IDENTIFICATION OF SOME STEREOISOMERIC DISACCHARIDES BY MASS SPECTROMETRY

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SUMMARY: The mass spectra of the trimethylsilyl derivatives of the disaccharides α-lactose, β-lactose, β-cellobiose and the equilibrium mixtures α, β-lactose and α, β-cellobiose have been recorded. A close investigation of peak intensity ratios shows persistent differences for the compounds investigated, allowing a new method of identification. The configuration of the anomeric C-atom of the reducing unit turned out to be the most important factor influencing the relative intensity of several peaks in the mass spectra.

The structure determination of oligomers as peptides (1, 2, 3), nucleotides (4, 5) and saccharides by mass spectrometry is still gaining interest. The analysis of oligosaccharides, however, offers special difficulties. Besides the identification of the constituent monosaccharides and their sequence, the position as well as the configuration of the glycosidic linkages are to be determined. Obviously, each of these structural aspects will have a more or less profound influence on the mass spectrum. Kochetkov et al. (6) observed that mass spectrometry can be used to determine the type of glycosidic link in trimethylsilyl (TMS) ethers of di- and trisaccharides. However, they stated that this technique is insensitive to the stereochemistry of the constituent monosaccharides, so that sequence determination is impossible. Kärkkäinen (7), investigating TMS-disaccharide alditols, as well as Johnson et al. (8), studying 1-phenylflavazole peracetates of oligosaccharides, concluded that the differences in the mass spectra of these

stereoisomeric compounds were too small to be used for sequence determinations. However, the results obtained by Heyns et al. (9, 10), showing that stereoisomeric permethylated monosaccharides can be distinguished from each other, stimulated us to investigate the mass spectra of some stereoisomeric disaccharides in more detail.

We have chosen the trimethylsilyl (TMS) derivatives for our study, because we used these derivatives also in gas-liquid chromatography (11) and proton magnetic resonance spectroscopy (12). In the present study we investigated the mass spectra of the TMS-derivatives of two disaccharides varying in the non-reducing unit and in the anomeric form : α-lactose, β-lactose, β-cellobiose and the anomeric mixtures [+] α, β-lactose and α, β-cellobiose. Because TMS-α-cellobiose was not available, its mass spectrum could not be recorded.

MATERIALS AND METHODS

The TMS-derivatives of α-lactose (4-O-β-D-galactopyranosyl-α-D-glucopyranose), β-lactose (4-O-β-D-galactopyranosyl-β-D-glucopyranose), β-cellobiose (4-O-β-D- glucopyranosyl-β-D-glucopyranose) and of the anomeric mixtures α,β-lactose (4-O-β-D-galactopyranosyl-D-glucopyranose) and α,β - cellobiose (4-O-β-D-glucopyranosyl-D-glucopyranose) were prepared as described before (12). The carbohydrates were purchased as α-D(+)-lactose monohydrate, β-lactose anhydrous and β-D-cellobiose from J.T. Baker Chemicals N.V., Calbiochem Inc. and Fluka A.G. respectively.

The ratio of the anomers in the equilibrium mixture of the anomeric pyranose forms has been determined by gas-liquid chromatography (11) and turned out to be 7 to 10 for TMS-α-lactose and TMS-β-lactose respectively in the anomeric lactose mixture, as well as for TMS-α-cellobiose and TMS-β-cellobiose in the anomeric cellobiose mixture.

The mass spectra have been recorded with an AEI MS9 or MS902 mass spectrometer using the direct introduction technique under the following conditions :

[+] See materials and methods.

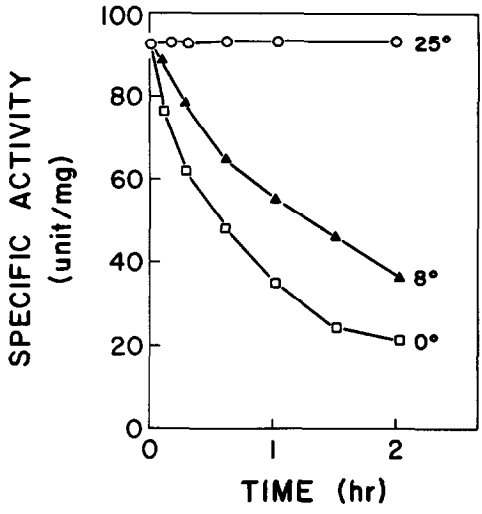


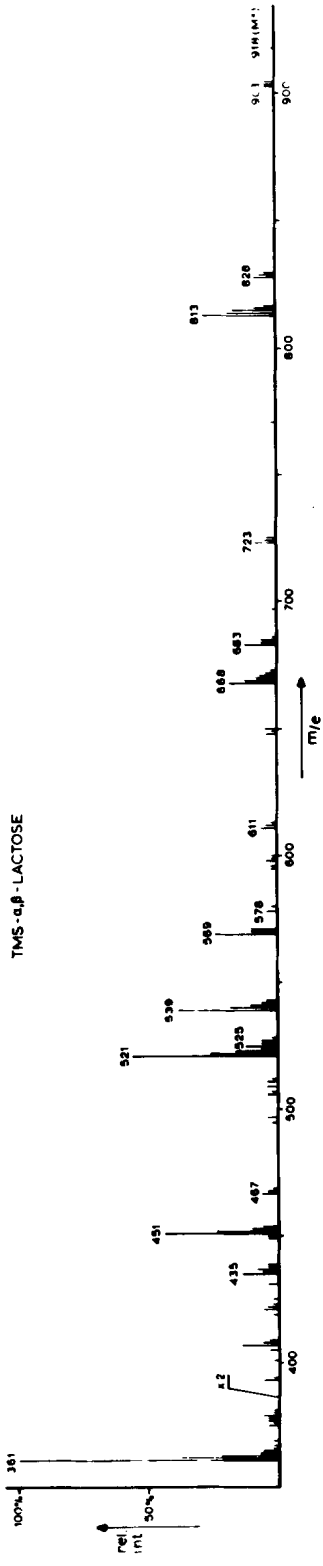
Fig. 1: Effect of temperature on the stability of phosphofructokinase. The enzyme at 0.01 mg/ml was incubated in 0.05 M glycylglycine at pH 8, 8 mM potassium phosphate, 2 mM ammonium sulfate, 25 mM dithiothreitol and 1 mM EDTA. The enzyme solution was incubated at 25° (o), 8° (▲) and 0° (□) and at given time interval aliquots were removed and assayed for enzyme activity as described in "Methods".

activity vs. time yields a biphasic time course of inactivation.

The cold inactivation depends on a variety of conditions such as protein concentration, pH of the medium, and the concentration and type of salt of the medium. For example, at 5 mg/ml or above the enzyme at 0° is completely stable for at least one day, but below 0.3 mg/ml the activity is lost very rapidly at this temperature. Thus, loss of the activity after 1 hour at 0° at protein concentrations of 30 μg/ml, 60 μg/ml, and 300 μg/ml is 70%, 55% and 35%, respectively.

The cold inactivation is enhanced by monovalent anions such as Cl^- or Br^-, but polyvalent anions such as phosphate or sulfate tend to protect against the inactivation. Fructose 1, 6-di-P also offers protection, but ATP enhances the rate of cold inactivation. The pH of the medium has a significant effect on the inactivation process. At 0° the enzyme is most stable at pH 8.3, but the stability decreased considerably above or below this pH.

As shown in Fig. 2 the cold inactivated phosphofructokinase can be reactivated by warming to 25°. In this experiment the enzyme which had been



A typical example of the mass spectrum of a TMS-aldohexosyl-(1→4)-aldohexose.

mixtures and the corresponding α or β compound, and between any lactose and any cellobiose will be demonstrated.

ANOMERS : The table shows that the two lactose anomers can be distinguished unequivocally by using all peak intensity ratios, the ratios 668/578[++], 578/539 and 569/521 excepted.

EPIMERS : For a distinction between the C4-epimers TMS-β-lactose and TMS-β-cellobiose all peak intensity ratios reported in the table can be used. Because of the equal extent of anomerization for TMS-α,β-lactose and TMS-α,β-cellobiose, in this case these anomeric mixtures can be considered as epimers. Therefore, all peak intensity ratios can also be used for a distinction between both anomeric mixtures. Taking the standard deviations into account, the ratios 578/569, however, appear to have a small overlap.

DIASTEREOISOMERS : The diastereoisomers TMS-α-lactose and TMS-β-cellobiose are distinguishable using all peak intensity ratios reported in the table.

ANOMERIC MIXTURE AND α OR β COMPOUND : In order to decide whether we are dealing with a β-compound or with an anomeric mixture, the intensity ratios 668/611, 611/578 578/569, 569/539, 539/521 and 521/451 can be used for TMS-lactose as well as for TMS-cellobiose. For a distinction between TMS-α-lactose and TMS-α,β-lactose also the intensity ratio 683/668 can be used.

LACTOSES AND CELLOBIOSES : The peak intensity ratios 668/578, 578/539 and 569/521 appeared to be very useful in order to distinguish the TMS-lactoses (α, β or α,β) from the TMS-cellobioses (β or α,β).

The differences between the peak intensity ratios of TMS-β-lactose and TMS-β-cellobiose are very similar to those for both anomeric mixtures. This might be expected on account of the equal extent of anomerization.

In general, it turned out that the anomers show greater differences in peak intensity ratios than the epimers. In the case of the ratios 668/578, 578/539 and 569/521, however, the epimers result in the greatest differences.

[++]The intensity ratio 668/578 stands for : the intensity of the fragment ion at m/e 668, devided by the intensity of the fragment ion at m/e 578 in the mass spectrum. This notation is used throughout the present paper.

fragment ions	TMS-α-lactose			TMS-β-lactose			TMS-β-cellobiose			TMS-α,β-lactose			TMS-α,β-cellobiose		
	R	s.d.	n	R	s.d.	n	R	s.d.	n	R	s.d.	n	R	s.d.	n
723/683	0.45	0.06	14	0.85	0.06	19	1.38	0.36	12	0.59	0.14	30	0.99	0.14	29
683/668	2.62	0.59	17	0.53	0.08	21	0.27	0.05	12	0.77	0.16	33	0.31	0.08	31
668/611	0.37	0.04	15	2.78	0.54	23	7.41	1.18	10	1.67	0.57	35	4.08	1.08	29
668/578	2.07	0.57	14	1.42	0.17	17	0.75	0.17	17	1.86	0.34	23	0.91	0.16	26
611/578	5.23	1.74	15	0.55	0.09	20	0.11	0.02	10	1.25	0.26	25	0.24	0.06	28
578/569	0.11	0.01	12	1.06	0.20	24	1.49	0.18	16	0.39	0.16	32	0.68	0.17	32
578/539	0.22	0.03	13	0.17	0.03	22	0.62	0.04	13	0.16	0.04	31	0.66	0.04	24
569/539	1.72	0.18	15	0.17	0.03	22	0.40	0.04	15	0.48	0.15	38	0.91	0.23	33
569/521	0.25	0.04	18	0.17	0.04	23	0.74	0.04	13	0.25	0.09	36	0.84	0.04	18
539/521	0.14	0.02	18	0.98	0.04	14	1.71	0.20	16	0.56	0.13	34	0.99	0.27	32
525/521	0.11	0.01	15	0.22	0.02	21	0.43	0.05	15	0.16	0.04	32	0.37	0.05	27
521/451	2.85	0.24	16	0.94	0.07	20	0.40	0.03	12	1.35	0.26	36	0.63	0.10	31

Mean intensity ratios R, the standard deviations s.d. and the number of determinations considered n, of some fragment ions of TMS-lactoses and TMS-cellobioses.

On the basis of these results, we conclude that the disaccharides investigated can be distinguished from each other by measuring one particular peak intensity ratio, while identification of μg amounts of stereoisomeric disaccharides is expected to be possible by comparing a number of peak intensity ratios with the spectra of a series of reference compounds.

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