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Note

Linkage analysis in disaccharides by electrospray mass spectrometry

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Analysis of the structures of complex carbohydrates requires knowledge of the identity, anomeric configuration, and sequence of the sugar residues, and identification of the reducing terminus and the positions of the glycosidic linkages.

Desorption-m.s. and f.a.b.-m.s. are powerful techniques for determining the sequence, the pattern of branching, and the molecular weight of oligosaccharides containing up to 30 sugar units, and the structure of the aglycon[1–4]. Negative-ion tandem-f.a.b.-m.s. can be used to discriminate between the linkage positions in underivatised oligosaccharides[5,6].

Electrospray (e.s.) ionisation has also been described[7–9]. Although most of the applications have been concerned with the determination of molecular weights and sequencing of proteins, some studies have shown that it can be applied to carbohydrates, and we now report its application in the linkage analysis of reducing disaccharides.

The negative-ion e.s.-mass spectra of (1→2)- (sophorose), (1→3)- (laminaribiose), (1→4)- (cellobiose), and (1→6)- (gentiobiose), β-linked glucodisaccharides shown in Figs. 1–4, respectively, reflect the positions of the linkages. Each mass spectrum contains peaks at *m/z* 341 for $(M-H)^-$ and at *m/z* 179 and 161 associated with fragmentation which involves the glycosidic linkages. In addition to these peaks, there are peaks at *m/z* 323 (sophorose, Fig. 1), 281 (cellobiose and gentiobiose, Figs. 3 and 4), 263 (sophorose and cellobiose Figs. 1 and 3), 251 (gentiobiose, Fig. 4), and 221 (sophorose, cellobiose, and gentiobiose, Figs. 1, 3, and 4). For laminaribiose, only the peaks at *m/z* 341, 179, and 161 are present (Fig. 2).

The peak at *m/z* 323 is due to loss of water from the $(M-H)^-$ ion, and those at *m/z* 281, 263, 251, and 221 are associated with fragments from the sugar rings which are diagnostic of the position of the linkage. These fragmentations are likely to involve the reducing moiety, since the non-reducing moieties are identical in the four disaccharides.

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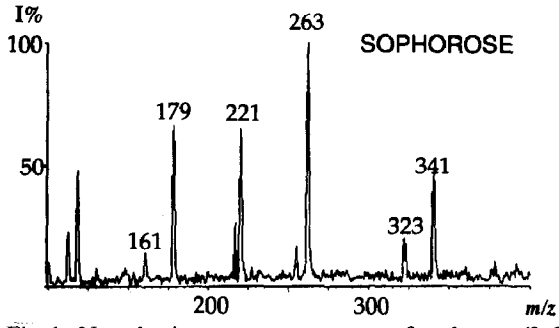

Fig. 1. Negative-ion e.s.-mass spectrum of sophorose (2-*O*-β-D-glucopyranosyl-D-glucopyranose).

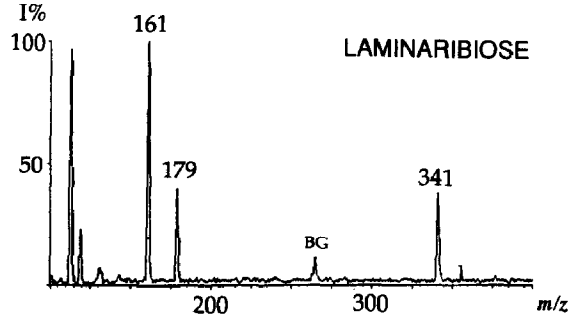

Fig. 2. Negative-ion e.s.-mass spectrum of laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucopyranose): BG indicates a background peak at *m/z* 265.

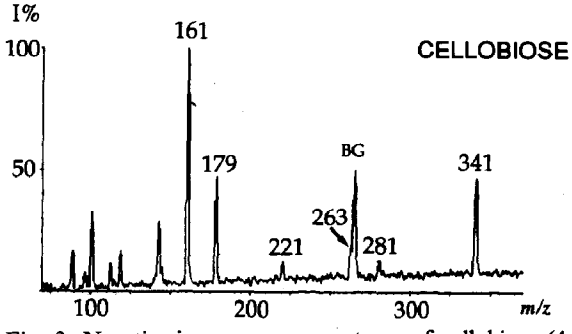

Fig. 3. Negative-ion e.s.-mass spectrum of cellobiose (4-*O*-β-D-glucopyranosyl-D-glucopyranose): BG indicates a background peak at *m/z* 265.

The structures and fragmentation pathways of the ions present in the negative-ion e.s.-mass spectra are summarised in Table I, using the system proposed by Domon and Costello[10] and illustrated in Fig. 5. The data do not allow unequivocal conclusions to be drawn and the structures and fragmentation pathways are tentative.

The negative-ion e.s.-mass spectrum of maltose (Fig. 6) is similar to that of cellobiose (Fig. 3), except for the higher intensity of the ion at *m/z* 221. Maltose and cellobiose differ only in the configuration at C-1′ and this, apparently, has little influence on the fragmentation of the $(M-H)^-$ ions as shown also in f.a.b.-m.s. studies[5,6].

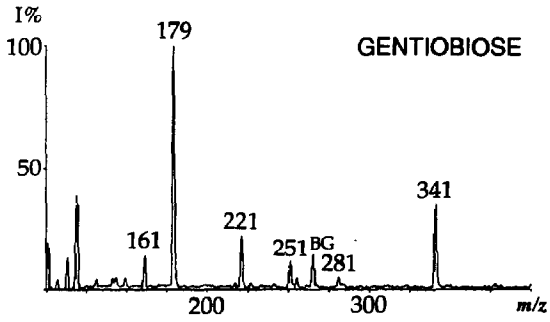

Fig. 4. Negative-ion e.s.-mass spectrum of gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucopyranose): BG indicates a background peak at *m/z* 265.

TABLE I

Structures and fragmentation pathways[a] in the negative-ion e.s.-mass spectra of disaccharides

Ion (m/z)	*Sophorose*	*Laminaribiose*	*Cellobiose*	*Gentiobiose*
341	C_2^-	C_2^-	C_2^-	C_2^-
323	B_2^-			
281			$^{0,2}A_2^-$	$^{0,2}A_2^-$
263	$(^{0,4}A_2 - H_2O)^-$			
251				$^{0,3}A_2^-$
221	$^{1,3}A_2^-$		$^{2,4}A_2^-$	$^{0,4}A_2^-$
179	C_1^-	C_1^-	C_1^-	C_1^-
161	B_1^-	B_1^-	B_1^-	B_1^-

[a] For symbolism used, see ref. 10.

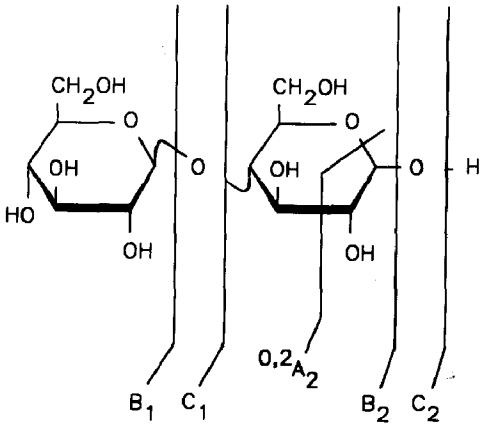

Fig. 5. Designation of the fragments in the negative-ion e.s.-mass spectrum of cellobiose: $^{0,2}A_2$ represents the fragment at *m/z* 281.

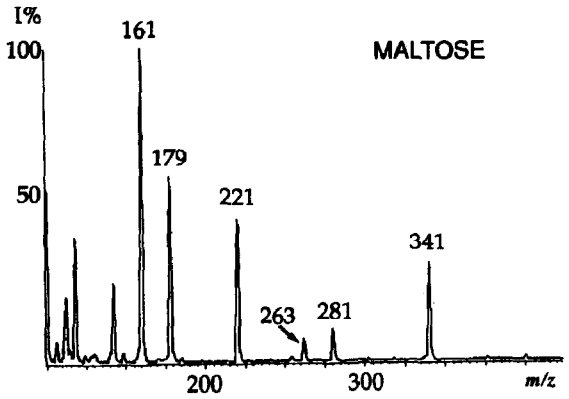

Fig. 6. Negative-ion e.s.-mass spectrum of maltose (4-*O*-α-D-glucopyranosyl-D-glucopyranose).

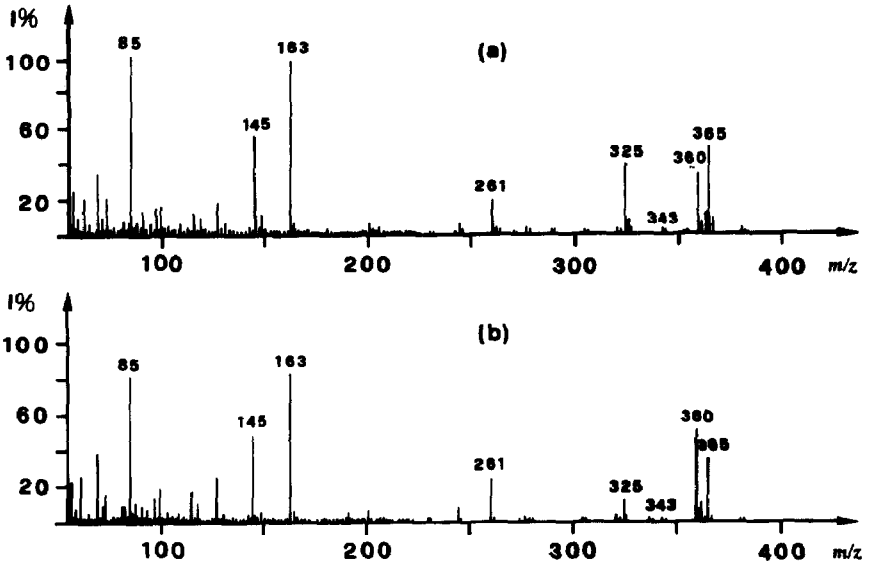

Fig. 7. Positive-ion e.s.-mass spectra of (a) cellobiose and (b) gentiobiose.

The positive-ion e.s.-mass spectra of cellobiose and gentiobiose (Fig. 7) are similar and do not allow linkage analysis.

Under the standard negative-ion e.s. conditions, selective deprotonation of HO-1, which is more acidic than the other hydroxyl groups, is to be expected, and this drives the fragmentation of the reducing moiety to give the ions that are diagnostic of the linkage position. In the positive-ion mode, protonation is likely to occur unselectively and several fragmentation patterns result. F.a.b.-m.s. data have been explained in the same manner[5,6].

By using e.s. ionisation, it is not necessary to resort to tandem mass spectrometry as in f.a.b. experiments[5,6]. Careful choice of the parameters is essential (see Experi-

mental) in order to obtain pseudomolecular ions with the appropriate internal energy to produce useful fragmentations.

EXPERIMENTAL

Materials. — All of the compounds analysed were obtained commercially and were used without purification.

Mass spectrometry. — A VG BioQ triple-quadrupole mass spectrometer with the standard e.s. source was used as a single stage instrument, *i.e.* ions were detected after they were separated by the first mass analyser. The extent of fragmentation was influenced greatly by the difference in potential between the last accelerating plate in the ion source and the skimmer cone before the mass analyser. Lower values of this potential favoured the detection of pseudomolecular ions, and higher potentials induced extensive fragmentation. A potential of 80 V was used for the negative-ion mass spectra and 150 V for positive-ion mass spectra. Negative-ion mass spectra were obtained by injecting $\sim 10\ \mu L$ of solutions (~ 10 mg/mL) in 10mM ammonium acetate–acetonitrile (1:1) in the flow of the same solvent delivered at $5\ \mu L$/min into the e.s. source by an h.p.l.c. pump. The signals lasted for a few minutes, and several scans could be collected which were summed to give the final mass spectrum. Positive-ion mass spectra were obtained under similar conditions.

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