PERACETYLATED 1-PHENYLFLAVAZOLES AS CARBOHYDRATE DERIVATIVES FOR MASS SPECTROMETRY

PART II. APPLICATIONS TO TRI-, TETRA-, AND PENTASACCHARIDES[1]

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ABSTRACT

Mass spectra of tri-, tetra-, and pentasaccharides, as their 1-phenylflavazole peracetates, contain readily identifiable molecular-ions and prominent fragment-ions that allow the sequential determination of the masses of the individual monosaccharide constituents. In addition, these spectra contain features that allow distinction between (1→4) and (1→6) glycosidic linkages at each linkage position.

INTRODUCTION

Although a few derivatives of tri- and tetrasaccharides have been subjected to mass spectrometric analysis[2], this method could make a far greater contribution to important biological problems involving oligosaccharide structure, were suitable derivatives available. The encouraging results obtained from mass spectrometric study of 1-phenylflavazole peracetate derivatives of mono- and disaccharides[1], prompted investigation of these derivatives with carbohydrates of higher molecular-weight.

Mass-spectral analysis of oligosaccharides involves some features that are common to oligopeptide and oligonucleotide analysis[3]. In particular, abundant and easily distinguishable sequence-ions are desirable. The 1-phenylflavazole derivatives, with the unique elemental composition of their heteroaromatic moiety, seemed well suited to fill this requirement; moreover, the ease with which glycosidic cleavage occurs in the mass spectra of carbohydrates virtually guaranteed that sequence ions would be prominent in these spectra.

EXPERIMENTAL

O-α-D-Glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose (panose), *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose (maltotriose), *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-

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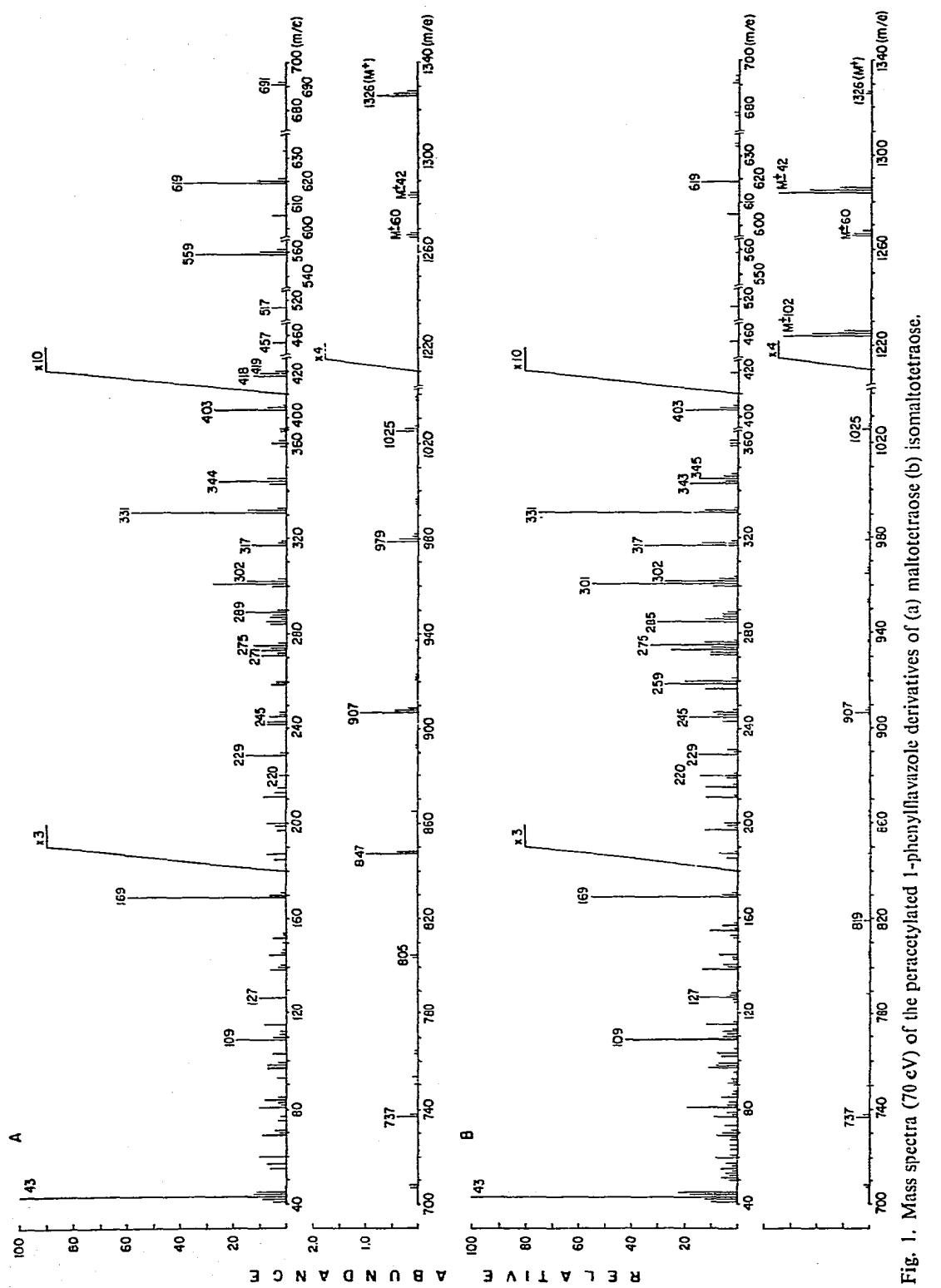

Fig. 1. Mass spectra (70 eV) of the peracetylated 1-phenylflavazole derivatives of (a) maltotetraose (b) isomaltotetraose.

(1→4)-D-glucose (maltotetraose), and *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose (maltopentaose) were purchased from commercial sources. *O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-D-glucose (4-*O*-β-D-glucosylgentiobiose) was a gift from Dr. I. J. Goldstein, University of Michigan Medical School. Cellodextrins, *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucose (cellotriose) and *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucose (cellotetraose) and isomaltodextrins, *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose (isomaltotriose) and *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose (isomaltotetraose) were obtained by acid hydrolysis of cellulose and dextran, respectively. *O*-α-D-Galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-D-glucose (manninotriose) was prepared by invertase-catalyzed hydrolysis of stachyose. In all cases, individual components in the hydrolyzates were isolated by chromatography on poly(acrylamide) gel.

Derivatization of the higher oligosaccharides was effected as for the disaccharides, except that the organic phase of a 1:3:3:6 benzene–pyridine–water–butyl alcohol system was used to develop the thin-layer plates. Mass-spectrometric analysis was effected as described in the preceeding paper[1]. The source temperatures varied from 210° for the trisaccharide derivatives to 260° for maltopentaose1-phenylflavazole peracetate.

DISCUSSION

Because of its aromatic nature, the presence of the 1-phenylflavazole group in carbohydrate derivatives is expected, upon electron impact, to generate relatively stable, hence relatively abundant, molecular ions. In order to test this contention in the case of trisaccharides, mass spectra of the peracetate of maltotriose* (**1**) and the 1-phenylflavazole peracetate of maltotriose (**2**) were compared. Compound **1** showed a molecular ion of only trace abundance (*ca.* 0.005% relative to the base peak, *m/e* 43), whereas the molecular ion of compound **2** was 2–3 orders of magnitude more abundant (1.3% relative abundance).

Mass spectra of maltotetraose 1-phenylflavazole peracetate (**3**) and isomaltotetraose 1-phenylflavazole peracetate (**4**) are reproduced in Fig. 1. The corresponding derivative of cellotetraose (**5**) gave a spectrum that closely resembled that of compound **3**. The spectrum of maltopentaose 1-phenylflavazole peracetate (**6**) has been previously reproduced[4]. In addition to compound (**2**), the five other trisaccharide 1-phenylflavazole peracetate derivatives (**7–11**) listed in Table I were studied. Because the trisaccharide spectra are predictable from the two tetrasaccharide spectra (Fig. 1) and because essential features appear in Fig. 2. and Table 1, the full spectra are not reproduced**. Molecular ions were observed for all derivatives, and their relative abundances are recorded in Table 1.

*Assayed as the mixture of α and β anomers produced by pyridine-catalyzed acetylation of maltotriose at room temperature.

**These spectra are presently available from the authors.

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TABLE I

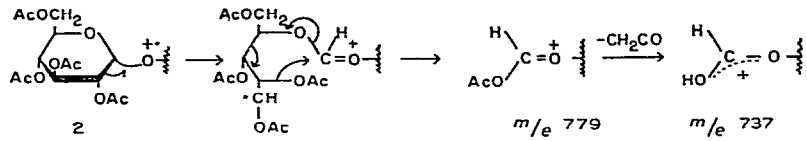
ASSIGNMENT OF LINKAGE POSITIONS OF THE FIRST GLYCOSIDIC BOND[a]

Parent sugar	M^+	$M^+ - 42/M^+$	$M^+ - 102/M^+$	m/e *418*	m/e *344*	m/e *317*[b]
Maltotriose (2), α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc	1.30	0.30	0.20	7.5	50.7	5.4
Cellotriose (7) ,β-D-Glc-(1→4)-β-D-Glc-(1→4)-D-Glc	1.07	0.075	0.37	0.56	9.0	1.0
Panose (9), α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc	0.60	0.53	0.63	3.3	45.1	7.8
4-*O*-β-D-Glucosylgentiobiose (8), β-D-Glc-(1→4)-β-D-Glc-(1→6)-D-Glc	0.41	2.07	0.54	0.0	1.5	4.2
Isomaltotriose (10) α-D-Glc-(1→6)-α-D-Glc-(1→6)-D-Glc	0.34	4.85	3.94	0.0	0.8	8.3
Manninotriose (11), α-D-Gal-(1→4)-α-D-Gal-(1→6)-D-Glc	1.0	11.4	11.2	0.0	1.1	22.9
Maltotetraose (3) ,α-D-Glc[(1→4)-α-D-Glc]$_2$-(1→4)-D-Glc	0.19	0.26	0.11	1.2	7.9	4.1
Cellotetraose (5), β-D-Glc-[(1→4)-β-D-Glc]$_2$-(1→4)-D-Glc	0.44	0.33	0.09	0.7	11.3	3.0
Isomaltotetraose (4), α-D-Glc[(1→6)-α-D-Glc]$_2$-(1→6)-D-Glc	0.025	17.2	16.4	0.0	0.1	11.8
Maltopentaose (6), α-D-Glc-[(1→4)-α-D-Glc]$_3$-(1→4)-D-Glc	0.01	1.3	1.0	0.6	0.5	2.3

[a]Ion abundances have been corrected for ^{13}C isotopic contributions. [b]See text for comments on this ion.

Most of the more-abundant ions in the spectra arise as a result of glycosidic cleavage. Thus, two groups of Type A ions[5] (originating with *m/e* 331 and *m/e* 649) occur in the trisaccharides, three such groups in the tetrasaccharides, and four groups (*m/e* 331, *m/e* 619, *m/e* 907, and *m/e* 1195) are seen for the pentasaccharide. The alternative mode of cleavage at each of the glycosidic linkages also gives rise to an abundant series of ions (*m/e* 403, 691, 979, and 1267), here termed Type Z ions, which in turn generate groups of daughter ions by multiple elimination of ketene and acetic acid. It is to be emphasized that the parent member of each Type A or Type Z group of ions is a sequence ion, and that sequence determination of the masses of the monomeric units from either end of the molecule is therefore feasible.

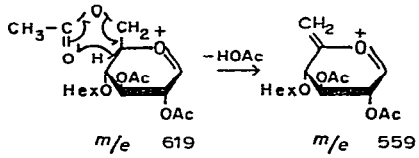
A third series of prominent ions appears in spectra of these compounds at $M^+ - (301 + 288n, n = 0, 1, 2...)$. The well known skeletal rearrangement[6] illustrated for compound **2** would account for these ions.



Scheme 1

The nature of the first* glycosidic linkage can be inferred (Table 1) by using criteria developed in analyzing the disaccharide spectra[1]. It is to be noted, however, that the abundance of *m/e* 317 is no longer reliable, the best criteria being the $M^+ - 42$, and the *m/e* 418 ions.

Unexpectedly, perhaps, the linkage positions of the second and higher glycosidic bonds can also be determined unambiguously. The feature used is the extent to which the primary, Type A ion can eliminate acetic acid**. The nice contrast between the behavior of those trisaccharides containing a (1→4)-linked second glycosidic bond, and the compounds in which this linkage is a (1→6)-, is illustrated in Fig. 2. The identity of the acetic acid lost has not been established by deuterium labelling of specific positions, but elimination across the C-5–C-6 bond (see illustration) seems reasonable because of the following features: (i) the process, as drawn, is not possible



Scheme 2

*Numbered from the flavazole-containing terminus.

This elimination was confirmed by the observation of prominent metastable peaks at *m/e* 505.4 in compounds **2, **3**, **5**, **6**, **7** and **8**; at *m/e* 791.0 in compounds **3**, **5** and **6**; and at *m/e* 1078.0 for compound **6**. It was further substantiated by the shift of *m/e* 619 to 640, and *m/e* 559 to *m/e* 577, in the spectrum of the trideuterioacetylated maltotriose 1-phenylflavazole[7].

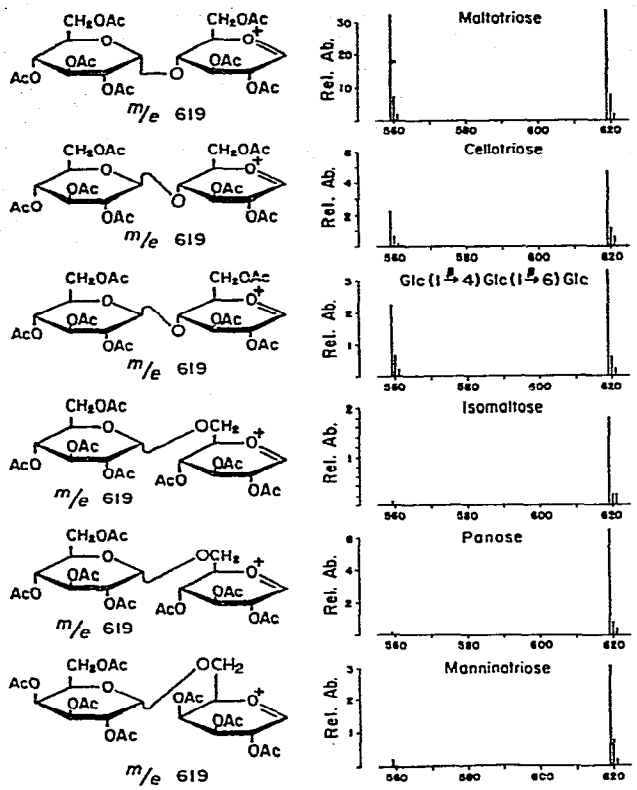


Fig. 2. Partial mass spectra of the 1-phenylflavazole derivatives of some trisaccharides, illustrating the assignment of the position of the second glycosidic linkage.

in the (1→6)-linked ions, (ii) the product ion is conjugated, and (iii) the electronegative oxonium ion may activate the C-5 hydrogen atom. It is not obvious why ions arising by a similar elimination across the C-4–C-5 bond in the (1→6)-linked, primary, Type A ions are relatively low in abundance. Their near non-appearance may be due to facile further fragmentations: in any event, whenever the linkage to the charge-containing pyranose ring is (1→4), a prominent daughter-ion, due to loss of acetic acid, appears; whenever the linkage to the charge-containing ring is (1→6) the secondary ion is of relatively low abundance. In Table 2, the validity of this criterion in all of the compounds examined is shown.

CONCLUSION

As with the mono- and disaccharide derivatives, mass spectra of the 1-phenylflavazole peracetate derivatives of tri-, tetra-, and pentasaccharides produce spectra that contain measurable molecular-ions; that for maltopentaose being of low abundance. Thus the pentasaccharide probably represents an upper limit to the complexity of sugars for which the molecular ion may in this manner be reliably detected. Criteria

TABLE II

ASSIGNMENT OF LINKAGE POSITIONS OF THE SECOND AND SUBSEQUENT GLYCOSIDIC BONDS

Trisaccharides	*2nd linkage*	m/e *559* / m/e *619*				
Maltotriose	α-D-(1→4)	0.94				
Cellotriose	β-D-(1→4)	0.49				
4-*O*-β-D-Glucosylgentiobiose	β-D-(1→4)	0.69				
Isomaltose	α-D-(1→6)	0.052				
Panose	α-D-(1→6)	0.031				
Manninotriose	α-D-(1→6)	0.065				
Tetrasaccharides	*2nd linkage*	m/e *847* / m/e *907*	*3rd linkage*	m/e *559* / m/e *619*		
Maltotetraose	α-D-(1→4)	0.40	α-D-(1→4)	0.89		
Cellotetraose	β-D-(1→4)	0.25	β-D-(1→4)	0.68		
Isomaltotetraose	α-D-(1→6)	0.086	α-D-(1→6)	0.071		
Pentasaccharide	*2nd linkage*	m/e *1135* / m/e *1195*	*3rd linkage*	m/e *847* / m/e *907*	*4th linkage*	m/e *559* / m/e *619*
Maltopentaose	α-D-(1→4)	2.56	α-D-(1→4)	1.72	α-D-(1→4)	1.12

for distinguishing (1→4) from (1→6) glycosidic linkages in these compounds are established. Although derivatives containing other types of glycosidic linkages have not as yet been studied, the extention of this method for the identification of additional linkage types seems entirely possible. Investigation of oligosaccharides containing deoxy or amino sugars might also prove interesting.

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