Tetrahedron Letters No. 26, pp. 2367-2370, 1971. Pergamon Press. Printed in Great Britain.

MASS SPECTROMETRY OF FRUCTOSE CONTAINING OLIGOSACCHARIDES

J.P. Kamerling and J.F.G. Vliegenthart
Laboratory of Organic Chemistry
University of Utrecht, The Netherlands

J. Vink and J.J. de Ridder
Laboratory of Analytical Chemistry
University of Utrecht, The Netherlands

(Received in UK 17 May 1971; accepted in UK for publication 26 May 1971)

In our studies on the structure determination of oligosaccharides by mass spectrometry, we observed that hexosyl oligosaccharides containing fructose units behaved differently to those consisting of aldohexoses only. The mass spectrum of pertrimethylsilyl-sucrose* (II) shows the presence of the fragment ion at m/e 437 ($C_{17}H_{41}O_5Si_4$) with a rather high abundance compared to the peak at m/e 435 (figure 1). In the spectra of TMS-disaccharides containing only aldohexoses the relative intensity of the peak at m/e 437 is always smaller than that of the peak at m/e 435 [1].

The usefulness of the fragment ion at m/e 437 or its analogue at m/e 815 as described below, for the characterization of fructose containing oligosaccharides may be illustrated by the 14 examples given in Table A.

In the mass spectra of TMS-aldohexosyl-(1 → 1)- or (1 → 2)-fructoses the peak at m/e 437 has a high intensity. For the components with the glycosidic bond located on the remaining positions viz. 1 → 3, 1 → 4, 1 → 5 or 1 → 6, the intensity of m/e 437 is about the same as in the TMS-aldohexosyl-aldohexoses. However, these fructose containing disaccharides are characterized by the presence of a peak at m/e 815 (M^+ minus $^{\cdot}CH_2OTMS$) being high in comparison to the peak at m/e 813 (M^+ minus $^{\cdot}CH_3$ minus TMSOH). The formation of m/e 815 and m/e 813 in these disaccharides seems to be equivalent to the formation of m/e 437 and m/e 435 in monosaccharides [2,3]. In the latter case the relative intensities of the peaks at m/e 437 and m/e 435 can be used to distinguish 2-ketohexoses from aldohexoses [4,5].

The formation of the fragment ion at m/e 437 in 1 → 1 disaccharides results from the cleavage of the bond between C1 and C2 of the fructose unit, corresponding to the elimination of $^{\cdot}CH_2OTMS$ in TMS-fructose [5]. For 1 → 2 disaccharides, we suggest the following fragmentation (figure 2), via pertrimethylsilyl-fructose as an intermediate.

TABLE A

	Carbohydrates	Relative intensities [a]				m/e 217
		m/e 435	m/e 437	m/e 813	m/e 815	m/e 204
I	β-D-Gp-(1→1)-D-Fru [b]	5	108	1	2	1.3
II	α-D-Gp-(1→2)-β-D-Fruf(=sucrose) [c]	1	37	0.1	0.1	7.4
III	α-D-Gp-(1→3)-D-Fru(=turanose) [d]	5	2	1	62	1.5
IV	β-D-Galp-(1→4)-D-Fru(=lactulose) [c]	7	3	4	168	0.9
V	α-D-Gp-(1→5)-D-Frup(=leucrose) [b]	5	7	5	102	0.5
VI	α-D-Gp-(1→6)-D-Fruf(=palatinose) [e]	8	4	3	96	1.1
VII	β-D-Fruf-(2→6)-D-G [b]	8	114	2	22	1.7
VIII	α-D-Galp-(1→6)-α-D-Gp-(1→2)-β-D-Fruf(=raffinose) [c]	1	57	0.2	0.3	1.3
IX	α-D-Gp-(1→2)-β-D-Fruf-(3→1)-α-D-Gp(=melezitose) [c]	1	0.7	0.5	27	1.6
X	α-D-Gp-(1→2)-β-D-Fruf-(1→2)-β-D-Fruf(=1-kestose) [b,f]	2	39	<1	<1	8.1
XI	α-D-Gp-(1→2)-β-D-Fruf-(6→2)-β-D-Fruf(=6-kestose) [b]	2	17	0.8	16	5.9
XII	β-D-Fruf-(2→6)-α-D-Gp-(1→2)-β-D-Fruf(=neokestose) [b]	2	12	0.4	0.8	8.8
XIII	β-D-Fruf-(2→1)-β-D-Fruf-(2→6)-D-G [b]	3	73	2	13	2.6
XIV	α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Gp-(1→2)-β-D-Fruf(=stachyose) [e]	1	37	0.2	0.3	0.6

[a] in percentages of m/e 361

[b] gifts (see acknowledgements)

[c] J.T. Baker Chemicals N.V.

[d] Pierce Chemicals Company

[e] EGA-Chemie K.G.

[f] In kestose X the intensities of m/e 813 and m/e 815 have been corrected for the isotopic contribution of the intense peak at m/e 811.

G = Glucose

Gal = Galactose

Fru = Fructose

p = pyranose

f = furanose

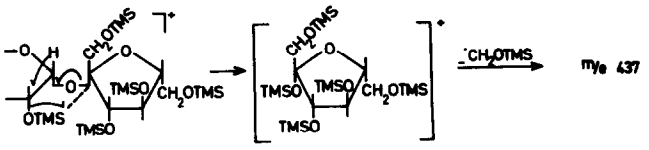

figure 2

However, an ion at m/e 540 corresponding to this intermediate was not present in a significant abundance.

The mass spectrum of TMS-β-D-fructofuranosyl-(2→6)-D-glucose (VII) also shows an intense peak at m/e 437. However, in the TMS-aldohexosyl-(1→6)-aldohexoses this peak has a low intensity (m/e 437 < m/e 435)[1]. These observations make it highly probable that bonding of fructose via C2 gives rise to the formation of an intense peak at m/e 437, independent of the position of the connection in the other carbohydrate unit (figure 3).

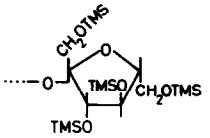

figure 3

On the basis of these results the mass spectra of tri- and tetra-saccharides (VIII to XIV) can be interpreted. The occurrence of the peak at m/e 437 in all these sugars with the exception of melezitose (IX) can be explained by the presence of a (x→2)-D-fructofuranose unit at one end of the molecule. The intense peak at m/e 815 ($C_{32}H_{75}O_{10}Si_7$) in IX, XI and XIII originates from a (x→2)-D-fructofuranose unit substituted by a hexose on one of the remaining positions of fructose. The mass spectrum of kestose X reveals the presence of a peak at m/e 815 of an unexpectedly low intensity. The other fragmentations in kestose X leading to a characteristic peak at m/e 811 (m/e 829 minus H_2O; metastable peak at 793,4; exact mass measurements give the molecular formulae $C_{33}H_{77}O_{10}Si_7$ (m/e 829) and $C_{33}H_{75}O_9Si_7$ (m/e 811)) are still under investigation.

In monosaccharides, pyranose and furanose ringforms can be distinguished on the basis of the ratio of the peak intensities at m/e 217 (TMSO-CH=CH-$\overset{+}{C}$H-OTMS) and m/e 204 (TMSO-$\overset{+}{C}$H-$\dot{C}$H-OTMS) being >1 for the furanose ring and <1 for the pyranose ring [4,5]. The intensities of the peaks at m/e 217 and m/e 204 in oligosaccharides result from contributions of each of the constituent monosaccharides, whereas the type of glycosidic linkage also plays a definite role [1]. The ratios of the intensities of the peaks at m/e 217 and m/e 204 of I to XIV are presented in Table A. Evidently, the

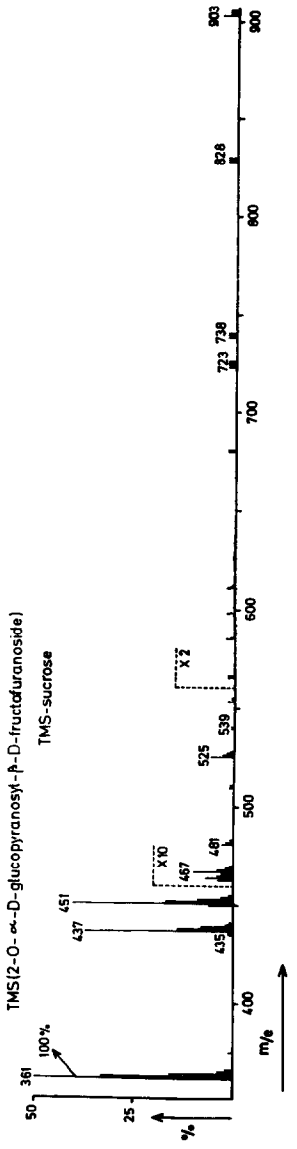

figure 1. 70 eV mass spectrum of TMS-sucrose. Only m/e values > 360 are given

fructofuranose unit greatly influences this ratio. When a greater series of these data is available, this ratio may be helpful in structural determinations.

In this paper we have shown the importance of the peaks m/e 437, m/e 815, m/e 204 and m/e 217 for the structural determination of fructose containing oligosaccharides. More details will be published elsewhere.

*Preparation of the trimethylsilyl derivatives: 0.5 mg of an oligosaccharide was dissolved in 1 ml of pyridine. Subsequently 0.2 ml of hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS) were added. After two hours at room temperature for disaccharides and 3 hours at 70°C for higher oligosaccharides 2 ml of hexane and 2 ml of water were added to the turbid mixture. The two layer system was strongly agitated and the water-layer was then removed. The hexane-layer was washed with 1 ml of water, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was dissolved in 1 ml of hexane. If the oligosaccharide did not dissolve easily in pyridine, a very small drop of water was added before the addition of pyridine. In this case 0.6 ml of HMDS and 0.3 ml of TMCS was used. Reducing oligosaccharides were anomerized in water during 48 hours at room temperature. 10 µl of this solution was used for mass spectrometry.

Acknowledgements: We thank Dr. F. Arcamone (Milan), Dr. F.H. Stodola (Peoria, Illinois), Dr. R.W.Bailey (Palmerston North), Dr. J.S.C. Bacon (Aberdeen), Dr. M. Trojna (Prag) and Dr. W. Kahl (Kraków) for the gifts of carbohydrates. This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

1. J.P. Kamerling, J.F.G. Vliegenthart, J. Vink and J.J. de Ridder, Tetrahedron, in press
2. D.C. DeJongh, T. Radford, J.D. Hribar, S.Hanessian, M. Bieber, G. Dawson and C.C. Sweeley, J. Am. Chem. Soc. 91, 1728 (1969)
3. N.K. Kochetkov, O.S. Chizhov and N.V. Molodtsov, Tetrahedron 24, 5587 (1968)
4. H.C. Curtius, M. Müller and J.A. Völlmin, J. Chromatog. 37, 216 (1968)
5. S. Karady and S.H. Pines, Tetrahedron 26, 4527 (1970)