THE MASS SPECTRA OF PERMETHYLATED DISACCHARIDES

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INTRODUCTION

Disaccharides have been investigated most of the time as their trimethylsilyl ethers, mainly because of their ease of preparation (1-3). However permethylderivatives of oligosaccharides have considerable adavantages, as has been pointed out by Das and Thayumanavan (4) and by Moor and Waight (5) in their systematic investigation of permethylated oligosaccharides. In the latter investigation Moor *et al*. described the differences between the spectra of only those disaccharides with a 1 - 1, 1 - 2, 1 - 4 or a 1 - 6 glycosidic bond.

In our investigation we measured the spectra of 22 permethylated disaccharides, most of the time as the pure alpha or beta anomer, with all possible linkages between them, so including the 1 - 3 and 1 - 5 glycosidic bond type. The names of the measured disaccharides are given in Table 1.

Moor *et al*. (5) did identify the different glycosidic linkages by looking at a couple of diagnostic masses and intensities. The diagnostic peaks they used are shown in Table 2.

RESULTS AND DISCUSSION

In Figs. 1 to 5 the spectra are given of a representative of each of the different possible glycosidic linkages, which will be discussed hereafter.

In the spectrum of the 1 - 6 linkage (Fig. 1) mass 353 is

TABLE 1.
Names of the measured permethylated disaccharides.

α-D-glucopyranosyl-(1-1)-α-D-glucopyranoside	αα-trehalose
β-D-glucopyranosyl-(1-1)-β-D-glucopyranoside	ββ-trehalose
β-D-glucopyranosyl-(1-2)- D-glucopyranoside	sophorose
α-D-glucopyranosyl-(1-2)- D-glucopyranoside	kojibiose
β-D-glucopyranosyl-(1-3)-α-D-glucopyranoside	α-laminaribiose
β-D-glucopyranosyl-(1-3)-β-D-glucopyranoside	β-laminaribiose
β-D-glucopyranosyl-(1-4)-α-D-glucopyranoside	α-cellobiose
β-D-glucopyranosyl-(1-4)-β-D-glucopyranoside	β-cellobiose
α-D-glucopyranosyl-(1-4)-α-D-glucopyranoside	α-maltose
α-D-glucopyranosyl-(1-4)-β-D-glucopyranoside	β-maltose
β-D-galactopyranosyl-(1-4)-α-D-glucopyranoside	α-lactose
β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside	β-lactose
α-D-glucopyranosyl-(1-5)-α-D-glucofuranoside	
α-D-glucopyranosyl-(1-5)-β-D-glucofuranoside	
β-D-glucopyranosyl-(1-5)-α-D-glucofuranoside	
β-D-glucopyranosyl-(1-5)-β-D-glucofuranoside	
β-D-glucopyranosyl-(1-6)-α-D-glucopyranoside	α-gentiobiose
β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside	β-gentiobiose
α-D-glucopyranosyl-(1-6)-α-D-glucopyranoside	α-isomaltose
α-D-glucopyranosyl-(1-6)-β-D-glucopyranoside	β-isomaltose
α-D-galactopyranosyl-(1-6)-α-D-glucopyranoside	α-melibiose
α-D-galactopyranosyl-(1-6)-β-D-glucopyranoside	β-melibiose

TABLE 2.
Diagnostic peaks used by Moor et al. (1975).

1-1 bond type: 101 is the base peak, 219 and 279 have low intensity.

	type of glycosidic linkage		
mass	1-2	1-4	1-6
380	1.2%	1.7%	0.1%
365	1.0%	-	-
353	0.4%	0.15%	5.5%
305	4.0%	4.9%	-
161	-	3.5%	-

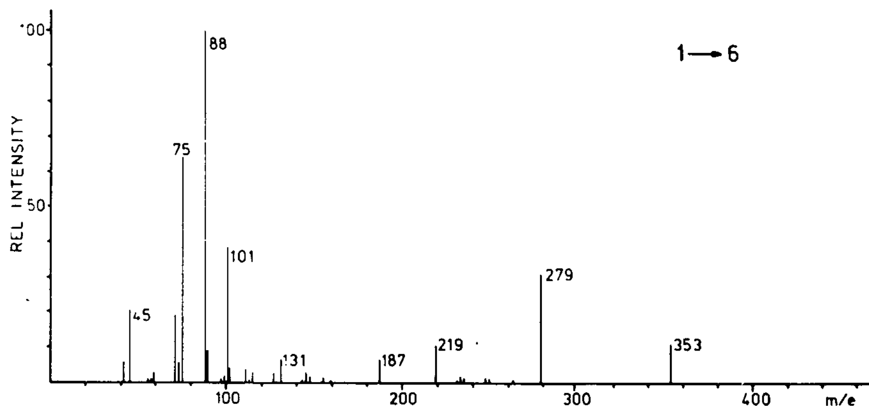

FIG. 1. The mass spectrum of β-D-glucopyranosyl--(1-6)-β-D-glucopyranoside.

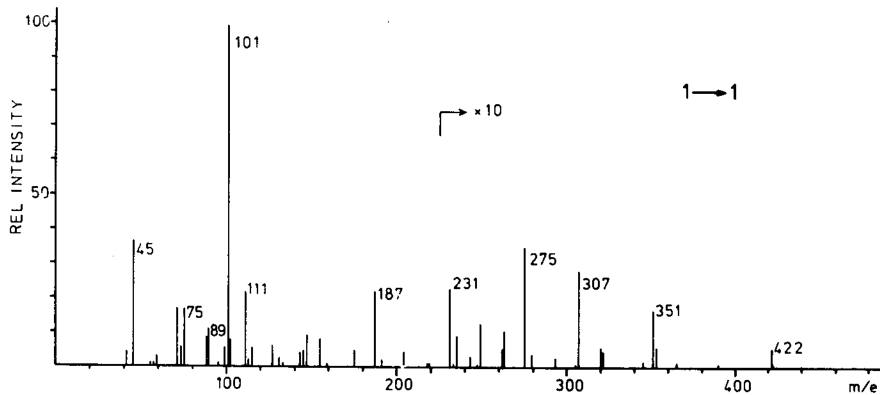

FIG. 2. The mass spectrum of α-D-glucopyranosyl--(1-1)-α-glucopyranoside.

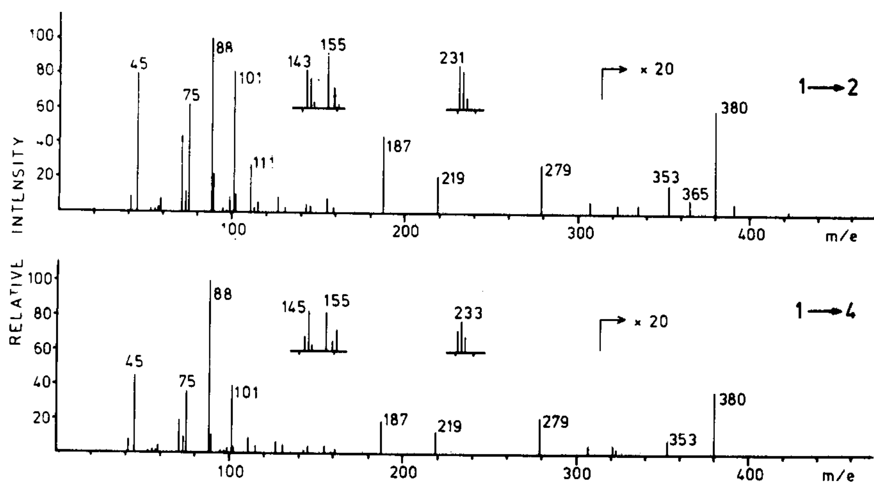

FIG. 3. The mass spectra of α-D-glucopyranosyl--(1-2)-D-glucopyranoside and β-D--glucopyranosyl-(1-4)-β-D-glucopyranoside.

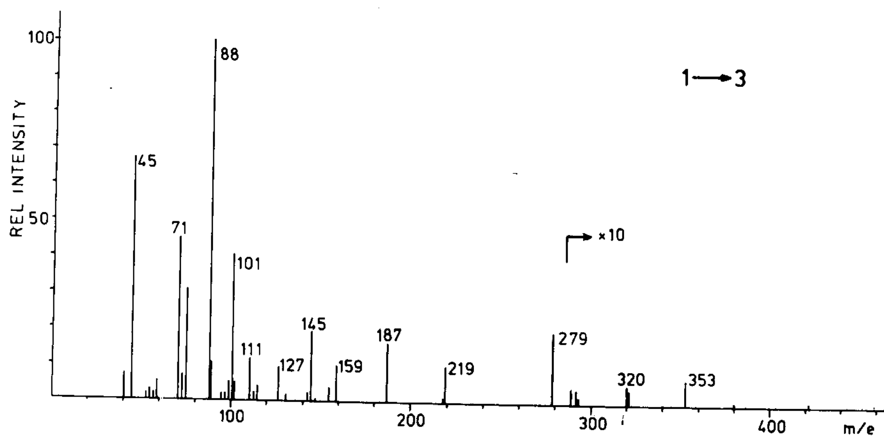

FIG. 4. The mass spectrum of β-D-glucopyranosyl--(1-3)-β-D-glucopyranoside.

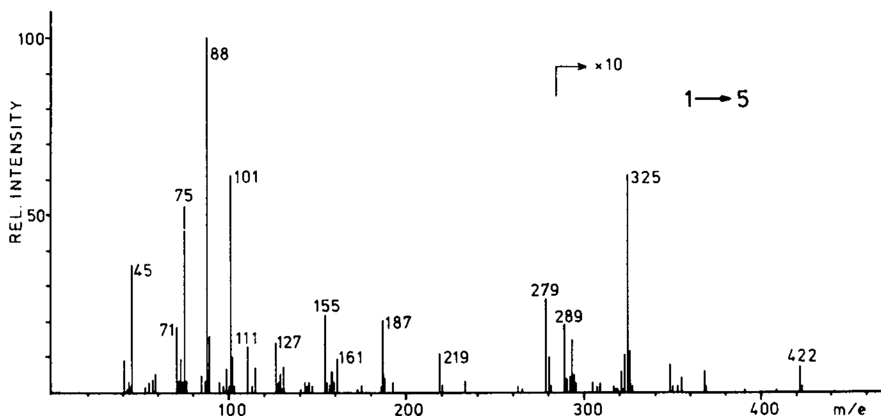

FIG. 5. The mass spectrum of β-D-glucopyranosyl-(1-5)-β-D-glucofuranoside.

characteristic. It appeared that in the beta-form the intensity of mass 353 was twice that in the alpha-form. In the lower mass region mass 131 was always larger than mass 127.

The 1 - 1 linkage (Fig. 2) is easily recognized by the base peak, which is mass 101 instead of mass 88 in all other bond types. In the higher mass region this bond type is recognized by the low intensity of mass 219 and 279 and the occurrence of the masses 351, 275 and in particular mass 307, since in the published spectrum of trehalose (Moor, 1975) only a peak at mass 305 was given. In the lower mass region the high intensity of masses 187 and 111 is characteristic.

The 1 - 2 and 1 - 4 glycosidic bond types (Fig. 3) are most difficult to discriminate, because their spectra are very similar. According to Moor et al. (5) the 1 - 2 and 1 - 4 both have a mass 380 and 305. We have noticed again that the intensity of these peaks is lower in the alpha-form than in the beta-form. In the alpha-form of the 1 - 4 type, mass 380 becomes so small that it could no longer be used to characterize the 1 - 4 bond type.

We also found mass 365 in the 1 - 2 type as described by Moor et al. (5) but its intensity is rather low to be a reliable criteria. To discriminate between the 1 - 2 and 1 - 4 bond type, we have to make use of rather subtle differences in peak patterns in the lower mass region.

The spectrum of the 1 - 3 bond type shows great resemblances

with the 1 - 2 and 1 - 4 bond types. The characteristic differences are the high intensity of masses 145 and 159.

The characteristics of the 1 - 5 glycosidic bond type are the high intensity of masses 155 and 161 and the presence of mass 325 not shown by any other bond type.

Our more extensive data enabled us to improve and to extend the discrimination scheme as given by Moor et al. (5).

As has been indicated by Vink (6) for monosaccharides and by Kamerling (7) for oligosaccharides, it is more reproducible to use carefully selected peak intensity ratios to discriminate spectra of compounds with closely related structures. Characterization of the glycosidic bond type by a few diagnostic peak intensities can lead to erroneous identification, so we have tried to select a couple of characteristic peak intensity ratios for each of the different glycosidic bond types. These ratios were taken as far as possible throughout the whole mass range to give the most reliable results.

Combining all the sometimes very distinct, sometimes very subtle differences between the spectra of disaccharides, we have come up with the following discrimination scheme represented in a flow chart in Fig. 6.

Starting at the upper left corner of the flow chart, we first decide which mass is the base peak, so we can discriminate 1 - 1 from all others. The three ratios 275/279, 351/353 and 145/147 can then be used to confirm the decision made. If the base peak is mass 88, the next branch point is the ratio 187/191; if this ratio is less than 50 we are dealing with a 1 - 6 bond type and we can check the ratios 127/131, 353/323 and 353/422 to confirm this decision.

If this ratio is larger than 50 we come to the next decision point: the peak intensity ratio 325/323, which is larger than 2 for the 1 - 5 bond. The identification of the 1 - 3 bond type is based on its high intensity for mass 159.

The last and most difficult identification between 1 - 2 and 1 - 4 is done with the intensity ratios of 155/161, 143/145, 111/115 and 175/218.

The use of pattern recognition techniques to discriminate these bond types is of course possible, but is only useful when one uses a set of features which are relevant from a spectroscopic point of view. This has been indicated amongst others by Vink (6) and Nägeli (8). So in this case one should use the above mentioned ratios to generate a decision vector and not just all the masses

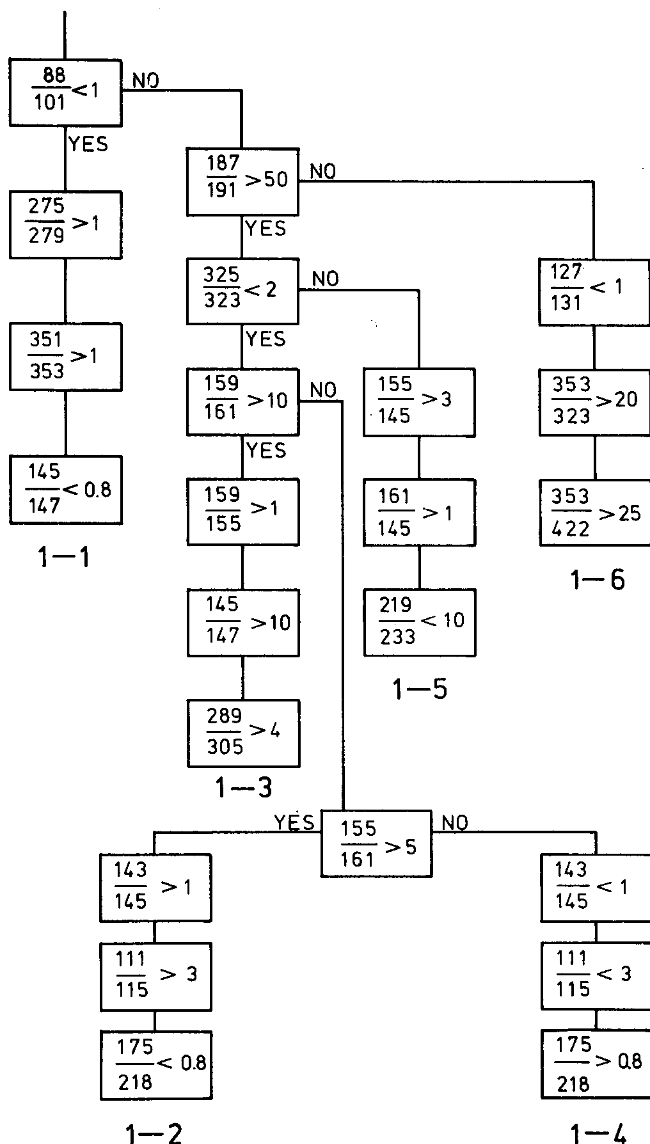

FIG. 6. Flowchart for discriminating between different glycosidic bond types.

and intensities. Besides, the ratio of the number of compounds to be classified to the number of dimensions used for the vectors must be at least 5 (8).

Of course it would be very interesting not only to be able to distinguish the spectra of these permethylated disaccharides, but also to be able to indicate how the different ions are formed and in this way perhaps to explain the differences in their spectra.

For this purpose we have measured the metastable transitions in the first field free region for all ions of interest by scanning the accelerating voltage with constant ESA-voltage.

Furthermore we measured the exact mass value of a large number of ions. We also had at our disposal four disaccharides which were perdeuteromethylate and two disaccharides which had a methoxy-group at carbon atom-1 of the reducing unit and OCD_3-groups on all other carbon atoms. The spectra of the latter compounds enabled us to determine how many OCH_3-groups a particular ion contains and whether carbon atom-1 is involved. In this way we were able to assign a plausible structure to most ions, but to explain their intensity differences is hardly possible. These data will be published elsewhere, but we would like to mention one peculiar example: the formation of mass 307 in trehalose.

Mass 307 appears to be $C_{14}H_{27}O_7$ from HRP-measurements, contains 6 methoxy-groups, is formed from the molecular ion and has a daughter

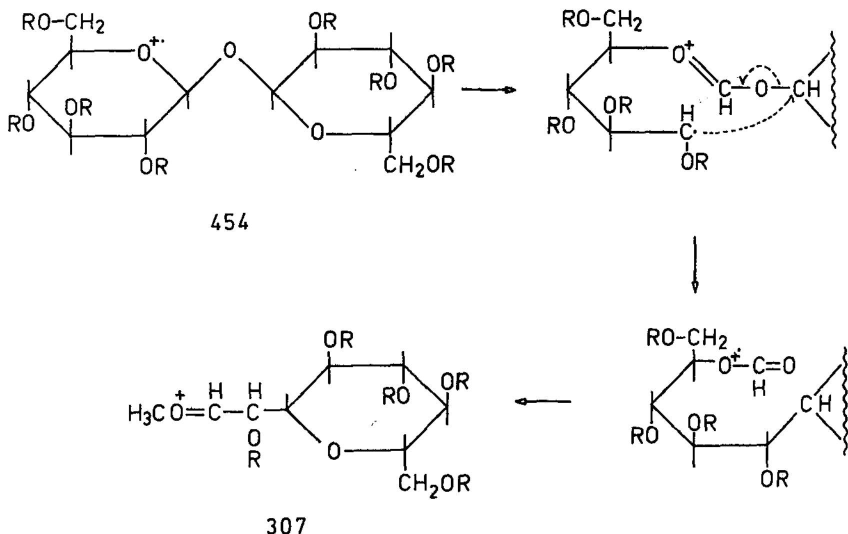

FIG. 7. The formation of mass 307 in trehalose.

ion at mass 275. This data could only be explained by a peculiar rearrangement reaction. After ring opening between C1 and C2 of the non-reducing unit, carbon atom-1 recombines with the C1 atom of the reducing unit. This intermediate then gives mass 307 by splitting off mass 147, which is also present in the spectrum of trehalose. This rearrangement reaction is depicted in Fig. 7.

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