Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

DEAE-cellulose carbonate form. A useful medium for fractionating polysaccharides*

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(Received June 12th, 1970; accepted for publication, September 12th, 1970)

In any investigation of polysaccharide structure, isolation of a homogeneous molecular species is important for further, meaningful experimentation. Fractionation of polysaccharides by the conventional methods[1–5] is often a laborious operation and its success varies largely with the complexity of the polysaccharide mixture. The role of detergent cations in the fractionation of both acidic and neutral polysaccharides is well-established[6–9], but it also has limitations. Column chromatography with cellulose anion-exchangers has given rise to procedures based on their borate[10], phosphate[10,11], hydroxide[10], acetate[12], and chloride[13] forms, but the potential of DEAE-cellulose (carbonate form) has, to the best of our knowledge, not been examined as a medium for polysaccharide fractionation. We now report on this technique.

During fractionation of a mixture of essentially protein-free, water-soluble polysaccharides from rape seed (*Brassica campestris*) meats, it was found that DEAE-cellulose (carbonate form) provides a versatile method for the separation of acidic and neutral polysaccharides; partial resolution of the acidic polysaccharide fraction also occurred, as evidenced by the change in carbohydrate composition. The procedure developed resulted in the separation of four polysaccharide fractions designated *W*, *1*, *2*, and *3* in yields of 20, 5, 43, and 12%, respectively. The polysaccharides isolated belong to two optically distinct groups. The water-eluted fraction *W*, on the basis of its sugar components, characteristic iodine-staining properties, $[\alpha]_D$, and resistance to amylases, appeared to be analogous to the so-called seed amyloid-type of polysaccharides[14–16]. Polysaccharide fractions *1*, *2*, and *3*, eluted with increasing concentrations of ammonium carbonate, apparently belong to the pectin or hemicellulose group. The polysaccharide species present in these latter fractions, in contrast to fraction *W*, contained uronic acids (acid hydrolysis and paper chromatography), and it was inferred that this component provided the basis of the fractionation. Sugar composition indicated that the major polysaccharide of the fractions eluted by carbonate was an acidic arabinogalactan.

Chromatography on DEAE-cellulose (CO_3^{2-}) thus provides an excellent method

*Contribution No. 151 of the Food Research Institute, Canada Department of Agriculture.

for the fractionation of the rape-seed polysaccharides, and seems to offer possibilities of resolution for other polysaccharide mixtures[17]. Its main advantage lies in its simplicity, mildness, ease of operation, and good recoveries of the fractionated materials.

EXPERIMENTAL

Paper chromatography was performed by the descending method on Whatman No.1 paper with ethyl acetate–pyridine–water (8:2:1) and detection with aniline hydrogen phthalate. Evaporations were carried out at 35° with a rotary evaporator. Rotations were measured on a Perkin–Elmer 141 polarimeter. DEAE-cellulose Whatman DE52, microgranular, presoaked, and with an ion-exchange capacity of 1 mequiv/g was used. The column effluent fractions were monitored by optical rotation and by the anthrone reagent (0.2%) in conc. sulphuric acid.

The rape-seed polysaccharide mixture was in the free acid form and had N, 0.86 and ash, 1.3%.

Preparation of the column. — A slurry of purified DEAE-cellulose[18] in 0.1 M sodium hydroxide was allowed to drip into a chromatography column (2.5 × 35 cm) half-filled with 0.1 M sodium hydroxide. After the DEAE-cellulose had settled to a height of 25 cm, it was washed with water until the effluent was neutral. The carbonate form was prepared by washing the column with 8–10 bed volumes of 0.5 M ammonium carbonate, followed by sufficient water to remove the excess of carbonate.

Operation of the column. — A solution of the polysaccharide fraction (200 mg) in water (10 ml) was applied to the top of the column which was washed with water (300 ml) followed by gradient elution with 0–0.5 M ammonium carbonate. The effluent was collected in 15-ml fractions at a flow rate of *ca.* 1 ml/min. The concentration and distribution of polysaccharide in the fractions were determined polarimetrically after spot-testing the fractions with anthrone reagent. The elution profile is given in Fig. 1.

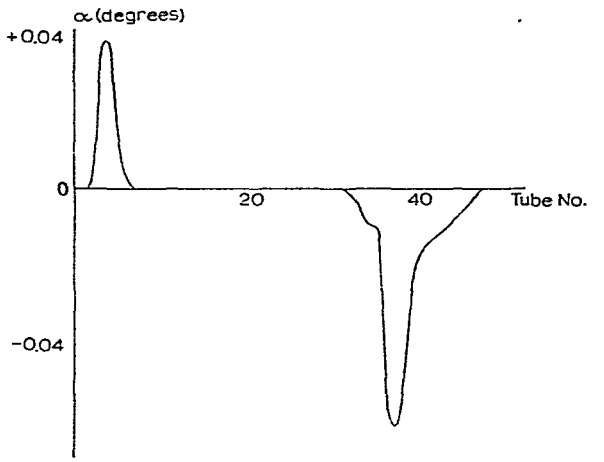

Fig. 1. Fraction profile of water-soluble, rape-seed meat polysaccharide on DEAE-cellulose (CO_3^{2-}).

The appropriate fractions were combined, deionized with Rexyn-101 (H^+) resin, and filtered, and the polysaccharides were recovered by freeze-drying. A portion (2 mg) of each fraction was hydrolysed with M sulphuric acid for 3 h at 100°. The hydrolysates were neutralized ($BaCO_3$) and filtered, and the solutions were chromatographed. The results of fractionation are shown in Table I.

TABLE I

FRACTIONATION OF WATER-SOLUBLE, RAPE-SEED MEAT POLYSACCHARIDES ON DEAE-CELLULOSE (CO_3^{2-})

Tube No.	*Fraction No.*	*Yield (g)*	$[\alpha]_D^{22}$ *(water) (degrees)*	*Sugars by paper chromatography*					
				Uronic acid	*Gal*	*Glc*	*Man*	*Ara*	*Xyl*
2–6	*W*	0.04	+31	−	major	major	trace	minor	major
32–34	*1*	0.01	−11.5	+	major	major	minor	major	trace
35–41	*2*	0.086	−42.1	+	major	trace	trace	major	trace
42–49	*3*	0.023	−5.2	+	major	trace	trace	major	major

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