A STUDY OF THE POLYSACCHARIDES FROM THE STEMS OF ALCEA HYRCANA

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The present work is a continuation of a study of the polysaccharides of plants of the family Malvaceae [1–4]. We studied the polysaccharides from the stems of Alcea hyrcana Grossh. (Hyrcanian hollyhock) collected in 1967 in Azerbaidzhan SSR in the period of mass flowering of the plants. Samples of polysaccharides were obtained separately from the bark (polysaccharide I) and from the bark-free stem (polysaccharide II) by extraction with water at room temperature. The polysaccharides were isolated and purified by precipitation from aqueous solution with ethanol (1 : 1) [2]. Some properties of the polysaccharides are given in the table.

Properties of the Polysaccharides Isolated from the Stems of the Hyrcanian Hollyhock

Source of polysaccharides	Yield of polysaccharides (% of the air-dried material)	Content, %			$[\alpha]_D^{20}$, deg (1.25 N NaOH)	Content, %					
		ash	calcium	magnesium		OCH_3 groups	uronic acid	glucose and galactose	arabinose	xylose	rhamnose
Bark of the stem	2.20	10.63	7.8	2.0	+17.8 (*c* 0.37)	0.46	45.7	24.5	9.9	4.9	14.8
Bark-free stem	1.60	8.07	4.3	0.8	+11 (*c* 0.20)	0.57	32.3	38.7	9.6*	6.3	12.9

*Contents of arabinose and mannose.

The isolated polysaccharides do not possess reducing properties nor give a color reaction with iodine. They do not undergo enzymatic cleavage under the action of saliva amylase, i.e., they do not contain a glucan type of starch.

The glycoside bonds in the molecules of the polysaccharides possess mainly the β-configuration (from the specific rotation and from the presence in the IR spectra of absorption bands in the 895–900 cm^{-1} region). A small amount of α-glycoside bonds is present (weak absorption band at 850 cm^{-1}).

The demineralization of the polysaccharides was carried out by treating aqueous solutions of them with KU-2 ion-exchanger (H^+ form). The process was monitored by means of IR spectroscopy; the spectra of the initial polysaccharides had a strong absorption band in the 1600-cm^{-1} region and a very weak band at 1740 cm^{-1}, while in the spectra of the completely deionized polysaccharides the first absorption band practically disappeared and the intensity of the second band (1740 cm^{-1}) increased sharply [5].

To determine their qualitative carbohydrate composition, samples of the polysaccharides were subjected to acid hydrolysis and the hydrolysates (after neutralization) were chromatographed on paper. In polysaccharide I we detected uronic acids (and glucuronolactone), rhamnose, xylose, arabinose, glucose, galactose, and traces of mannose. Polysaccharide II was found to contain the same carbohydrates, but the amount of mannose was slightly greater and that of arabinose smaller. The quantitative content of monosaccharides was determined densitometrically (see table).

It was established by paper electrophoresis that the hydrolysates of both polysaccharides contained galacturonic and glucuronic acids and glucuronolactone (for the quantitative amounts of uronic acids, see table).

The attempts which we made to fractionate the isolated polysaccharides by means of cetyltrimethylammonium bromide (Cetavlon) and by precipitation with ethanol of various concentrations did not lead to the desired results. The Cetavlon treatment yielded two fractions for each polysaccharide: an acid fraction, precipitated by the Cetavlon,

and a neutral fraction, which remained in solution under these conditions. However, the qualitative carbohydrate compositions of the fractions obtained and of the initial polysaccharides were completely identical.

EXPERIMENTAL

Chromatographic analysis of the carbohydrates on paper was carried out in the following solvent systems: 1) ethyl acetate–pyridine–water (8 : 2 : 1), 2) ethyl acetate–acetic acid–water (18 : 7 : 8), 3) n-butanol–ethanol–water (4 : 1 : 5), and 4) n-butanol–acetic acid–water (4 : 1 : 5). The developers were aniline hydrogen phthalate and an alkaline solution of silver nitrate [6]. Type M ["slow"] chromatographic paper of the Volodarskii Leningrad Factory was used.

Paper electrophoresis was carried out on an OE-202 apparatus for vertical electrophoresis (1500 V, 4 mA, 3 hr, 1% aqueous solution of acetic acid; developer, aniline hydrogen phthalate).

The specific rotations were measured on a Pepol-60 instrument, and the IR spectra were recorded on a UR-10 spectrophotometer. The samples for analysis were prepared by molding the polysaccharides with KBr.

The quantitative contents of the monosaccharides were determined densitometrically by means of an ERJ-65 instrument (Carl Zeiss, Jena), the content of uronic acids by the method of Baker et al. [7], Ca^{++} and Mg^{++} in the ash by complexonometric titration, and the amount of OCH_3 groups by a published method [8]. The aqueous and aqueous ethanolic solutions of the hydrocarbons were concentrated in a rotary evaporator at 35–40° C.

Isolation of polysaccharides from the bark and stems. The bark was separated from the dry stems, after which the two types of raw material were ground separately and 400-g samples were covered with water (bark 1 : 10, bark-free stems 1 : 15). After standing 2–3 hr the extract was squeezed out through gauze and centrifuged. The isolation of polysaccharides from the same portion of plant material was repeated. Then the centrifugates were combined and the polysaccharides were isolated by precipitation from aqueous solution with ethanol (1 : 1), washed with ethanol, and dried to constant weight. The yield of polysaccharide from the bark (polysaccharide I) was 2.2%, and from the stems (polysaccharide II), 1.6% (of the weight of the air-dried material). The polysaccharides were purified by two reprecipitations from aqueous solutions with ethanol.

Complete acid hydrolysis of polysaccharides I and II. A solution of 0.05 g of polysaccharide I in 2.5 ml of 1 N H_2SO_4 was heated with constant stirring in a boiling water bath for 30 hr. The hydrolysates were neutralized with dry $BaCO_3$ and the precipitate was filtered off and washed with hot water until the reaction for carbohydrates (phenol and H_2SO_4) was negative. The filtrate and the wash waters were combined, passed through KU-2 cation-exchanger (H^+ form), and evaporated to small volume.

Polysaccharide II was hydrolyzed under the same conditions. The paper chromatographic separation of the hydrolysates of polysaccharides I and II was carried out in systems 1, 2, 3, and 4, and paper electrophoresis in 1% acetic acid solution.

Demineralization of the isolated polysaccharides I and II. A solution of 2 g of polysaccharide I in 0.5 *l* of water was treated with 75 g of KU-2 cation-exchange resin (H^+ form). The resulting mixture was stirred for 4 hr. Then the solid matter was filtered off and washed with water, after which the solution and the wash waters were subjected to freeze drying. The yield of demineralized polysaccharide I was 1–1.2 g.

Demineralized polysaccharide II was obtained under the same conditions.

The complete demineralization of the polysaccharides was confirmed by a comparison of the IR spectra of the initial polysaccharides and those that had been treated with the KU-2 resin (H^+ form).

Action of amylase on polysaccharides I and II. A solution of 0.129 g of polysaccharide I in 58 ml of water was treated with 3.8 ml of a 0.2 M solution of sodium acetate and 1.3 ml of a freshly prepared solution of amylase (2 ml of saliva was diluted with water to 4 ml, the precipitate was separated off by centrifuging, and the solution was used as the amylase preparation [9]). The mixture was stirred and kept in the thermostat at 34–35° C for 15 hr. Then it was heated in a boiling water bath for 5 min (to inactivate the enzyme) and filtered, and the polysaccharide was precipitated with ethanol. The precipitate was filtered off, washed with ethanol (4 × 20 ml), and dried. Yield 0.074 g.

The filtrate was evaporated to small volume and chromatographed on paper in systems 1 and 4; no glucose was detected.

The amylase treatment of polysaccharide II was carried out similarly, and again no glucose was found among the amylase degradation products.

Fractionation of polysaccharides I and II with Cetavlon. A solution of 0.70 g of polysaccharide I in 700 ml of water was treated with 100 ml of 2.5% Cetavlon solution. The precipitate was separated by centrifuging, washed (repeatedly) with water, and dissolved in 100 ml of 10% NaCl solution (with gentle heating). The resulting solution was treated with 100 ml of ethanol. The precipitate (acid fraction) was filtered off, washed with ethanol, and dried to constant weight. Yield 0.58 g.

The filtrate and the wash waters were combined, dialyzed against water for 24 hr, and evaporated. On the addition of ethanol, a precipitate deposited which was filtered off, washed with ethanol, and dried to constant weight. Yield 0.03 g.

The isolated fractions ("acid" and "neutral") were hydrolyzed with 2 N H_2SO_4 for 16 hr in a boiling water bath. The solution was treated in the usual way and chromatographed in the presence of markers, using solvent system 1. Polysaccharide II was fractionated similarly.

CONCLUSIONS

1. Water-soluble polysaccharides have been isolated from the bark and stems of Alcea hyrcana Grossh. with yields of 2.2% and 1.6%, respectively; they contain uronic acids, glucose, arabinose, and xylose.

2. The quantitative amounts of these monosaccharides in samples of the isolated polysaccharides have been determined.

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