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Carbohydrate analysis with ion chromatography using Eurokat stationary phases

Preparative separation of monosaccharides and their fluorinated derivatives

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ABSTRACT

The interaction of weakly acidic monosaccharides with a polystyrene sulphonate in the H^+ form resulted in unexpected selectivity for epimeric aldohexoses, deoxyaldohexoses and deoxyfluoroaldohexoses. This led to a new application of high-performance liquid chromatography. The separation is dominated solely by the electrostatic interaction between carbohydrate oxygen atoms and H^+ of the stationary phase. Column heating was not required. High flow-rates in excess of 7 ml/min were possible, thus allowing preparative separations. Examples include 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose and 2-deoxy-2-fluoro-D-galactose.

INTRODUCTION

Synthetic fluorinated carbohydrate analogues (*e.g.*, Fig. 1) offer unique advantages for studies on the carbohydrate biochemical pathways by non-invasive techniques using ^{19}F NMR spectroscopy or positron emission tomography from ^{18}F-labelled compounds. For example, the replacement of the hydroxyl group at the C-2 of D-galactose with fluorine permitted studies of the *in vivo* metabolism of 2-deoxy-2-fluoro-D-galactose [1]. It was confirmed by *in vivo* studies that 2-deoxy-2-fluoro-D-galactose has potential as a UTP-depleting chemotherapeutic agent in D-galactose-metabolizing tumours such as hepatocellular carcinoma. The compound also may be used at tracer doses as a diagnostic agent, *in vivo* or *in vitro* pinpointing defects of enzymes in galactosaemia [1].

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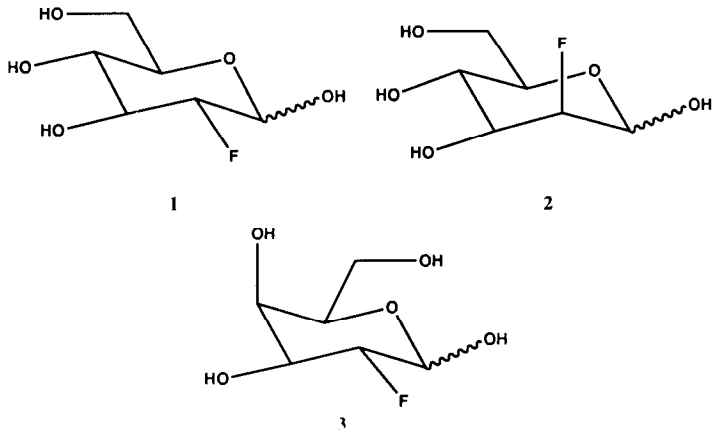

Fig. 1. Fluorination of tri-O-acetyl-D-galactal or -glucal using fluorine diluted to 5% in neon yielded compounds 1, 2 and 3. The reaction is the most straightforward preparation of 2-deoxy-2-fluoro-D-galactose (3), but its isolation and purification was previously tedious.

The chemical and biochemical aspects of these fluorinated carbohydrates and their syntheses by various chemical procedures have been described [2].

It must be noted that particular efforts are necessary in the preparation and purification of fluorinated carbohydrates. These difficulties in synthetic chemistry persisted in chromatography. As fluorinated carbohydrates are used as diagnostic agents in the human body when labelled with the radioactive isotope ^{18}F, it was of crucial importance to provide very pure compounds. Thus a chromatographic procedure was required for the analytical and preparative separation of such mixtures. Consequently, our studies were focused on efficient analytical procedures for the separation of ^{18}F-labelled tracers, on reliable quality control procedures for deoxyfluoroaldohexoses and on practical methods for the isolation of deoxyfluoroaldohexoses on a semi-preparative scale. A high selectivity for epimeric compounds was required, allowing the determination of 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose and 2-deoxy-2-fluoro-D-talose in multicomponent mixtures. The eluent should preferably be compatible with physiological fluid and high flow-rates should be possible.

EXPERIMENTAL

Fluorinated carbohydrates were prepared routinely by fluorination of unsaturated 3,4,6-tri-O-acetyl derivatives using molecular fluorine diluted in an inert gas [3]. The acidic aqueous solution of products, after hydrolysis with 1 *M* hydrochloric acid, was neutralized using AG1-X8 anion-exchange resin (Bio-Rad Labs.) in the OH^- form. Chromatography was run on a Waters 600 multi-solvent delivery system using manual injection (U6K injector). Detection was usually effected with a Waters 410 differential refractometer at 35°C. The columns used were 30 cm × 8 mm I.D. and 25 cm × 32 mm I.D., packed by Eurochrom Knauer (Berlin, Germany) with an improved 9% cross-linked polystyrene sulfonate resin in the H^+ form as stationary

phase. Standard chromatographic conditions employed were 0.8 ml/min with water at room temperature in analytical applications and 7 ml/min with water in preparative applications. Low carbohydrate concentrations were detected by a pulsed amperometric detector (Dionex) with post-column addition of 0.5 *M* sodium hydroxide solution at 1ml/min; the detector settings were $E_1 = 0.06$ V, $E_2 = 0.6$ V and $E_3 = 0.8$ V with pulse durations of 300, 120 and 300 ms respectively.

RESULTS

Analytical and preparative chromatography of raw material, which was obtained by fluorination of 3,4,6-tri-O-acetyl-D-galactal is shown in Fig. 2. The preparative run yielded 680 mg of pure 2-deoxy-2-fluoro-D-galactose and 400 mg of pure 2-deoxy-D-galactose (Fig. 2a); the sample load was 2.5 g of raw material in 4 ml of water. The retention time for 2-deoxy-2-fluoro-D-galactose was 27 min. Re-injection of an aliquot under analytical conditions gave the chromatogram in Fig. 2b. A specific identity test by ^{1}H NMR confirmed that a purity of $\geqslant 98\%$ was obtained for 2-deoxy-2-fluoro-D-galactose. It was the first time that this compound could be obtained without further purification steps in such quality from the above-mentioned preparation procedure.

Although fluorine is considered in carbohydrates to be an isosteric substitute for hydrogen, the differences in hydrogen bonding and electronegativity make it more like a hydroxyl group. The strong electrostatic interaction of the fluorine substituent at C-2 with the anomeric effect suggested a variety of conformationally clearly different molecules, to which ligand-exchange chromatography with H^+ as counter ion

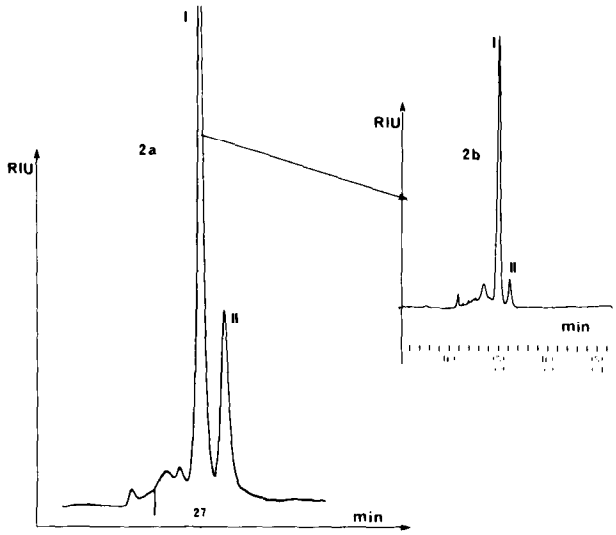

Fig. 2. (a) Preparative and (b) analytical high-performance liquid chromatography of 2-deoxy-2-fluoro-D-galactose, peak I. Peak II is 2-deoxy-D-galactose. As known from previous works [3], 2-deoxy-2-fluoro-D-idose and 2-deoxy-2-fluoro-D-gulose are minor impurities in the reaction mixture. These compounds are not mentioned here. Their enrichment is possible using Eurokat H^+ and peak shaving techniques.

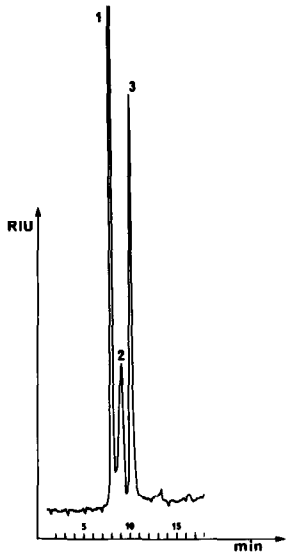

Fig. 3. Ligand-exchange chromatography of a mixture of epimeric 2-deoxy-2-fluoro-D-aldohexoses on a commercial 300 × 8 mm I.D. Eurokat H^+ column at room elution with water at 0.8 ml/min. Peaks: 1 = 2-deoxy-2-fluoro-D-glucose (capacity factor, $k' = 0.47$); 2 = 2-deoxy-2-fluoro-D-mannose ($k' = 0.69$); 3 = 2-deoxy-2-fluoro-D-galactose ($k' = 0.84$).

may apply. This could be demonstrated in this work. The separation of the epimeric aldohexoses and fluorodeoxyaldohexoses was dominated by hydrogen-bonding interactions between carbohydrate oxygen and carbohydrate fluorine atoms and H^+ of the stationary phase. The contribution of size-exclusion mechanisms to the separation was neglected in this investigation with monosaccharides as the only components. As mutarotation is catalysed by H^+, and the rates of partitioning between the stationary phase and the mobile phase are fast when only hydrogen bonding is involved, no column heating is required for speeding up the exchange kinetics. An excellent selectivity (Fig. 3) was obtained for the three 2-deoxy-2-fluoroaldohexoses in Fig. 1.

CONCLUSIONS

A convenient resin-based procedure for analytical and preparative carbohydrate separation by high-performance liquid chromatography has been developed. The direction and stereospecificity of interaction with the stationary phase is dominated by hydrogen bonding. The method is characterized by its simplicity despite its broad applicability. Tedious column conditioning does not interfere with routine analytical applications. The column material also allows preparative carbohydrate separations at a high flow-rate. The eluate is compatible with physiological fluid and can be recommended for radiopharmaceutical and pharmaceutical preparations. The application should therefore be in accordance with the EEC Directive on finished product specifications for drug compounds.

Excellent selectivity has been achieved for epimeric 2-deoxy- and 2-deoxy-2-fluorohexoses, but the procedures applies also to other fluorinated derivatives. The k' values are given in Fig. 3.

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