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Note

High-performance liquid chromatographic separation of methyl glycosides

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In previous work[1] we noted the ability of reversed-phase high-performance liquid chromatography (HPLC) to resolve the anomers of partially methylated monosaccharides. In some cases the resolution of anomers was quite marked, and indicated an important role for the anomeric centre in the separation process. It was considered that the methyl glycosides might be capable of resolution, and the present work shows that in many cases this is so.

EXPERIMENTAL

The HPLC system consisted of the following Waters Assoc. (Milford, MA, U.S.A.) instruments: M6000 pump, U6K injector, R401 refractive index detector, and radial compression module RCM100. The column was a Waters "Dextropak" plastic cartridge, 10 × 1 cm, which for use was compressed in the RCM100. The eluent was distilled water. This is a specialist reversed-phase column developed for the separation of carbohydrate oligomers with water as eluent[2], but which also separates methyl glycosides well. Samples reported here were run at a flow-rate of 2 ml/min, though some trials were run also at lower flow-rates in attempts to achieve better resolution. Standard methyl glycosides were provided by Professor S. J. Angyal and Dr. J. D. Stevens (School of Chemistry, University of New South Wales, Kensington, Australia). Methanolic hydrogen chloride solution (1 %, w/v) was made by adding acetyl chloride to dry methanol. Glycoside formation was carried out by dissolving the dried, pulverised sugar (100 mg) in the above solution (20 ml). The reaction flask was shaken continuously at room temperature. Samples (3 ml) were removed at intervals, and the acid was removed by treatment with Amberlite IRA-400 (HCO_3^-) resin. The methanol was removed in a rotary evaporator at 40°C, and the sample was dissolved in water, filtered (0.45 μm, Millipore) and injected into the chromatograph.

Initially the pure standard of each available glycoside was injected. Appropriate standard mixtures were then made up and injected to show the resolution achieved. Finally the glycosides formed in methanolic HCl were injected in sequence to determine the formation pattern.

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RESULTS AND DISCUSSION

Fig. 1 shows that the four methyl glycosides of ribose are completely resolved. As expected[3], during glycoside formation the furanosides appeared quickly (b). Substantial amounts of the pyranosides are formed only on prolonged shaking (d), at least under the experimental conditions used. Note that free ribose is apparently resolved into anomers. This observation was checked several times with ribose from different sources and was confirmed.

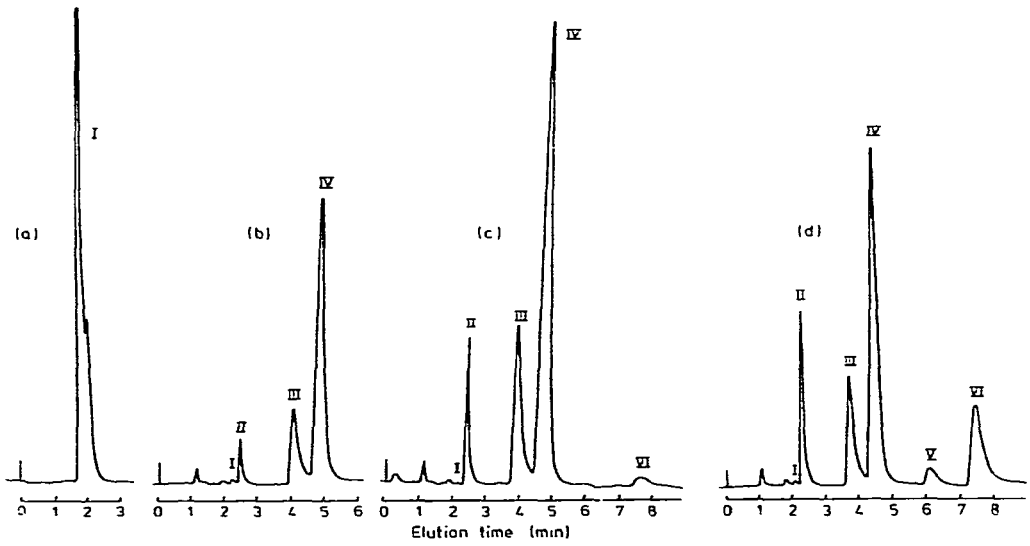

Fig. 1. Separation of methyl glycosides of D-ribose on a Dextropak column. Eluent, water. Flow-rate, 2 ml/min. (a) Ribose before glycosylation; (b) 1 h glycosylation; (c) 4 h glycosylation; (d) 24 h glycosylation. Peaks: I = D-ribose; II = methanol; III = methyl-α-D-ribofuranoside; IV = methyl-β-D-ribofuranoside; V = methyl-α-D-ribopyranoside; VI = methyl-β-D-ribopyranoside.

Xylose also showed partial resolution into two peaks. It is possible that the second peaks for ribose and xylose are furanose forms, as substantial amounts of these are found in ribose and xylose solutions.

Fig. 2 shows that the four glycosides of glucose are also resolved. A substantial amount of glucose is still present after 24 h. This amount of residual free sugar was greatest in the aldohexoses glucose, galactose and mannose, less in fructose, and least in the aldopentoses.

Fig. 3 shows the formation and separation of the methyl fructosides. In keeping with previous observations[4] the methyl α-D-fructopyranoside was formed in quite low amounts.

Table I shows the retention times of glycosides of all the sugars studied. In all cases the furanosides formed most quickly.

Some generalizations concerning elution order can be made:

(a) In the aldohexose series the pyranosides elute before the furanosides, and in general are close together.

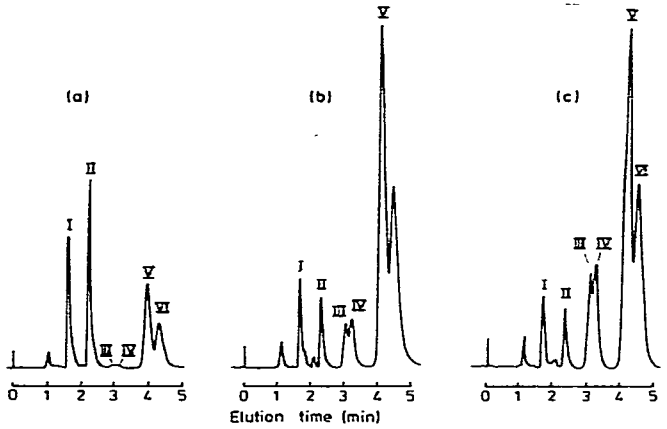

Fig. 2. Separation of methyl glycosides of D-glucose on a Dextropak column. Eluent, water. Flow-rate, 2 ml/min. (a) 2.5 h glycosylation; (b) 8 h glycosylation; (c) 24 h glycosylation. Peaks: I = D-glucose; II = methanol; III = methyl-α-D-glucopyranoside; IV = methyl-β-D-glucopyranoside; V = methyl-α-D-glucofuranoside; VI = methyl-β-D-glucofuranoside.

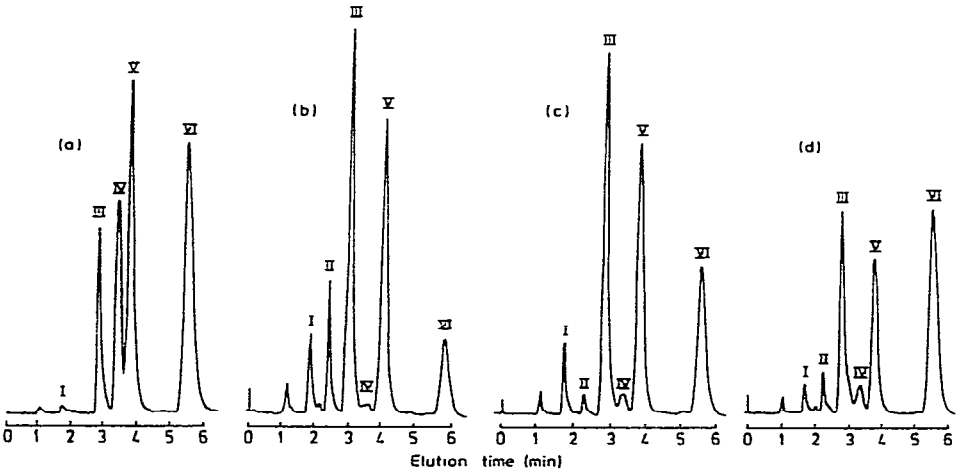

Fig. 3. Separation of the methyl glycosides of D-fructose on a Dextropak column. Eluent, water. Flow-rate, 2 ml/min. (a) Standard mixture; (b) 1 h glycosylation; (c) 4 h glycosylation; (d) 24 h glycosylation. Peaks: I = D-fructose; II = methanol; III = methyl-β-D-fructofuranoside; IV = methyl-α-D-fructopyranoside; V = methyl-α-D-fructofuranoside; VI = methyl-β-D-fructopyranoside.

(b) In the aldopentose series, the furanosides elute first, and are in general well resolved.

(c) Though not enough reference compounds are available to prove the point, the general tendency (Table I) is that the α anomer elutes before the β anomer of the same ring form, the only provable exception being the fructofuranosides.

The glycoside resolutions possible with this column should allow:

(a) Convenient study of relative rates of formation of the various glycosides.

(b) Quick optimisation of conditions to form maximum amounts of a chosen glycoside, using the present or other[5] methods of glycosylation.

TABLE I
RETENTION TIMES OF METHYL GLYCOSIDES
Column, Dextropak. Eluent, water. Flow-rate, 2 ml/min. a = Standards available, but not resolved; b = standards not available.

Sugar	*Methyl glycoside*		*Retention time (min)*
D-Glucose	α-Pyranoside		3.0
	β-Pyranoside		3.2
	α-Furanoside		4.1
	β-Furanoside		4.4
D-Fructose	β-Furanoside		2.8
	α-Pyranoside		3.4
	α-Furanoside		3.8
	β-Pyranoside		5.5
D-Galactose	α-Pyranoside, β-Pyranoside	a	2.5
	α-Furanoside, β-Furanoside	b	3.1, 3.5
D-Mannose	α-Pyranoside		4.2
	β-Pyranoside, α-Furanoside, β-Furanoside	b	5.0, 5.3
D-Ribose	α-Furanoside		3.7
	β-Furanoside		4.4
	α-Pyranoside		6.1
	β-Pyranoside		7.4
D-Xylose	α-Furanoside, β-Furanoside	b	3.5, 3.75
	α-Pyranoside		4.5
	β-Pyranoside		5.3
L-Arabinose	α-Furanoside, β-Furanoside	b	3.3, 3.7
	α-Pyranoside, β-Pyranoside	a	4.5

(c) Isolation of small amounts of a chosen glycoside. The Dextropak column can handle single sample loadings of up to 8 mg.

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