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Note

DEAHP-starch and DEAHP-cellulose, new ion exchangers for fractionating polysaccharides and other biopolymers

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DEAE-cellulose and DEAE-Sephadex, which have been extensively used in a variety of separation procedures, are commonly produced by the reaction of diethyl-2-chloroethylammonium chloride with a suitable support in an alkaline medium. Synthesis of this intermediate, however, is more complex and involves higher production costs[1] than the preparation of diethyl-2,3-epoxypropylamine[2] used in our laboratory for etherification of cross-linked starch and cross-linked microcrystalline cellulose. The resulting O-(3-diethylamino-2-hydroxypropyl) (DEAHP) derivatives were tested for their ability to fractionate polysaccharide mixtures.

It is the aim of the present paper to describe the preparation of DEAHP ion exchangers and to prove their ability to separate the components of polysaccharide mixtures.

EXPERIMENTAL

Materials and methods

Potato starch and microcrystalline cellulose cross-linked with epichlorohydrin in aqueous alkali medium[3,4] were used as basic supports. Pectin of *Salix alba* L. bark[5] and crude D-gluco-D-mannan from *Populus monilifera* H. wood[6] were the materials used for fractionation. Diethyl-2,3-epoxypropylamine was prepared by the reaction of epichlorohydrin with diethylamine[2].

One mole of the cross-linked polysaccharide (starch or cellulose) was activated by treatment with 0.005–1.5 mole of sodium hydroxide (0.4–17.5% solution) at 20° for 30 min. The activated polymer was then etherified by stirring it with 0.2–1.5 mole of diethyl-2,3-epoxypropylamine at 20° for 30 min and then at 50–60° for 2–6 h. When the reaction was completed, the ion exchanger was washed with water until neutral and then with dry acetone, and was finally dried at room temperature. Ion-exchange capacity of the DEAHP-adducts thus prepared was 0.5–3 mequiv./g, according to the reaction conditions applied.

For the separation of the components of polysaccharide mixtures, DEAHP-starch and DEAHP-cellulose with ion-exchange capacities of 2.1 and 1.4 mequiv./g and swelling volumes of 7 and 3.5 ml/g, respectively, were used. The ion exchangers were tested in glass columns fitted with PTFE pistons for sample introduction.

Column dimensions were 80 × 2 cm (DEAHP-starch) and 23 × 2.5 cm (DEAHP-cellulose). The column effluent fractions were monitored by measurement of optical rotation and by the phenol–sulphuric acid test[7]. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter on aqueous solutions at *ca.* 21°. For quantitative evaluation of sugars, gas chromatography of the corresponding alditol trifluoroacetates[8] was applied. Gas–liquid chromatography (GLC) was performed on a Hewlett-Packard 5750 G instrument, using a column (305 × 0.3 cm) of 1% (w/w) of XE-60 on 80–100 mesh Gas-Chrom Z, and a programmed temperature range 120–160° at 2°/min, with nitrogen as carrier gas at a flow-rate of 25 ml/min.

Fractionation of pectin on DEAHP-starch

DEAHP-starch (30 g) was swollen in 5 m*M* sodium formate (pH 5.5) and packed in a chromatographic column; 7 ml of 5 m*M* sodium formate solution (pH 5.5) that contained 600 mg of pectin was applied to the column and allowed to enter the bed slowly. After 3 h of adsorption, the polysaccharide fractions were eluted with 1.0 l of 5 m*M* sodium formate (pH 5.5) followed by gradient elution with 2.0 l of 5–600 m*M* sodium formate (pH 5.5), and with 1.0 l of 0.3 *M* sodium hydroxide. The effluent was collected in 3-ml fractions at a flow-rate of *ca.* 18 ml/h. The appropriate fractions were combined, de-ionized with Ionenaustauscher I (H^+) resin, evaporated to dryness, redissolved in water and recovered by freeze-drying. A portion (3 mg) of each fraction was hydrolyzed with 90% formic acid for 7 h at 100 °C and, after reduction with sodium borohydride, analyzed by GLC. The results for the fractionation are given in Table I.

Fractionation of crude D-*gluco*-D-*mannan on DEAHP-cellulose*

An aqueous solution (8 ml) containing 500 mg of hemicelluloses was adsorbed on to a column of DEAHP-cellulose (30 g, carbonate form). The column was washed with 800 ml of water followed by gradient elution with 1.0 l of 0–1 *M* ammonium carbonate, and was finally eluted with 1.0 l each of 0.1 and 0.3 *M* sodium hydroxide. The effluent was collected in 5-ml fractions at a flow-rate of *ca.* 30 ml/h. The appropriate fractions were combined and analyzed as described above. The results for the fractionation are shown in Table II.

RESULTS AND DISCUSSION

Sodium hydroxide present in the reaction of starch or cellulose with diethyl-2-chloroethylammonium chloride is consumed even during the etherification and also during the conversion of diethyl-2-chloroethylammonium chloride into free amine. These facts necessitate the use of an excess of sodium hydroxide in the reaction mixture, which, however, results in deterioration of rheological properties, especially those of starch.

On the other hand, only an amount of sodium hydroxide sufficient to catalyze the etherification is necessary with diethyl-2,3-epoxypropylamine and is not consumed during the reaction. The rheological properties of the polymers are therefore essentially unaffected, thus making possible further reduction in time in the manufacture of the final products.

Moreover, the synthesis of diethyl-2,3-epoxypropylamine is less expensive

TABLE I

FRACTIONATION OF PECTIN FROM THE BARK OF *SALIX ALBA* L. ON DEAHP-STARCH

Fraction	*Eluent*	*Yield (%)*	$[\alpha]_D$ (°)	*Molar ratios of monosaccharides*							*Uronic acids**
				Galactose	*Glucose*	*Mannose*	*Arabinose*	*Xylose*	*Rhamnose*	*Fucose*	
Pectin	–	100	+ 62	1.0	0.4	0.2	2.9	0.3	0.4	0.1	++
A	5 m*M* Sodium formate	8.4	– 21	1.0	0.9	1.0	8.1	1.8	–	–	–
B	5-600 m*M* Sodium formate	8.6	– 41.4	1.0	0.3	0.3	5.0	0.2	0.1	Trace	+
C		7.0	– 33.3	1.0	0.1	Trace	2.8	Trace	0.3	0.2	+
D		17.8	+ 34	1.0	Trace	Trace	3.0	0.1	0.4	0.1	++
E		15.3	+ 92	1.0	0.3	Trace	2.2	0.1	0.6	0.2	++
F		25.1	+ 128	1.0	0.2	–	2.8	0.3	0.8	0.1	+++
G		7.6	+ 161.4	1.0	0.4	0.2	2.2	0.2	0.5	0.2	+++
H	0.3 *M* Sodium hydroxide	8.9	+ 42.5	1.0	1.5	0.47	1.2	0.5	0.1	Trace	++

* +++, Present in large amouts; ++, moderate; +, present; –, absent.

TABLE II

FRACTIONATION OF CRUDE D-GLUCO-D-MANNAN FROM THE WOOD OF *POPULUS MONILIFERA* H. ON DEAHP-CELLULOSE

Fraction	*Eluent*	*Yield (%)*	$[\alpha]_D$ (°)	*Molar ratios of monosaccharides*			*Uronic acids**
				Glucose	*Mannose*	*Xylose*	
Mixture	—	100	− 36	1.0	4.1	0.3	+
I	Water	42.6	− 26	1.0	4.0	—	—
II	0–1 *M* Ammonium carbonate	26.0	− 29	1.0	4.2	Trace	+
III	0.1 *M* Sodium hydroxide	18.3	− 46.6	1.0	3.9	0.9	++
IV	0.3 *M* Sodium hydroxide	11.8	− 53.2	1.0	3.1	1.3	+++

* +++, Present in large amounts; ++, moderate; +, present; —, absent.

and simpler compared with that of diethyl-2-chloroethylammonium chloride.

The pectin investigated is a very complex mixture of polysaccharides as is evident from the results for its fractionation shown in Table I. The isolated polysaccharides belong to two groups with distinctly different optical activities. The 5 m*M* sodium formate eluted fraction (A) appeared, on the basis of its sugar composition and optical rotation, to be composed largely of L-arabinan. This polysaccharide was found to be associated with pectic substances from a number of sources, *e.g.*, sugar beet[9] and fruit peel[10,11], and, recently, pure L-arabinan has been isolated from the hot aqueous extract of willow bark holocellulose[12] in this laboratory. L-Arabinan was probably also the main polysaccharide component in fractions B and C, but like the other neutral polysaccharides it was contaminated with an increasing amount of the acidic polymers. Polysaccharide fractions D, E, F and G apparently belong to the pectin group, and it was inferred that the content of uronic acids, degree of esterification and molecular size and shape of the individual pectinic acids provided the basis of fractionation. Polysaccharide mixture H, eluted with 0.3 *M* sodium hydroxide, was difficult to interpret owing to its complexity and possible modification of the polysaccharides.

The D-gluco-D-mannan examined contained a small amount of (4-O-methyl-D-glucurono)-D-xylan (*ca.* 4.7%), which could not be removed by any of the precipitation methods. Fractionation on DEAHP-cellulose (carbonate form) yielded 42.6% of a pure polysaccharide (fraction I, Table II) by elution with water. The unusually high molar ratio of D-glucose to D-mannose (1:4) in the isolated polymer uncommonly found in hardwood D-gluco-D-mannan[13] is remarkable.

Polysaccharide fractions II, III and IV, eluted with increasing concentrations of ammonium carbonate and sodium hydroxide, were evidently mixtures of both D-gluco-D-mannan and (4-O-methyl-D-glucurono)-D-xylan.

Obviously, chromatography on DEAHP-starch and DEAHP-cellulose is well suited to the fractionation of polysaccharides, and seems to offer possibilities of resolution for other natural polymeric materials.

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