ALKALI-SOLUBLE POLYSACCHARIDES OF THE STEMS OF *Silphium perfoliatum*

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The results are given of an investigation of a polysaccharide complex of the stems of the cup rosinweed isolated by alkaline extraction of the stems. The complex contains 71% of polysaccharide, 2% of ash substances, and 20% of "crude" protein. The carbohydrate moiety consists of a glucuronoxylan. The complex is not separated into its carbohydrate and protein constituents under the conditions of gel filtration and electrophoresis but the maxima of the elution curves of the components of the complex in gel filtration do not coincide, which indicates the absence of a strong bond between the protein and the carbohydrate.

The polysaccharides of wild herbs have been studied comparatively little [1]. The present paper gives the results of an investigation of an alkali-soluble polysaccharide complex isolated from the cleaned and comminuted stems of the cup rosinweed. After the elimination of accompanying substances by treatment with ether, ethanol, water, and an aqueous solution of ammonium oxalate, the residue was extracted with a 6% aqueous solution of caustic potash in an atmosphere of nitrogen for 72 h. The alkaline extract of the polysaccharide complex was treated with ethanol and was dialyzed. Yield 2.5%.

The precipitate obtained was analyzed. The product contained 71% of polysaccharide, 2% of ash substances, and 3.2% of nitrogen or 20% of "crude" protein.

To evaluate the interrelationship between the polysaccharide and the protein, the complex was fractionated on DEAE-cellulose and Sephadexes G-100 and G-200. The amounts of protein (by Lowry's method) and of carbohydrates (by the anthrone method) in the individual samples were determined. The protein component was scarcely separated from the polysaccharide, but their maxima did not coincide, which shows the absence of a strong chemical bond between these components.

Similar results were obtained in an attempt to fractionate this complex by electrophoresis.

A quantitative characterization of a hydrolysate of the polysaccharide part of the complex by chromatography showed the presence of xylose and uronic acids in a ratio of 1:10. Rechromatography of the uronic acids showed the presence of D-glucuronic, 4-O-methyl-D-glucuronic, aldobiuronic, and aldotriuronic acids.

These results show that the complex includes a glucuronoxylan containing not only D-glucuronic acid but also its 4-O-methyl derivative, which is characteristic for the xylans studied previously [2].

Periodate oxidation of the polysaccharide took place extremely slowly and constancy of the consumption of periodate was achieved only after five days. After the sodium tetrahydroborate reduction of the polyaldehyde obtained from the oxidation of the polysaccharide, followed by hydrolysis of the polyol, glycerol and xylose were found in the hydrolysate in a molar ratio of 8:1 by paper chromatography, which shows the presence of a $1 \rightarrow 4$ bond and the branched nature of the polysaccharide.

In parallel, the xylan was methylated by Hakomori's method. The completeness of methylation was checked by thin-layer chromatography on Al_2O_3 plates. The methylated product was hydrolyzed, and the composition of the hydrolysate was studied. On a chromatogram two main spots were detected corresponding to 3-O-methylxylose and 2,3-di-O-methylxylose, and trace amounts of 2,3,4-tri-O-methylxylose and of a methylated uronic acid. The molar ratio of 3-O-methylxylose to 2,3-di-O-methylxylose was 1:10.

Consequently, the carbohydrate part of the alkali-soluble complex is an ordinary glucuronoxylan and contains the fragments

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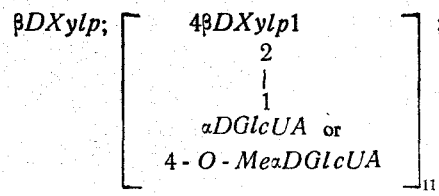

EXPERIMENTAL

The rosinweed stems (50 g) were comminuted and extracted with ether, with 82% ethanol, and with 0.5% ammonium oxalate solution.

The alkali-soluble complex was isolated by extraction with 6% KOH in an atmosphere of nitrogen for three days. The complex was precipitated from the alkaline extract with ethanol. It was purified by three reprecipitations from alkali followed by dialysis. Yield 1.25 g.

The xylan was methylated by the Hakomori method [3]. The completeness of methylation was checked on plates coated with Al_2O_3 and by the IRS method.

Hydrolysis of the Methylated Xylan and Its Characterization. The methylated product was subjected to formolysis with 90% HCOOH at 100°C for an hour and then to hydrolysis with 0.25 M H_2SO_4 for 14 hours. The hydrolysate was characterized by paper chromatography. The methylated sugars were identified from their R_f values and by comparison with markers, and their quantitative amounts were determined iodometrically [4].

The uronic acid was separated by rechromatography on paper. The solvent was ethyl acetate–acetic acid–formic acid–water (18:3:1:4) and the chromogenic agent was aniline phthalate.

Chromatography on DEAE-cellulose in the phosphate form [5]; column 2 × 25 cm; eluents: 0.5 M NaH_2PO_4 and 0.03 M NaOH. The fractions were monitored by the anthrone method and by the Lowry method. The fractions corresponding to a peak were combined, dialyzed, concentrated, and hydrolyzed [6].

Gel Filtration of Sephadexes. The gel was prepared by a standard method [7]. Sephadexes G-100 and G-200 were used. The eluent was 4% NaOH.

Electrophoresis was performed in an EFA-1 instrument, with the deposition of 5-7% solutions of the complex. The process was carried out in borate buffer, pH 11, with a potential gradient of 12-15 V per cm, a current strength of 15-20 mA, and a time of 6 h. Staining was carried out as described by Greisler [8].

SUMMARY

1. An alkali-soluble protein-polysaccharide complex not separated under the conditions of gel filtration and electrophoresis has been isolated from the stems of Silphium perfoliatum.

2. The carbohydrate moiety of the complex consists of a glucuronoxylan.

LITERATURE CITED

1. M. S. Dudkin, Khim. Drev., 8, 3 (1976).
2. M. S. Dudkin, N. G. Shkantova, and M. A. Parfent'eva, Khim. Prir. Soedin., 697 (1973).
3. S. Hakomori, J. Biochem., 55, 205 (1964).
4. E. L. Hirst, L. Haugh, and J. K. Jones, J. Chem. Soc., 928 (1949).
5. Yu. S. Ovodov and T. F. Solov'eva, Khim. Prir. Soedin., 581 (1962).
6. T. F. Solov'eva, L. V. Arsenyuk, and Yu. S. Ovodov, Khim. Prir. Soedin., 201 (1969).
7. R. L. Whistler and M. L. Wolfrom, Methods in Carbohydrate Chemistry, Academic Press, New York Vols. I-V (1962-1965).
8. A. E. Greisler, Lab. Delo, No. 6, 48 (1960).