An Approach to Oligosaccharide Sequencing by Mass Spectrometry

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Summary The mass spectra of some di- to penta-saccharides as their 1-phenylflavazole peracetates were measured, and relationships between spectra and structures established.

PROGRESS in peptide sequencing by mass spectrometry,[1] and recent steps towards oligonucleotide analysis by this method,[2] indicate the possibility for oligosaccharide sequencing. Sugars, because of their involatility and low thermal stability, are unsuitable for mass spectrometric analysis. The spectra of numerous derivatives of mono- and di-saccharides have been reported,[3] but apart from a few studies on trisaccharides[4] and a single study on a tetrasaccharide,[5] the mass spectra of higher oligosaccharides have not been measured.

Selected ions in the mass spectra of 1-phenylflavazole peracetates of some disaccharides[a]

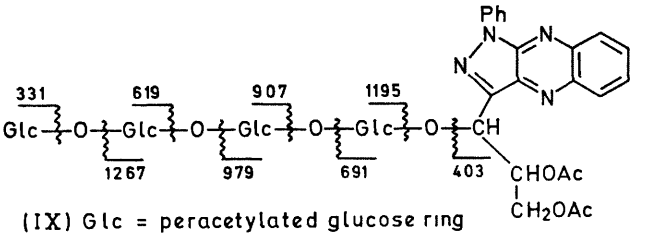
Disaccharide	Linkage	$M.^+$	$M.^+-42$	*m/e* 419	*m/e* 403	*m/e* 344[b]	*m/e* 331	*m/e* 317	*m/e* 301	*m/e* 169
Maltose (I)	1→4	0 8	0	1 6	8 5	6 4	17	0 9	14	43
Cellobiose (II)	1→4	4 5	0	1 3	6 5	2 4	23	1 0	12	54
Lactose (III)	1→4	1 8	0	1 0	7 9	4 4	40	0 5	14	31
Gentiobiose (IV)	1→6	1 8	0 8	0 2	4 8	0 1	10	10	20	38
Isomaltose (V)	1→6	1 4	1 5	0	4 5	0 9	11	11	19	38
Melibiose (VI)	1→6	1 1	1 8	0	6 0	0 3	21	12	19	20

[a] All data are for ion abundances relative to *m/e* 43 = 100% Spectra were all recorded under identical conditions at 170° using an AEI MS 9 mass spectrometer

[b] Corrected for contribution of the ^{13}C isotope of *m/e* 343

In our approach an aromatic group is incorporated into the molecule in order (i) to stabilize the molecular ion, and (ii) to allow sequencing by directing the fragmentation of the oligosaccharide [6] The peracetates of the 1-phenylflavazoles[7] were suitable derivatives moreover their fluorescence was an advantage in small scale experiments (1 μmole of maltose, for example, was successfully derivatized and analysed)

The mass spectra of the 1-phenylflavazole peracetate derivatives of six disaccharides (Table) were measured first to assess the value of this derivative in providing molecular ions and in allowing deductions of structure Molecular ions were of adequate abundance the major fragment ions arose by glycosidic and allylic cleavages accompanied by further acetic acid and keten eliminations and 1 → 4 and 1 → 6 glycosidic linkages could be readily distinguished using any one of a number of ions or ion groups which included (i) *m/e* 418—420, (ii) $M.^+ - CH_2CO$



(IX) Glc = peracetylated glucose ring

and $M.^+ - CH_2CO - CH_3CO_2H$ (iii) *m/e* 316—318, (iv) *m/e* 273—276 and (v) *m/e* 344 †‡ In line with previous results for carbohydrates,[3] epimeric compounds could not be distinguished by their mass spectra

The 1-phenylflavazole peracetate derivatives of maltose, maltotriose (VII), maltotetrose (VIII), and maltopentose (IX) all gave detectable molecular ions that of the pentasaccharide (M = 1614) had an abundance of 0 01%

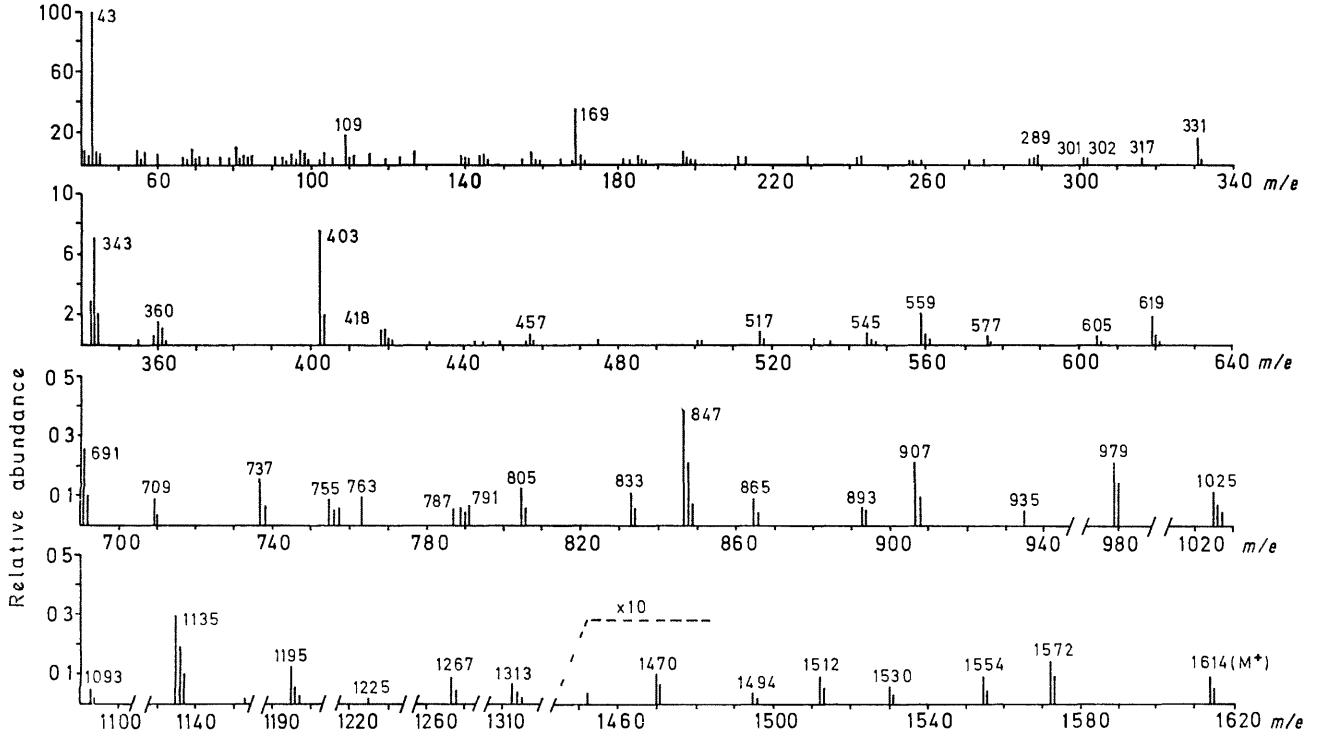


FIGURE *The 70ev mass spectrum of the pentasaccharide* (IX)

† Rationalization of these differences is straightforward for the most part and will be given elsewhere

‡ Complete spectra of all the compounds discussed are available upon request

relative to the *m/e* 43 base peak (Figure). Identical bond cleavages occurred in compounds (VII)—(IX) as shown by the fact that ions differing by 288 mass units were common to the three spectra. The major cleavage processes for the maltopentose derivative are indicated; particularly significant is the "sequential" fragmentation of the glycosidic linkages, starting from either end of the molecule. The ions indicated, together with their daughters due to acetic acid and/or keten loss, constituted virtually all the major ions above *m/e* 350 in the spectrum.[8]

In addition to the molecular weight of an oligosaccharide and the sequence of masses of its constituent monosaccharides—items which our results suggest are available from the mass spectra of oligosaccharides—the stereochemistry of the monosaccharide units and the positions of the glycosidic linkages are of interest. It is not yet possible to determine the stereochemistry of monosaccharides by mass spectrometry;[3] while there are some distinguishing features in the mass spectra‡ of the peracetylated 1-phenylflavazole derivatives of manninotriose (X) and isomaltotriose(XI) there is no evidence that configuration can be inferred from these spectra. The position of the glycosidic linkage is easily determined in disaccharides and a comparison of the spectra‡ of the derivatives of maltotriose (VII), manninotriose (X), isomaltotriose (XI), panose (XII), maltotetrose (VIII), and isomaltotetrose (XIII) suggested that some of the same criteria could be used to determine the position of the first glycosidic linkage§ in higher oligosaccharides. In particular, the $M.^+ - 42/M.^+$ abundance ratio is high when the first glycosidic linkage is 1 → 6 [18, 9, and 30, for compounds (X), (XI), and (XIII)], but only 0·4, 0·3 and 0·4 for (VII), (VIII), and (XII), which have 1 → 4 linkages. The nature of the second and subsequent glycosidic linkages can be determined from the ease with which acetic acid is lost by the fragment ions belonging to the sequence which does not retain the flavazole [*viz. m/e* 619, 907, and 1195 in compound (IX)]. In every case these ions lose acetic acid readily, if, and only if, the lowest numbered glycosidic linkage (adjacent to that cleaved in forming the ion in question) is 1 → 4. (Sequence ion — AcOH)/sequence ion ratios of 1·0—2·5 were observed for 1 → 4 linkages but the ratios for 1 → 6 linkages were 0·04—0·10.

We are modifying our procedure by using permethylation. Since our method is limited to oligosaccharides which can form 1-phenylflavazoles we are testing other heteroaromatic groups as derivatives.

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§ Numbering is made from the 1-phenylflavazole peracetate end of the molecule.

[1] D. W. Thomas, B. C. Das, S. D. Gero, and E. Lederer, *Biochem. Biophys. Res. Comm.*, 1968, **32**, 199; J. H. Jones, *Quart. Rev.*, 1968, **22**, 302.

[2] J. J. Dolhun and J. L. Wiebers, *J. Amer. Chem. Soc.*, 1969, **91**, 7755, and references therein.

[3] For leading references see (a) N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydrate Chem.*, 1966, **21**, 39; (b) N. K. Kochetkov, O. S. Chizhov, and N. V. Molodtsov, *Tetrahedron*, 1968, **24**, 5587; (c) J Kärkkäinen, *Carbohydrate Res.*, 1969, **11**, 247; (d) D. C. DeJongh, T. Radford, J. D. Hriber, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 1969, **91**, 1728.

[4] V. Kovacik, S. Bauer, and J. Rosik, *Carbohydrate Res.*, 1968, **8**, 291. See also reference 3(b).

[5] D. C. DeJongh, S. D. Hribar, S. Hanessian, and P. W. K. Woo, *J. Amer. Chem. Soc.*, 1967, **89**, 3364.

[6] For a somewhat related approach to disaccharides, see J. Karliner, *Tetrahedron Letters*, 1968, 3545.

[7] Prepared using *o*-phenylenediamine and phenylhydrazine and purified using cellulose t.l.c. See *Methods Carbohydrate Chem.*, 1963, **2**, 136.

[8] Loss of 301 mass units and (301 + 288) mass units from the molecular ion also gave fairly abundant ions which probably arise by C-6 acetate migration to C-1 in the terminal monosaccharide. This is a common rearrangement in sugar derivatives; compare K. Heyns and D. Muller, *Tetrahedron*, 1965, **21**, 3151.