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Note

Separation of acidic polysaccharides from *Ulmus glabra* Huds. on Mono P™

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The common elm, *Ulmus glabra* Huds, was in former days a tree of great value in Norway[1–3]. The inner bark of the elm contains special mucilage-containing sacs, and this mucilage was the reason for the interest in this tree. The powdered inner bark of elm was used as a wheat flour substitute when the harvest was poor. The powder had gelling and thickening properties and was believed to be of nutritional value. The inner bark of elm has also a long tradition as a medicinal remedy. The mucilage was thought to have strengthening, healing and astringent properties, and was used against ulcers, burns and inflamed surfaces. The viscosity and gelling properties of the mucilage led to its use as a regulator of the digestive system. The inner bark of Slippery Elm (*U. fulva*) has been an official drug in the United States Pharmacopoea[4]. The structure of the total mucilage isolated from *U. fulva* has been studied by Beveridge *et al.*[5] and Hirst *et al.*[6], whereas only preliminary studies of the structure of that from *U. glabra* have been performed[7].

Our aim was to study the structure of the mucilage isolated from the inner bark of *U. glabra*. Preliminary experiments indicated that the mucilage consisted of more than one polymer, and this paper reports a separation procedure for these polymers based on the column Mono P™.

EXPERIMENTAL

Preparation of the elm extract for chromatography

The inner bark of common elm (*U. glabra* Huds.) (200 g) was extracted with ethanol–water (3:1) at 80°C to remove low-molecular-weight material and coloured matter. The carbohydrate polymer was then extracted at 100°C with water, dialysed against distilled water and lyophilized. The crude extract thus obtained (14 g) was further purified after redissolution, which was achieved when the solution was kept at 100°C for 4 h. The solution obtained after centrifugation at 1800 *g* and filtration through Whatman GF/A glassfibre paper was then applied to a DEAE-Sepharose CL-6B column.

Preliminary separation on DEAE-Sepharose CL-6B

A column (60 × 5 cm I.D.) of DEAE-Sepharose CL-6B was converted into the chloride form and washed with distilled water prior to application of the partly

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purified elm extract (2 g). The column was coupled to a peristaltic pump P-1 (Pharmacia, Uppsala, Sweden), and fractions of 8.8 ml were collected in an Ultrorac 7000 fraction collector (LKB, Bromma, Sweden). The column was eluted at 1 ml/min, first with water (500 ml), then by gradient elution using 0 to 1 *M* sodium chloride for elution of the acidic polymers (Fig. 1). The eluent was monitored at 206 nm in a 2158 Uvicord SD detector, (LKB) with a Cole-Parmer (K8387-321) recorder (Chicago, IL, U.S.A.). The fractions were tested for carbohydrate content by the phenol–sulphuric acid method[8].

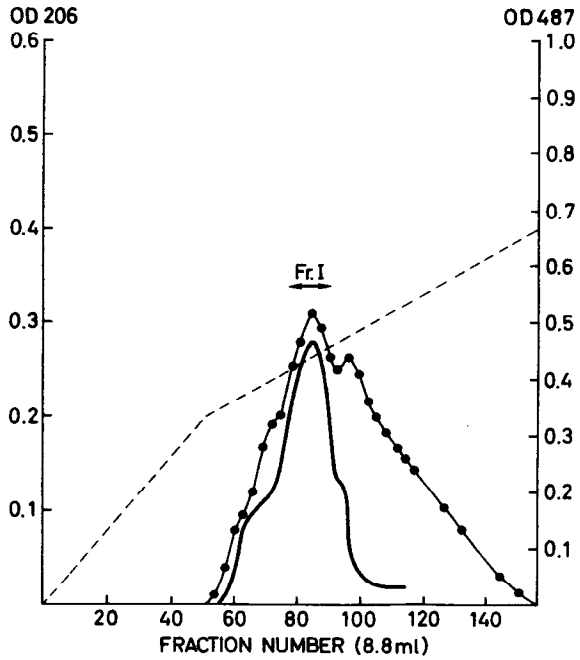

Fig. 1. Chromatography of the mucilage from common elm on a DEAE-Sepharose CL-6B column. Curves: ——— = carbohydrate profile[8]; ●—● = optical density at 206 nm (OD 206) (corrected for the absorption due to the salt gradient); – – – = salt gradient (0–1 *M* sodium chloride).

Separation and isolation of elm polysaccharides on a Mono P column in the fast protein liquid chromatography (FPLC) system

The anion-exchange column, Mono P, was fitted in the FPLC system (Pharmacia) consisting of two P-500 pumps, a gradient programmer GP-250, a V-7 valve, a UV-1 monitor at 214 nm, a FRAC-100 fraction collector and a two-channel recorder with event marker, REC 482. For analytical purposes 2 mg in 500 μl were injected, whereas for preparative purposes 10 mg in 5 ml were injected onto the column via a superloop. The column was eluted at 1 ml/min, using 0–1 *M* sodium chloride in 15 m*M* phosphate–sodium chloride buffer (pH 7). The elution programme is shown in Fig. 2. Fractions of 1 ml were collected and tested for carbohydrate content as above.

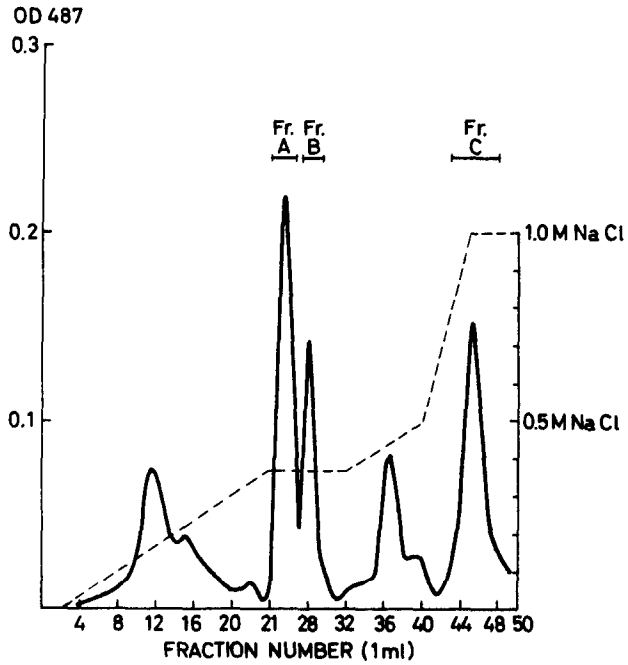

Fig. 2. Elution profile (carbohydrate) of Fr. I from Fig. 1. applied on a Mono P column in the FPLC system. The dashed curve represents the 0–1 *M* sodium chloride elution programme.

Quantitative determination of the carbohydrate composition

The samples (100–200 μg) were subjected to methanolysis with 1 *M* or 6 *M* hydrochloric acid in anhydrous methanol for 24 h at 80°C[9]. The reagent was removed with nitrogen and the methylglycosides dried *in vacuo* prior to conversion into the corresponding trimethylsilylethers.

The samples were subjected to gas chromatography analysis on a Carlo Erba 4200 Fractovap chromatograph (Milan, Italy) with a 430 LT programmer (Milan, Italy). The chromatograph was fitted with a Grob split-splitless injector, flame ionization detector, LCD Chromatography Integrator, Model 308/9 (Shannon, Ireland) and a REC-481 recorder (Pharmacia). The column was a DB-5 fused-silica capillary column (15 m × 0.32 mm I.D.) film thickness, 0.25 μm (Durabond™, J&W Scientific, U.S.A.). The carrier gas was hydrogen at 50 cm/sec. The samples were analysed under the following conditions: injector temperature, 230°C; detector temperature, 230°C; column temperature, 140°C when injecting, followed by an increase of 1°C/min to 170°C, then 6°C/min to 190°C and kept at 190°C for 5 min.

Molecular weight estimation by high-performance liquid chromatography (HPLC)

The HPLC system consisted of a LKB 2150 HPLC pump, a Rheodyne 7125 injector fitted with a 100-μl loop (Cotati, CA, U.S.A.), DuPont high-performance size-exclusion chromatography (HPSEC) columns SE 60, SE 100, SE 500, SE 1000 coupled in series (Wilmington, DE, U.S.A.), Optilab 5902 interference refractometer (Tecator AB, Höganäs, Sweden) and a Cole-Parmer K8387-32 recorder. The column

temperature was maintained at 30°C by a Thermomix 1441 (Braun, F.R.G.) thermostat in a water-bath. Dextrans T 70, T 250 and T 500 (Pharmacia) were used as standards. The eluent was 0.05 *M* acetate buffer (pH 4.7), and the flow-rate was 0.25 ml/min.

RESULTS AND DISCUSSION

The inner bark of *U. glabra* contains 7% water-soluble carbohydrate of polymeric nature. As the polymeric substances was difficult to dissolve after freeze-drying, redissolution was partly achieved when heating at 100°C for 4 h. This treatment might result in cleavage of a few glycosidic linkages, thus giving fragments with a somewhat lower molecular weight than that of the native polymer.

After centrifugation and filtration the extract was applied on a DEAE-Sepharose CL 6B column. The acidic polymers were eluted from the column by a salt gradient elution. As shown in Fig. 1, carbohydrate material was first eluted with 0.5 *M* sodium chloride, and total exclusion was achieved at 0.8 *M* sodium chloride. The elution profile clearly shows that several polymeric fractions of an acidic nature are present in the material extracted from the inner bark. However, it was not possible to obtain pure fractions on this column, and other media were tested.

The fraction marked Fr. I (Fig. 1) was first applied to a Mono Q™ column fitted in the FPLC system. This matrix is a strong anion-exchanger[10], and gave no separation of the material applied. The Mono P column, primarily developed for chromatofocusing of proteins[10], was then tested as a medium for the separation of acidic polysaccarides. Initial trials with 2 mg of polymer in 580 µl indicated that separation of the polymer into various fractions was possible under the conditions given in Fig. 2. As shown, at least nine different polymers were present in the fraction applied to the column. The column capacity allowed a sample load of 10 mg in 5 ml for preparative purposes. Larger amounts resulted in overlapping, particularly between fractions A and B (Fig. 2). Fractions A, B and C were rechromatographed in order to obtain pure polymers. The fractions thus obtained were analysed for carbohydrate composition and molecular weight (Table I).

TABLE I

CARBOHYDRATE COMPOSITION* AND MOLECULAR WEIGHT** OF VARIOUS FRACTIONS OF THE POLYSACCHARIDES ISOLATED FROM THE INNER BARK OF *U. Glabra*

Rha = Rhamnose; 3-O-Me-Gal = 3-O-methylgalactose; Gal = galactose; GalA = galacturonic acid; GlcA = glucuronic acid.

Fraction	*Carbohydrate ratio (%)*					$\overline{MW}$
	Rha	*3-O-Me-Gal*	*Gal*	*GalA*	*GlcA*	
I	20	7	56	17	trace	–
A	25	0	46	26	trace	475 000
B	34	0	32	28	trace	710 000
C	16	11	22	38	14	1 120 000

* Determined by fused-silica capillary gas chromatography of the derivatives of the corresponding methylglycosides.

** Estimated by HPLC with reference to dextrans.

The results show that the sugar 3-O-methylgalactose only is present in fraction C, which, as expected, contains the highest amount of uronic acids, both galacturonic and glucuronic. Only trace amounts of the latter are present in fractions A and B, which were also devoid of 3-O-methylgalactose. Fractions A and B are quite similar with respect to carbohydrate composition. As they are separated on the Mono P column, it may be that the uronic acid of fraction A is esterified to a somewhat higher degree than that in fraction B, thus resulting in a slight difference in the binding capacity to the matrix of the column. The position of the uronic acids can be different, and also the shape of the molecule. The molecular weights ($\overline{MW}$) of the fractions (Table I, Fig. 3) are given with reference to standard dextrans with a different molecular structure from the polymers isolated from the inner bark of the elm. As the shapes of these polymers probably differ from those of the dextrans, there is a great deal of uncertainty as to the real molecular weight of the fractions, but they certainly are different in size as well as in carbohydrate composition.

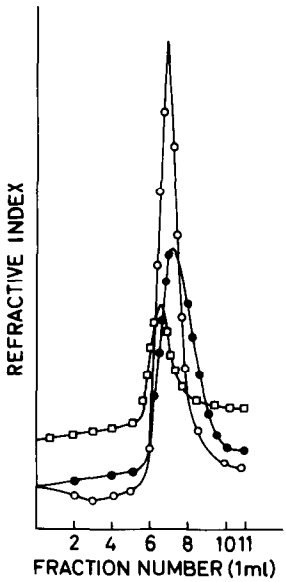

Fig. 3. Gel permeation chromatography of fractions A (●—●), B (○—○) and C (□—□) on HPSEC columns.

CONCLUSION

The results obtained on separation of polymers from the inner bark of common elm (*U. glabra*) clearly show that the chromatofocusing column, Mono P, is suitable for separation of acidic polysaccharides of pectin-like nature with minor apparent differences as to the carbohydrate composition.

ACKNOWLEDGEMENT

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