Chemistry of Natural Compounds, Vol. 31, No. 5, 1995

BRIEF COMMUNICATIONS

ISOLATION AND CHARACTERIZATION OF THE POLYSACCHARIDE OF *Sophora japonica* FRUIT

L. A. Gorbacheva, V. I. Grishkovets, G. A. Drozd, and V. Ya. Chirva

UDC 547.917

In recent years there has been a revival of interest in the polysaccharides of plants in connection with the detection in them of an immunological action on human and animal organisms. Literature information on the immunostimulating polysaccharides of higher plants has been generalized in a review [1]. We have detected and described [2] the immunostimulating action of a total extract and of the polysaccharide complex (PSC) of the fruit of *Sophora japonica* L. (Fabaceae) (Japanese pagoda tree). In the present communication we describe the isolation and general characteristics of the PSC of the Japanese pagoda tree introduced into the Crimea.

The PSC was isolated by extracting the fruit (600 g) with boiling water (3 × 2 liters) and then freeing the aqueous extract from resinous, waxy, and fatty substances by treatment with chloroform (3 × 1 liter). To eliminate phenol glycosides, the extract was then treated with butanol (3 × 1 liter), followed by centrifugation, concentration of the supernatant in vacuum to 1 liter, and precipitation of the PSC with 4 liters of isopropanol. Additional purification of the PSC was achieved by dissolving it in 500 ml of water and reprecipitating it with 2 liters of ethanol. The yield of PSC was 12 g. Part of the product obtained was additionally deionized to eliminate Ca^{2+} and Mg^{2+} cations by treating the PSC solution with KU-2-8 cation-exchange resin in the H^+ form (10 g per 1 g of PCS). Then the solution was filtered, dialyzed against distilled water, and subjected to freeze-drying.

The PSC was fractionated by precipitation with ethanol [3]. The bulk of the PSC (90%) precipitated in a relatively narrow range of alcohol concentrations: from 60 to 70 vol.-%, which showed a fairly high homogeneity of the PSC obtained in this way.

After the acid hydrolysis of the PSC (2N CF_3COOH, 100°C, 2 h), TLC on Silufol ($CHCl_3-CH_3OH-25\%\ NH_3$, 100:50:15) permitted the identification of arabinose, glucose, and galacturonic acid. The PSC gave a precipitate with salts of heavy metals (Cu^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}), which confirmed its acid nature. The IR spectrum of the PSC obtained (1 mg/100 mg of KBr) showed bands at 1740 and 1630 cm^{-1}, assigned to the $\nu_{C=O}$ stretching vibrations in $-COOCH_3$ and $-COOH$ groups, and a number of others: 3400 (ν_{OH}), 2800-3000 (ν_{C-H}), 1000-1100 (ν_{C-O}), 1300-1500 (ν_{C-H}), 1240-1260 (ν_{C-O-C}), which is typical for the IR spectra of pectin substances [4]. Protopectin (polygalacturonic acid) was obtained from the PSC by comparatively mild acid hydrolysis (1N CF_3COOH, 100°C, 1 h) in the form of a precipitate amounting, after separation by centrifugation and drying, to 85% of the weight of the PCS taken, which corresponds to a neutral sugars content of about 15%.

The amounts of free (K_f) and bound (K_e) carboxyl groups in the purified PSC were determined titrimetrically [5]. The calculated mean value of K_f was 22%, and that of K_e 2%. The total amount of carboxyl groups, K_t, was 24%. The degree of esterification of the pectin ($K_e/K_t \cdot 100\%$) was 8.35%. These results were confirmed by a semiquantitative analysis of the IR spectrum of the PSC, in which the ratio of the areas of the $\nu_{C=O}$ bands in ester (1740 cm^{-1}) and carboxyl (1630 cm^{-1}) groups was about 1:10.

An approximate evaluation of the molecular mass of the PSC was made on the basis of measurements of the viscosity of solutions of the PSC in water (VPZh-2 viscometer, with a 0.56 mm diameter capillary) and by calculation from the Staudinger equation $\eta_{sp} = KMc$ for a 0.25% solution [6] or from the Mark–Houwinkn–Kuhn equation $[\eta] = KM^a$ [6] for

Simferopol' State University, and Kursk State Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 748-749, September-October, 1995. Original article submitted March 10, 1995.

 0009-3130/95/3105-0626$12.50 ©1996 Plenum Publishing Corporation

0.25, 0.5, and 1.0% solutions. The values found were η_{sp} (for a 0.25% solution) = 0.038 and $[\eta]$ = 0.14. From this, with $a = 1$ and $K = 1.1 \cdot 10^{-5}$ we found the value $M \cong 13{,}000$, which corresponds to a degree of polymerization of about 70.

REFERENCES

1. G. F. Moiseeva and V. G. Belikov, Farmatsiya, 79 (1992).
2. G. A. Drozd and L. A. Gorbacheva, Farmatsiya, No. 1, 34 (1994).
3. B. Hottenroth, Die Pectine und ihre Verwendung, Munich (1961), p. 217.
4. M. P. Filippov, Infrared Spectra of Pectin Substances [in Russian], Shtiintsa, Kishinev (1978).
5. V. V. Arasimovich, S. V. Baltaga, and N. P. Ponomareva, Methods for the Analysis of Pectin Substances, Hemicelluloses, and Pectinolytic Enzymes in Fruit [in Russian], Izd-vo Akad. Nauk MSSR, Kishinev (1970).
6. A. A. Tager, The Physical Chemistry of Polymers [in Russian], Khimiya, Moscow (1978).
7. S. G. Kovalenko and O. D. Kurilenko, Ukr. Khim. Zh., **31**, 175 (1965).