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Note

High-pressure liquid chromatography of isomeric oligosaccharides that form part of the complex-type carbohydrate chains of glycoproteins

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The high-pressure liquid chromatography (h.p.l.c.) of sialo-oligosaccharides using a system that is capable of separating structural isomers has been previously reported[1]. This chromatographic technique was applied successfully to biosynthetic studies, facilitating the identification of the various products obtained *in vitro* by the action of ovine submaxillary gland[2], fetal calf liver[3], and human placenta[4] sialyltransferases. Likewise, studies of the specificity of other glycosyltransferases, such as D-galactosyl- or *N*-acetyl-D-glucosaminyl-transferases, would also benefit from a similar rapid and unambiguous identification of the transferase products. Several neutral oligosaccharides that form part of the *N*-glycosyl-linked, complex-type carbohydrate chains of glycoproteins, *e.g.*, α_1-acid glycoprotein[5], have been synthesized[6–10]. These oligosaccharides contain 2-acetamido-2-deoxy-D-glucosyl residues linked at various positions, thus yielding various sets of structural isomers (Table I).

TABLE I

RELATIVE RETENTION TIMES IN H P L C. OF OLIGOSACCHARIDES

Oligosaccharide	*Abbreviation*	R_{LNT}[a]
1	Di-2	0.37
2	Di-6	0.47
3	Tri-2	0.77
4	Tri-6	0.85
5	Tri-2,4	0.74
6	Tri-2,6	0.85
8	Penta-2,4	1.08
9	Penta-2,6	1.12
10	Hepta	1.30

[a]Retention times relative to lacto-*N*-tetraose (**7**). For conditions, see Experimental section

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It appeared that every set of isomers could be resolved effectively on a column of Lichrosorb-NH_2 (Figs. 1 and 2) by use of a mobile phase and an elution program similar to those applied previously for the separation of sialo-oligosaccharides[1]. As expected, retention times increased with molecular size (Table I). Recently, Turco[11] observed that branching of high-mannose-type oligosaccharides increases the retention time, which is in agreement with our experience with sialo-trisaccharides[1]. However, such an observation should not be generalized, as shown by the relative retention times of the four trisaccharides [Tri-2 (**3**), Tri-6 (**4**), Tri-2,4 (**5**), and Tri-2,6 (**6**)] given in Table I. Firstly, the retarding influence of branching can be compensated by the elution-accelerating effect of the replacement of a

hexose by an *N*-acetylhexosamine residue[11–13]. Secondly, the positions of the branching point also have a significant effect, as shown by comparing the mobilities of the two branched trisaccharides Tri-2,4 (**5**) and Tri-2,6 (**6**) and of the pentasaccharides Penta-2,4 (**8**) and Penta-2,6 (**9**) (Table I). It appears that oligosaccharides having a (1→6) linkage are invariably eluted later than the corresponding isomers having a (1→2) or (1→4) linkage. Therefore, the presence of a (1→6) linkage rather than a branching point might be the structural feature that is associated with an increased elution time.

The ability to separate isomeric substances renders this technique ideally suitable for studies on the specificity of glycosidases and glycosyltransferases, where it is of extreme importance to be able to discriminate between different types of substitution. Similarly, this technique may be of value in structural studies of oligosaccharides.

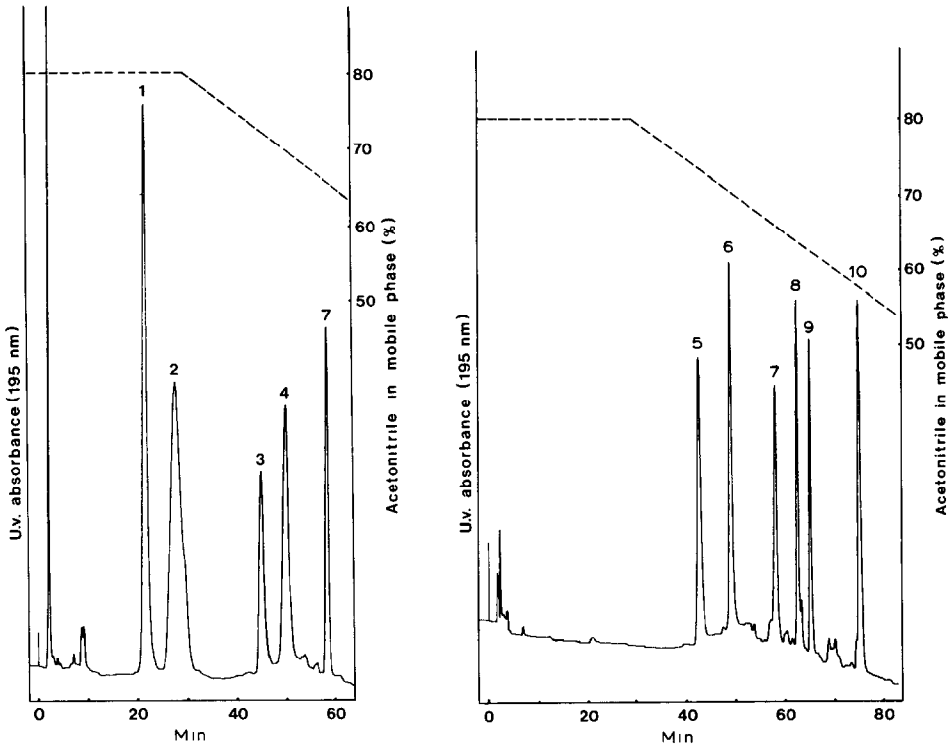

Fig. 1. High-pressure liquid chromatography separation of linear oligosaccharide isomers: Di-2 (**1**), Di-6 (**2**), Tri-2 (**3**), and Tri-6 (**4**). The retention times relative to lacto-*N*-tetraose (**7**) are given in Table I. Chromatography was performed as described in the Experimental section.

Fig. 2. High-pressure liquid chromatography separation of branched oligosaccharide isomers. Tri-2,4 (**5**), Tri-2,6 (**6**), lacto-*N*-tetraose (**7**), Penta-2,4 (**8**), Penta-2,6 (**9**), and Hepta (**10**). The retention times relative to lacto-*N*-tetraose (**7**) are given in Table I. Chromatography was performed as described in the Experimental section.

EXPERIMENTAL

Oligosaccharides. — Lacto-*N*-tetraose (**7**) was a kind gift of Dr. V. Ginsburg, National Institutes of Health, Bethesda, Maryland. The other oligosaccharides that were used in this study were all synthesized as described before[6–10].

High-pressure liquid chromatography. — The separations were performed with a Perkin–Elmer Series 2 Liquid Chromatograph, equipped with a Rheodyne 7105 injection valve and with a Perkin–Elmer LC 75 variable-wavelength detector operating at 195 nm. The analytical column (4 × 250 mm) was prepacked with Lichrosorb-NH_2 (particle size 5 μm, Merck). The separation of the oligosaccharides was accomplished by an isocratic elution with a mixture of acetonitrile (Lichrosolve grade) and de-ionized, distilled water containing 15mM potassium phosphate (pH 5.2) during the first 30 min, after which a linear gradient was applied increasing the buffer content by 0.5% per min. The flow rate was 2 mL/min throughout this study. The working pressure was 5–10 MPa. Samples (10–20 μL) containing 2–3 nmol of oligosaccharide were injected. When injecting 0.3 nmol of oligosaccharide the signal-to-noise ratio was 4:1.

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