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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ISOLATION OF ^{14}C-LABELLED GLUCO-OLIGOSACCHARIDES, MONOSACCHARIDES AND SUGAR DEGRADATION PRODUCTS ON ION-EXCHANGE RESINS

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SUMMARY

Gluco-oligosaccharides, monosaccharides and sugar degradation products in biomass hydrolysates were isolated in milligram amounts by means of high-performance liquid chromatography on various ion-exchange stationary phases. Also, radioactively labelled carbohydrates and sugar decomposition products that had been obtained on hydrolysis of ^{14}C-marked biomass were isolated and are thus available as reference samples.

INTRODUCTION

The isolation of radioactive and non-labelled oligomeric sugars, monosaccharides and sugar degradation products is a major area of applied analysis in the medicine and food chemistry fields and also in biomass research. Further, the application of ^{14}C-labelled gluco-oligosaccharides could be of great importance for the determination of the structures of products of biological degradation reactions and also in the elucidation of reaction mechanisms, *e.g.*, in organic sugar synthesis. Moreover, the isolation of gluco-oligomeric carbohydrates of > 2 degree of polymerization (d.p.) offers the possibility of using these fractions as calibration standards.

In the literature, it is mainly the separation of oligosaccharides by means of gel permeation chromatography (GPC) that has been dealt with[1–7]. This method, however, has the disadvantage of being time consuming. There are several ways of separating the oligosaccharide group by high-performance liquid chromatography (HPLC), *e.g.*, the use of either propylaminosilane-modifed silica as the stationary phase with acetonitrile–water as the eluent or of octadecyl-modified silica[8–15]. In both procedures, the products of carbohydrate rearrangements or degradation cannot be separated in one analytical step, and in most instances where organic solvents are employed these can produce adverse effects in subsequent processing, *e.g.*, in lyophilization.

For the determination of oligosaccharides, monosaccharides and sugar decomposition products, the use of strong cation exchangers loaded with Ca, Pb, H or Ag and water as the mobile phase is appropriate[16–21].

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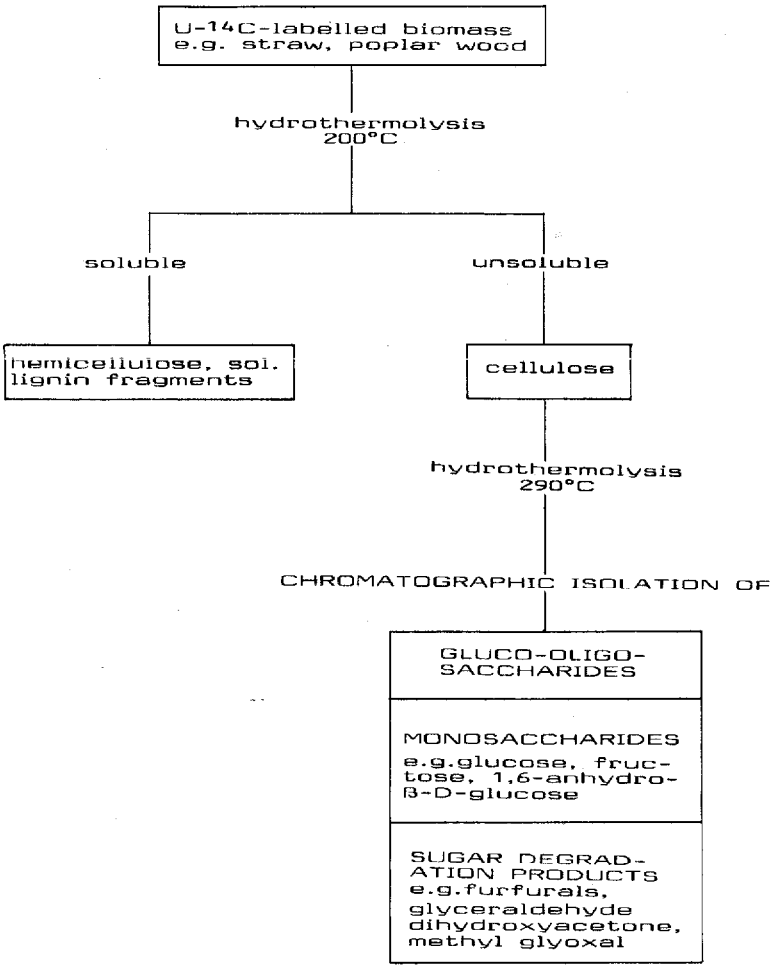

Scheme 1. Chromatographic isolation of biomass products.

When radioactively labelled lignocellulosic biomass, *e.g.*, poplar wood cultivated in a $^{14}CO_2$-gassed plant growth chamber, or commercially available U-^{14}C-labelled carbohydrates are subjected to hydrothermal degradation, the semi-preparative separation technique mentioned above allows the resulting labelled compounds to be isolated (Scheme 1)[22–25].

In this work, semi-preparative Ag- and H-loaded ion-exchange columns were used for separating the gluco-oligosaccharide moiety and the decomposition products of sugars present in biomass degradation solutions and for the fractionation of these compounds.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model 1330 pump (Bio-Rad Labs., Rich-

mond, CA, U.S.A.), an injection valve with a 20-μl, 100-μl and 1-ml loops (Valco Instruments, Houston, TX, U.S.A.), a Model 1770 differential refractive index detector (Bio-Rad Labs.), a solid scintillation detector (Isomess, F.R.G.), a column oven unit, a CR3A integrator (Shimadzu, Kyoto, Japan) and a Model 7000 Ultorac fraction collector (LKB, Bromma, Sweden).

Chromatographic isolation and analyses of fractions

For the semi-preparative isolation of the gluco-oligosaccharides and 1,6-anhydro-β-D-glucose, a 70% Ag-loaded sulphonated poly(styrene–divinylbenzene) copolymer ion-exchange column (300 × 10 mm I.D.) was used. In order to isolate the products of further sugar decomposition, *e.g.*, methylglyoxal, dihydroxyacetone and furfural, an H-loaded sulphonated poly(styrene–divinylbenzene) copolymer ion-exchange column (300 × 10 mm I.D.) (Bio-Rad Labs.) was employed. A refractive index detector was used. U-^{14}C-labelled compounds were monitored by means of a scintillation detector equipped with an yttrium silicate solid scintillation cell. Doubly distilled, degassed water was used as the mobile phase. The solutions were fractionated and lyophilized.

The purity of the isolated fractions was checked by comparing their retention times on different stationary phases and also by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The chromatographic conditions for each column system are given in the figure captions.

Preparation of ^{14}C-labelled poplar wood

^{14}C-labelled poplar plants were grown under a $^{14}CO_2$-containing atmosphere for a whole vegetation period. The specific activity of the poplar wood was 82 μCi/g (absolute dry).

The growth chamber was manufactured by Brown Boweri (Mannheim, F.R.G.) and allowed operation from −5 to 45°C. The relative humidity could be varied between 30 and 35%. The $^{14}CO_2$ gas supply was controlled by a microprocessor.

Hydrothermal degradation

The hydrothermolysis of lignocellulosic biomass, *i.e.*, percolation with pressurized water, was effected in the range 270–290°C[26]. The degradation solutions were fractionated. For chromatographic isolation of the compounds concerned the main fractions were used directly and the others after pre-concentration steps.

RESULTS AND DISCUSSION

Semi-preparative isolation on a silver-loaded ion exchanger

The wide range of biomass degradation and sugar decomposition products offers the possibility of the preparative isolation of radioactively labelled compounds that are commercially unavailable. Depending on the reaction temperature and percolation conditions, a higher proportion of gluco-oligomers or glucose degradation compounds than glucose is obtained. The gluco-oligomers reflect the extent to which the cellulose moiety of the original cellulose-containing material sample has been degraded.

In order to achieve the semi-preparative of gluco-oligomers, 1,6-anhydro-β-

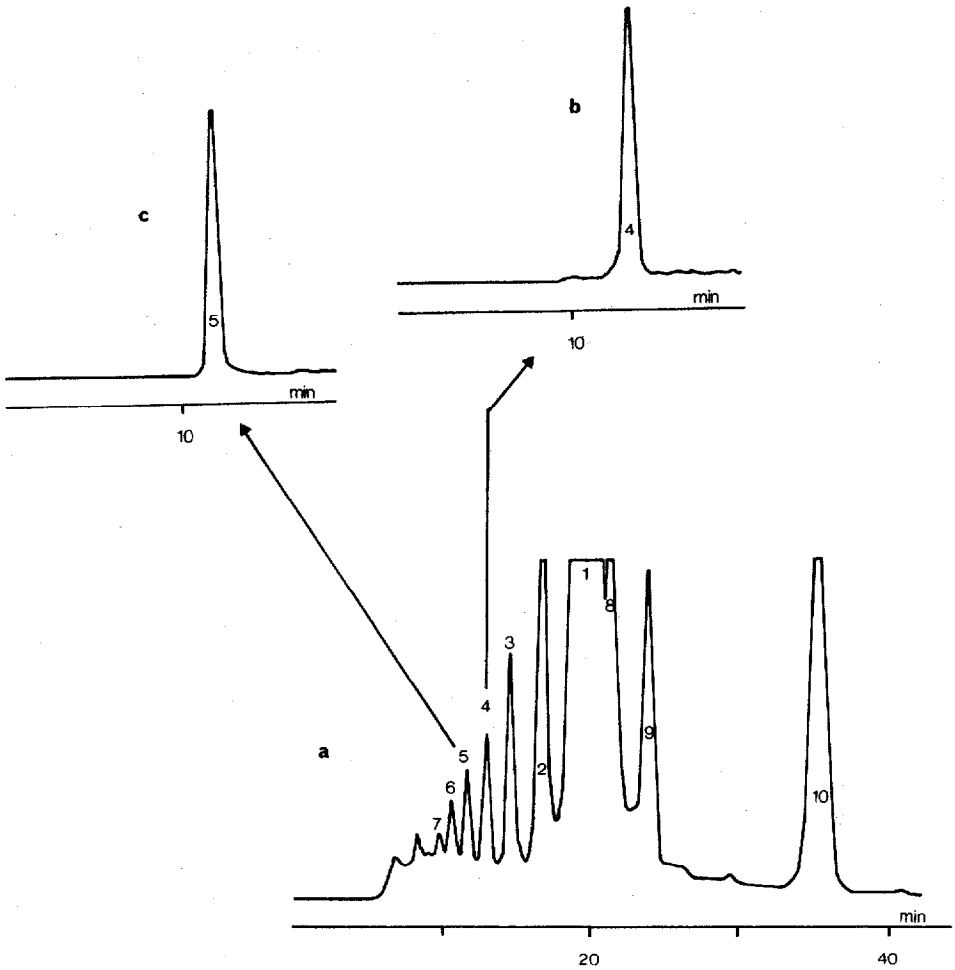

Fig. 1. (a) Semi-preparative HPLC separation of gluco-oligomers, 1,6-anhydro-β-D-glucose and hydroxymethylfurfural in poplar wood hydrolysates. (b) Isolated cellotetraose fraction. (c) Isolated cellopentaose fraction. Conditions: column, 70% Ag-loaded ion exchanger; mobile phase, water; flow-rate, 1.0 ml/min; column temperature, 85°C; detection, refractive index. Peaks: 1 = glucose; 2 = cellobiose; 3 = cellotriose; 4–7 = gluco-oligomers, numbers indicate d.p.; 8 = fructose; 9 = 1,6-anhydro-β-D-glucose; 10 = hydroxymethylfurfural.

D-glucose and sugar degradation products, a column was packed with an Ag-loaded ion-exchange resin. Following injection of 1 ml of degradation solution, gluco-oligomers up to d.p. 7 were isolated. Fig. 1a shows the semi-preparative separation of a poplar wood sample degraded hydrothermally at 290°C. Subsequently, the fractions were checked for purity by applying them directly on the Ag-loaded ion-exchange column. Fig. 1b shows the fractionated cellotetraose and Fig. 1c cellopentaose.

In addition, 1,6-anhydro-β-D-glucose (peak 9) could be isolated in the same analytical run as the oligosaccharides. Owing to the importance of this substance in stereoselective syntheses, its isolation from effluents of hydrothermal biomass decomposition by chromatography appears to be of particular interest.

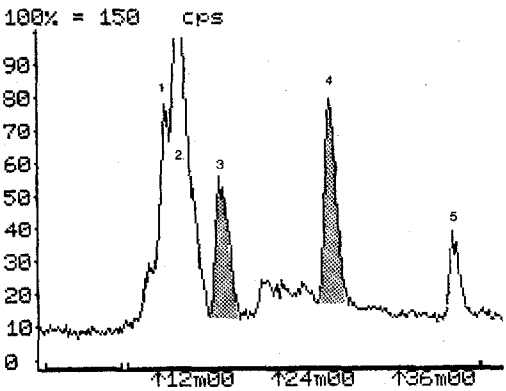

Fig. 2. Semi-preparative separation of ^{14}C-labelled sugar degradation products in poplar wood hydrolysate. Conditions: column, H-loaded ion exchanger; mobile phase, water; flow-rate, 1.0 ml/min; column temperature, 85°C; detection, solid scintillation; injection volume: 1 ml. Peaks: 1 = cellobiose; 2 = glucose; 3 = methyl glyoxal; 4 = HMF; 5 = furfural.

Further products resulting from carbohydrate decomposition, such as hydroxymethylfurfural (HMF; peak 10) can likewise be fractionated in the same step of the analysis, and isolated preparatively. This chromatographic separation with its analysis time of about 40 min can be compared with GPC separation methods in terms of yield. GPC, however, has the disadvantage of being time consuming, requiring 8–22 h for similar problems[27].

As only water is used as the mobile phase, the preparation of pure products from the individual aqueous fractions is easy, *e.g.*, by means of lyophilization. From hydrothermal biomass degradation solutions after a single pre-concentration step, gluco-oligosaccharides up to celloheptaose (*e.g.*, cellotriose up to 25 mg/ml), 1,6-anhydro-β-D-glucose up to 40 mg/ml and furfurals could be isolated in this way.

Semi-preparative isolation on a hydrogen-loaded ion exchanger

Although the H-loaded ion-exchange materials fail to separate the gluco-oligosaccharides, they do allow a semi-preparative separation to be achieved between monosaccharides and sugar decomposition products, *e.g.*, dihydroxyacetone, glyceraldehyde, methylglyoxal and furfurals. The products of carbohydrate cleavage and rearrangements can be obtained in even higher yields if the degradation temperature is increased (up to *ca.* 290°C).

Fig. 2 shows a separation profile of the effluent of a ^{14}C-labelled poplar wood degradation on an H-loaded sulphonated polystyrene–divinylbenzene copolymer as stationary phase with water as the mobile phase. The detection system used was a solid scintillation device. The shaded peaks correspond to the fractions collected. These compounds were isolated and lyophilized. In order to determine the degree of purity, methyl glyoxal (Fig. 3) and hydroxymethylfurfural (Fig. 4) were subjected to ion-exchange chromatography once again.

As with the gluco-oligomers, isolation of the purified compounds in milligram amounts can be achieved by lyophilization, *e.g.*, ^{14}C-labelled hydroxymethylfurfural up to 75 mg/ml. In order to isolate ^{14}C-labelled sugar conversion and degradation products, commercial U-^{14}C-labelled carbohydrates were hydrolysed. Hence the

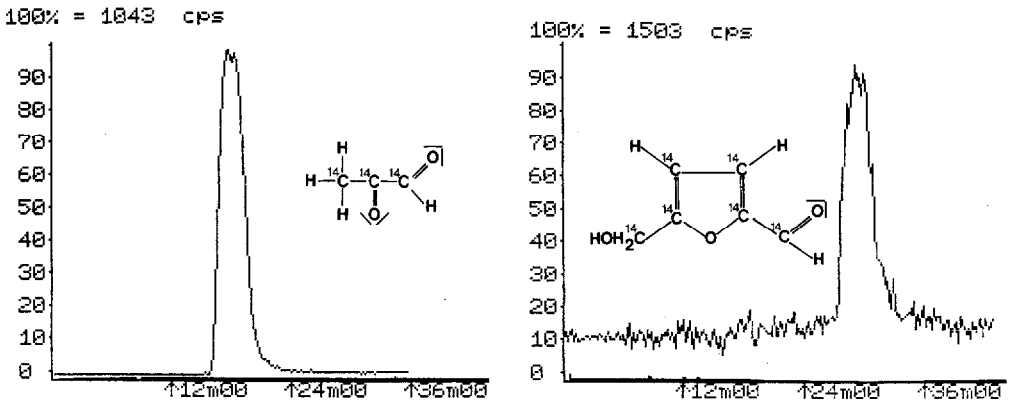

Fig. 3. Chromatogram of the isolated ^{14}C-labelled methyl glyoxal fraction. Chromatographic conditions as in Fig. 2; injection volume, 100 μl.

Fig. 4. Chromatogram of the isolated ^{14}C-labelled hydroxymethylfurfural fraction. Chromatographic conditions as in Fig. 2; injection volume, 10 μl.

products of these reactions can be isolated in the same way.

Finally, it is worth mentioning that radioactively labelled carbohydrate-derived compounds can profitably be employed for mechanistic investigations, kinetic studies, organic synthesis, as standards and for identification purposes.

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