structures was so successful,[1] that it seemed desirable to use this technique for investigating lower oligosaccharides in spite of the extremely low volatility even of lower oligosaccharides. Preliminary experiments with methyl ethers of disaccharides showed, that their mass spectra are sufficiently characteristic and can be used for direct elucidation of structural details.[2]

As small-scale methylation is tedious, the use of oligosaccharide methyl ethers for determination of structure is impractical, but the trimethylsilyl ethers (TMS)[3] are simple to prepare in good yields and they are very suitable for use in a microprocedure. Despite their relatively high molecular weights TMS-derivatives are sufficiently volatile[3] and are very suitable for mass spectrometry.

The mass spectra of two series of TMS-derivatives have been described—(i) glucosyl-glucose with different types of glucosidic bonds and (ii) glycosyl(1 → 6) glucoses differing by nonreducing monosaccharide units.

This investigation demonstrates that the nature of the intermonosaccharidic bond and the nonreducing monosaccharide structure influence the mass spectra. In addition, the mass spectrum of a trisaccharide was studied.

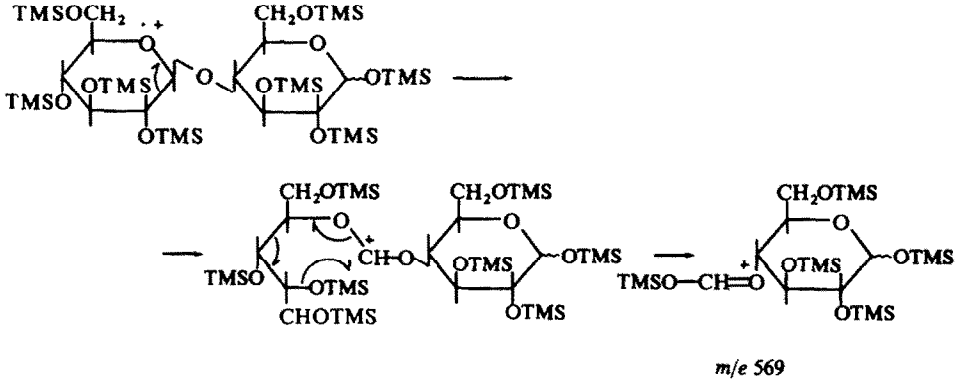
RESULTS AND DISCUSSION

The mass spectra of disaccharide methyl ethers[2] has shown, that their fragmentation can be described in the first approximation as the independent rupture of the both monosaccharide units—nonreducing unit "b" and reducing unit "a". The structures of the ions arising from unit "b" depend on the structure of unit "b" and independent of the type of intermonosaccharidic glycosidic bond, but the structures of ions arising from "a" unit depend the type of substitution and are diagnostic for the position of the intermonosaccharidic glycosidic bond. Fragmentation of TMS-derivatives of disaccharides under electron impact can be considered in a similar manner.

Mass spectra of glucosyl-glucoses

Partial mass spectra of four TMS-ethers of glucosyl-glucose-sophorose (1 → 2), laminaribose (1 → 3), cellobiose (1 → 4) and gentiobiose (1 → 6) are given in Table 1. The main section of the peaks occupy the same position in all the spectra, although in some cases the relative intensities are different. The intensity of the M^+-ion peaks at *m/e* 918 is very small and sometimes this peak disappears. The ions M—CH_3, M—CH_3—TMSOH and M—CH_3—2TMSOH (for hexosyl-hexoses at *m/e* 903, 813 and 723 respectively) are more intense. Replacement of one of hexosyl residues by pentosyl or deoxyhexosyl (see later) causes these peaks to shift to lower *m/e* values. The peak of molecular ion and M—CH_3, M—CH_3—TMSOH and M—CH_3—2TMSOH peaks can be used for detection of mol wts of disaccharides.

There is the relatively intense peak at *m/e* 569 in all the mass spectra. The ion must contain intact "a" unit (467 mu) because formation of this ion does not depend on the position of the intermonosaccharide bond. Additional 102 mu represent evidently TMSO—CH grouping retained after splitting of ring "b". Hence, the structure of this ion can be presented as TMS—O—CH=$\overset{+}{O}$—Hex, where Hex is tetra-O-trimethylsilylhexopyranosyl and is analogous to the ion with *m/e* 279 in the mass spectra of methylated disaccharides.[2] During formation of this ion rearrangement probably takes place, which consists in migration of TMSO-grouping from C_3 to C_1, e.g.



m/e 569

The two different sets of ions corresponding to the peak at *m/e* 451 are formed (a) by splitting of ring "a" and (b) splitting of the ions with *m/e* 569, mentioned above

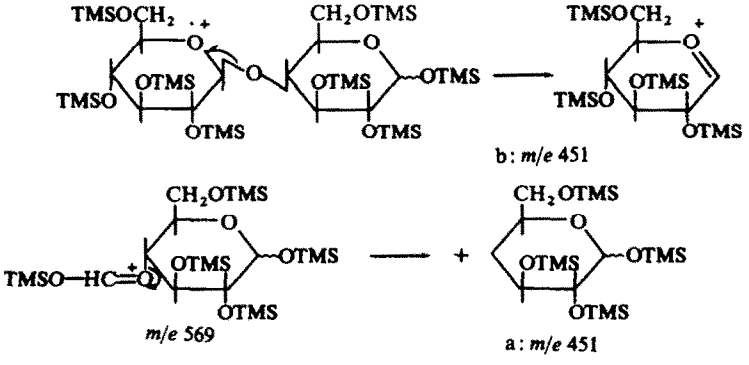
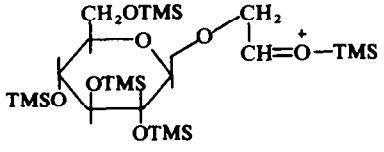


TABLE 1. PARTIAL MASS SPECTRA OF OLIGOSACCHARIDE TMS-DERIVATIVES

m/e	Relative abundance, % $\sum_{250}^{M}$									
	glI $\xrightarrow{\beta}$ 2Gl	glI $\xrightarrow{\beta}$ 3Gl	glI $\xrightarrow{\beta}$ 4Gl	glI $\xrightarrow{\alpha}$ 4Gl	glI $\xrightarrow{\beta}$ 6Gl	glI $\xrightarrow{\alpha}$ 6Gl	glI $\xrightarrow{\beta}$ 6Gal	arI $\xrightarrow{\alpha}$ 6Gl	rhI $\xrightarrow{\alpha}$ 6Gl	glI $\xrightarrow{\alpha}$ 4Gl $\xrightarrow{\beta}$ 6Gl
961	—	—	—	—	—	—	—	—	—	0·60
947	—	—	—	—	—	—	—	—	—	0·20
918	0·20	0·01	—	—	0·19	0·03	—	—	—	—
903	0·60	0·05	—	—	0·50	0·06	—	—	—	—
830	—	—	—	—	—	—	—	—	0·03	—
815	—	—	—	—	—	—	—	—	0·08	—
813	0·90	0·38	0·37	0·17	0·20	0·22	0·06	—	—	0·17
725	—	—	—	—	—	—	—	—	0·06	—
723	0·40	0·21	0·21	0·19	0·56	0·11	0·20	—	—	0·17
683	0·14	0·34	0·16	0·10	—	—	—	—	—	0·05
668	0·34	—	0·71	0·82	—	—	—	—	—	0·20
583	—	—	—	—	9·5	3·7	8·0	—	—	—
578	0·47	—	0·93	0·69	—	—	—	—	—	0·20
569	2·6	1·2	0·64	0·68	11·1	6·3	4·2	4·9	7·6	5·7
495	—	—	—	—	—	—	—	—	4·8	—
481	—	—	—	—	—	—	—	5·9	—	—
451	3·2	3·0	2·5	2·0	7·8	7·0	11·0	3·3	4·8	7·8
363	—	—	—	—	—	—	—	—	11·8	—
361	24	6·2	32	27	6·1	16	10	4·6	3·8	9·5
349	—	—	—	—	—	—	—	3·6	—	—
273	—	—	—	—	—	—	—	—	3·6	—
259	—	—	—	—	—	—	—	11·3	—	—

There is a "metastable" peak at *m/e* 358·5 corresponding to the second transformation. The peak at *m/e* 361 corresponds to two isometric ions formed from ions with *m/e* 451 by splitting of TMSOH molecules. The fact that two different sets of ions both correspond to the peaks at *m/e* 451 and *m/e* 361, one of them arising from ring "a" and the other from ring "b" becomes evident from the mass spectra of pentosyl-hexoses and desoxyhexosyl-hexoses (see later).

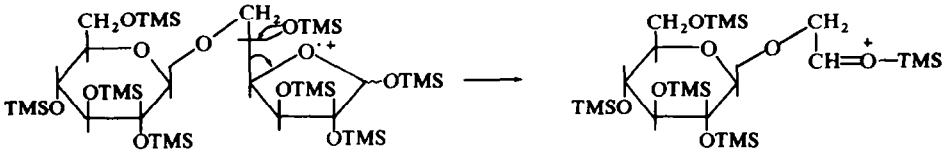
As seen from the Table 1, the mass spectra of each of the listed disaccharides have the following characteristic features. There is a very abundant fragment at *m/e* 583 in the mass spectrum of TMS-gentiobiose, which is absent from mass spectra of hexosyl-hexose with other types of glycosidic bonds (1 → 2, 1 → 3 or 1 → 4). The mass spectra of all other hexopyranosyl 1 → 6 hexoses contain this peak, although its intensity varies considerably. As we have demonstrated recently,[2,3] this fragment characteristic for hexosyl 1 → 6 hexoses must contain intact ring "a" (451 mμ) and fragment of ring "b", including its C_6-atom (132 mu). The following structure corresponds to this requirement:



m/e 583

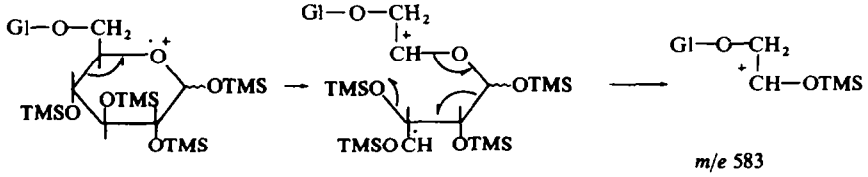
The structure of this ion is confirmed by the fact that corresponding peaks in the mass spectra of 6-deoxyhexosyl-hexose (see later) and pentosyl hexoses are 88 and 102 mu lower (correspondingly at *m/e* 495 and *m/e* 481).

There are different ways this ion may be formed. If during silylation ring "a" takes the furanose form, simple rupture of the C_4—C_5 bond could lead to formation of this ion:



m/e 583

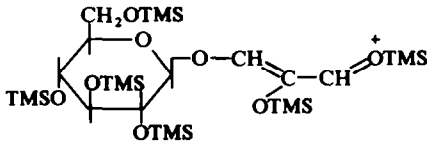
As there is no data supporting formation of considerable quantities of the furanose form during silylation, the possibility that the ion under consideration forms as a result of the following rearrangement of the pyranose form of disaccharide should not be excluded.



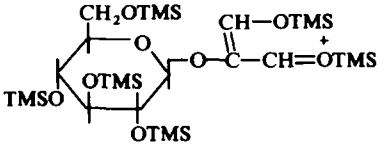
m/e 583

The question how this ion arises still remains open.

Formation of ion with *m/e* 683 is characteristic for glucosyl-glucoses with 1 → 2, 1 → 3 and 1 → 4 bonds (Table 1). This ion must contain intact ring "a" (451 mu) and fragment of the ring "b", consisting of C_2, C_3 and C_4 (232 mu). The following structures are in accordance with such requirements:

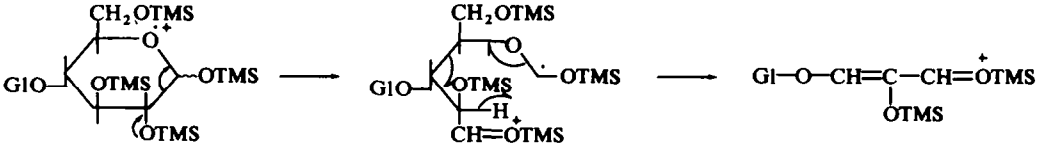


m/e 683 (from 1 → 2 or 1 → 4)



m/e 683 (from 1 → 3)

Formation of this ion can be presented by analogy with formation of B_2-ion for methylated methyl glycosides (cf.[4, 5]) as follows:



m/e 683

There are two additional relatively intense characteristic peaks at *m/e* 668 and 578 in the mass spectra of the glucosyl (1 → 4) or (1 → 2) glucoses. "Metastable" peak at *m/e* 501 is present, corresponding to the transition 668 → 578 (calc. 500·6). Although structures of these ions still are not proved unequivocally, their presence allows 1 → 2 or 1 → 4 disaccharides to be distinguished from 1 → 3 disaccharides.

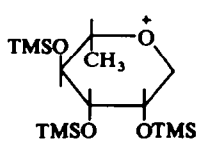
Although the mass spectra of 1 → 2 and 1 → 4 disaccharides contain almost the same set of peaks, the intensity of some of the peaks is very different. Thus, for example, the peak at *m/e* 569, in the mass spectrum of TMS-sophorose has relative abundance 2·6%, while in the mass spectrum of TMS-cellulose only 0·6%.

The characteristic peaks listed above make it possible to distinguish unambigously all the types of intermonosaccharidic bonds in glucosyl-glucoses (1 → 2, 1 → 3, 1 → 4 and 1 → 6). As the configuration of carbohydrate derivatives has very little effect on its fragmentation pattern,[1] the data presented may be used to recognize the glycosidic bond positions in any hexosyl-hexose.

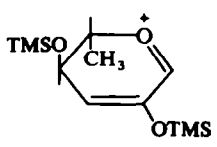
Mass spectra of glycosyl (1 → 6) *glucoses*

Partial mass spectra of TMS-derivatives of melibiose, α-L-rhamnopyranosyl-D-glucose and α-L-arabinopyranosyl-D-glucose are presented in Table 1. In the mass spectra of the disaccharides studied, a shift observed in some of the peaks is characteristic of a new monosaccharide residue, replacing a nonreducing hexose unit. For example, the peaks of all ions containing ring "b" are shifted 88 mu to the lower *m/e* values in the mass spectrum of 6-O-α-L-rhamnopyranosyl-D-glucose: M^+-peak shifts to 830, M—15— to 815, M—15—TMSOH— to 725, etc. Besides the

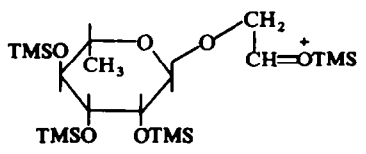
peaks at *m/e* 451 and 361, there are very intense peaks at *m/e* 363 and 273, corresponding to the rhamnosyl unit. Instead of the peak at *m/e* 583, as it is to be expected, the peak at *m/e* 495 appears:



m/e 363



m/e 273



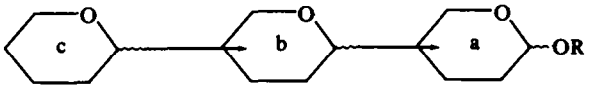
m/e 495

The peaks of ions retaining ring "a" intact (e.g. *m/e* 569) remain at their former position. Analogously in the mass spectrum of the TMS derivative of 6-O-α-L-arabinopyranosyl-D-glucose all corresponding peaks are shifted 102 mu to the lower *m/e* values. Thus by means of mass spectrometry the nature of both the reducing and nonreducing residues in the disaccharide molecule may be recognized if they have different masses. If however both monosaccharidic units have the same mass and type of fragmentation, as e.g. in melibiose and 6-O-β-D-glucopyranosyl-D-galactose, then at present mass spectrometry is of doubtful value in establishing the sequence of monosaccharides.

Mass spectrum of trisaccharide

The mass spectrum of the TMS-derivative of 6-O-β-D-[(4-O-α-D-glucopyranosyl)-D-glucopyranosyl]-D-glucose presented in the Table 1 reveals valuable information concerning the trisaccharide structure.

As the independent rupture of three monosaccharide units "a", "b" and "c" is evident, the structure and *m/e* value of ions, formed by rupture of ring "c", depend



only on the structure of this monosaccharide unit, while *m/e* values and abundance of the ions arising from rings "a" and "b" also characterize the type of intermonosaccharidic bonds c → b and b → a.

Fragmentation of ring "b" must give the same ions, as fragmentation of disaccharide c → b. Fragmentation of ring "a" must lead to the ions having mass numbers different from that of corresponding ions of disaccharide "b → a" by the mass of ring "c" (378 mu for tetra-O-TMS-hexosyl unit).

In the mass spectrum of the trisaccharide studied the peaks at *m/e* 683, 668 and 578 are characteristic of disaccharides with a 1 → 4 bond (see above) and therefore show the presence of a 1 → 4 bond between "c" and "b" rings. In addition, the peak at *m/e* 961, corresponding to the peak at *m/e* 583 (961 − 378 = 583) in the mass spectra of the disaccharides with a 1 → 6 bond, shows that "b" and "a" rings are connected by a 1 → 6 bond. Thus, the mass spectrum elucidates the whole structure of this trisaccharide, since the nature of monosaccharide units (hexoses), types of glycosidic bonds and their sequence are now known [c(1 → 4)b (1 → 6)a].

It is clear, that similar possibility exist for the study of other homooligosaccharides. Mass spectrometric elucidation of structure of heterooligosaccharides with monosaccharide units of different mass will be a simple task and branching of oligosaccharide chains should present no difficulty (cf.[6]), but recognition of constituent stereoisomeric monosaccharide sequences by mass spectrometry does not seem possible.

Considering the possibility of using mass spectrometry for the structural elucidation of more complex oligosaccharides (tetra-, penta-, hexa- etc.), probably the increased molecular weight of these compounds will create technical difficulties and coupled with decreasing volatility, the precise determination of masses above 1500 will entail considerably more calculations.

EXPERIMENTAL

Trimethylsilyl ethers of sophorose, laminaribiose, cellobiose, maltose, gentiobiose, melibiose, 6-O-β-D-glucopyranosyl-D-galactose,[7] 6-O-α-L-rhamnopyranosyl-D-glucose,[7] 6-O-α-L-arabinopyranosyl-D-glucose[7] and 6-O[β-D-(4-O-α-D-glucopyranosyl-D-glucopyranosyl]-D-glucose[7] were homogeneous GLCC[8] and obtained with some modifications[7] according to Sweely *et al.*[8] The mass spectra were measured on a RMU-6D instrument at 175° and ionizing potential 70 eV.

[1] N. K. Kochetkov and O. S. Chizhov, *Adv. Carb. Chem.* **21**, 39 (1966).
[2] O. S. Chizhov, L. A. Polyakova and N. K. Kochetkov, *Dokly Akad. Nauk SSSR* **158**, 685 (1964).
[3] O. S. Chizhov, N. V. Molodtsov and N. K. Kochetkov, *Carb. Res.* **4**, 273 (1967).
[4] N. K. Kochetkov and O. S. Chizhov, *Tetrahedron* **21**, 2029 (1965).
[5] K. Heyns and D. Müller, *Ibid.* **21**, 55 (1965).
[6] D. C. DeJongh, S. D. Hribar, S. Hanessian and P. W. K. Woo, *J. Am. Chem. Soc.* **89**, 3364 (1967).
[7] N. K. Kochetkov, A. Ya. Khorlin and A. F. Bochkov, *Tetrahedron* **23**, 693 (1967).
[8] C. C. Sweely, R. Bently, M. Makita and W. Wells, *J. Am. Chem. Soc.* **85**, 2497 (1963).
[9] J. Tsusue and T. Yamakawa, *J. Biochem.* **58**, 587 (1965).