POLYSACCHARIDES OF PANAX GINSENG

Yu. S. Ovodov and T. F. Solov'eva

Khimiya Prirodnykh Soedinenii, Vol 2, No. 5, pp. 299-303, 1966

At the present time an intensive chemical investigation of plants of the family araliaceae is being carried on. Most of the papers concerned give the results of a study of the physiologically active glycosides [1-6]. The polysaccharides of the araliaceae have not been studied previously; the oligosaccharides of ginseng are being studied by Japanese chemists [6].

In the present communication we discuss the isolation and general characteristics of the polysaccharides of Panax ginseng G. A. Mey (ginseng). The total polysaccharide fraction TPF (40%) was isolated by extracting ginseng roots with hot water. With iodine, this fraction gives a blue color, its hydrolyzate contains glucose, and it undergoes partial splitting with salivary amylase, which indicates the presence of a glucan of the starch type. The glucan has $[\alpha]_D^{20}$ + 184° C; when isolated from one of the extraction stages it is colored blue by iodine. On hydrolysis, the glucan forms only glucose.

Acid hydrolysis of the TPF gave galacturonic acid, glucose, galactose, and arabinose, which were identified by paper chromatography. In addition, the galactose was characterized as the phenylosazone and the galacturonic acid was identified by oxidation with bromine water to mucic acid.

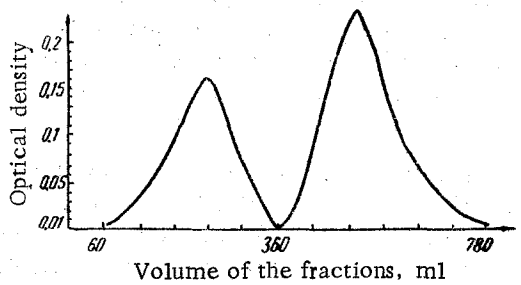

Fig. 1. Elution curve for the chromatography of the pectin on DEAE-cellulose

To remove the glucan, the TPF was treated with salivary amylase. The polysaccharide fraction isolated after the amylolysis process did not give a color with iodine, contained 5% of methoxy groups, and gave galacturonic acid, galactose, and arabinose on hydrolysis and, consequently, was a pectin [7, 8].

By means of cetyltrimethylammonium bromide (Cetavlon), the pectin was separated into two fractions: an acid fraction, precipitated by Cetavlon, and a neutral fraction which was not precipitated.

The acid fraction contained 80-90% of the total amount of pectin; on acid hydrolysis it formed galacturonic acid, galactose, arabinose, and xylose, and it contained about 70% of the galacturonic residues.

Hydrolysis of the neutral fraction formed the same monosaccharides but the galacturonic acid content of the hydrolyzate was very small in comparison with that of the neutral monosaccharides.

The acid fraction was additionally separated by means of DEAE-cellulose and DEAE-Sephadex. The elution curve of the DEAE-cellulose chromatography had two peaks (Fig. 1). Both peaks contained polysaccharides giving galacturonic acid, galactose, arabinose, and xylose on acid hydrolysis. The polysaccharide of the first peak was greatly enriched with neutral monosaccharides, while that of the second peak was a polyuronide with a smaller content of neutral monosaccharide.

The elution curve of gel filtration on DEAE-Sephadex A-50 (neutral) contained three peaks (Fig. 2). The first peak corresponded to a polysaccharide enriched in neutral monosaccharides. The polysaccharide corresponding to the second peak consisted of a polygalacturonide containing a very small amount of neutral monosaccharides; when it was dialyzed, a polygalacturonide precipitated. A similar phenomenon has been reported previously [9]. The third peak corresponded to a neutral polysaccharide whose acid hydrolysis gave galactose, arabinose, and xylose. The amount of polysaccharides corresponding to the first and third peaks was very small, and the bulk of the acid fraction of the pectin consisted of a polygalacturonide with a small amount of neutral monosaccharides.

Experimental

The chromatography was carried out with Whatman No. 3 paper and in the following solvent systems (by volume): 1) ethyl acetate-glacial acetic acid-pyridine-water (5:1:5:3), 2) ethyl acetate-pyridine-water (8:2:1), and 3) ethanol-water-concentrated ammonia (35:13:2) containing 0.03% of Thymol Blue.

The spots were revealed with: 1) aniline hydrogen phthalate; 2) hydroxylamine followed by treatment with a 1% solution of ferric chloride in dilute hydrochloric acid (hydroxamic acid test) [10].

Fractionation was carried out on DEAE-cellulose (Cl^--form, capacity 0.61 mg-eq/g) treated by the method of Neukom et al. [11], and on DEAE-Sephadex A-50 (neutral) of the Swedish firm Pharmacia Uppsala. The fractions were monitored by Smith's method [12].

The galacturonic acid content of the polysaccharide fractions was determined by Anderson and Garbutt's method [13]. All the solutions were evaporated in vacuum at 40°–50° C.

Isolation of the TPF. Ten grams of the air-dry comminuted roots which had been defatted and extracted with methanol was covered with hot water and heated in a boiling water bath for 3 hr. The mixture was cooled and centrifuged. The solution was treated with four volumes of methanol, and the precipitate which deposited was separated off, washed with methanol and ether, and dried in the air. The operation was repeated 14 times. This gave 4.5–5.0 g of total polysaccharide fraction (about 40% of the weight of the roots).

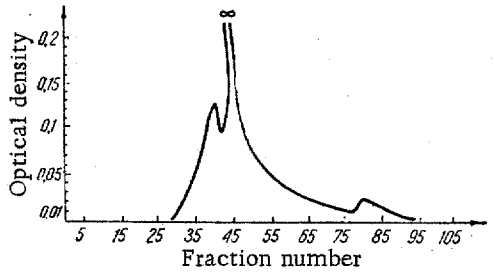

Fig. 2. Elution curves of the gel-filtration of the pectin on DEAE-Sephadex A-50.

Acid hydrolysis of the polysaccharide fractions. A. A mixture of 5 mg of the polysaccharide and 0.4 ml of 3% nitric acid containing a small crystal of urea was heated in a sealed tube for 13 hr at 120° C. The solution was chromatographed on paper in systems 1 and 2.

B. A mixture of 5 g of TPF and 350 ml of 85% formic acid was boiled for 30 hr, filtered, and evaporated to dryness. The residue was covered with 60 ml of 1 N sulfuric acid and was heated for 1.5 hr on a boiling water bath. The reaction mixture was filtered and neutralized with barium carbonate. The precipitate was filtered off and the solution was evaporated to small bulk and poured into methanol (4-5 volumes). The barium salt of galacturonic acid which precipitated was separated off, washed with methanol, and dried in the air. Yield 0.12 g. The combined filtrates were evaporated. This gave about 3 g of a crude mixture of neutral monosaccharides.

C. This mixture (3 g) was transferred to a column of cellulose (2.5 × 48 cm) and eluted first with butan-1-ol 2/3-saturated with water and then with water-saturated butan-1-ol + 5% of ethanol and, finally, with methanol. The separation was followed by paper chromatography in systems 1 and 2. The fractions were evaporated. The yield of glucose was about 1.5 g, that of galactose 0.10 g and that of arabinose 0.25 g. The D-glucose obtained had mp 150° C (from alcohol), $[\alpha]_D^{20}$ +52° C (in water); the galactose had mp 163° C (phenylosazone, mp 186°–188° C; and the arabinose had mp 155° C. Mixtures of these materials with authentic samples gave no depressions of the melting points.

D. A solution of 100 mg of the barium salt of the galacturonic acid in 20 ml of distilled water was treated with the cation-exchanger Amberlite IR-120 (H^+-form), filtered, and chromatographed in systems 1 and 2, and the product was shown to be identical with an authentic sample of galacturonic acid. A solution of the uronic acid was saturated with bromine and left for 48 hr at room temperature; the excess of bromine was eliminated by aeration and the solution was evaporated until a precipitate of mucic acid deposited. Yield 50 mg, mp 218°–219° C. Its identity with an authentic sample of mucic acid was established by a mixed melting point test, comparison of the IR spectra, and chromatography in systems 1 and 3. For chromatography in system 1, the mucic acid was first converted into the lactone by prolonged boiling with water. The spots were revealed by reagent 2 [10].

Amylolysis of the TPF. 5 ml of saliva was diluted with water to 10 ml, the precipitate was separated off by centrifuging, and the solution was used as a preparation of the enzyme amylase [14].

A solution of the polysaccharide (1 g) in 460 ml of distilled water was treated with 30 ml of 0.2 M sodium acetate and 10 ml of freshly-prepared amylase solution. The resulting mixture was incubated at 37° C for 15 hr and was then heated in a boiling water bath for 5 min (to inactivate the enzyme) and filtered; the filtrate was evaporated to small volume and poured into methanol. The precipitate which deposited was separated off, eluted with methanol, and dried. This gave a fraction of pectic substances. Yield 0.3–0.6 g, $[\alpha]_D^{20}$ +205° C (c 0.5; in water).

Found, %: anhydrogalacturonic acid 55.0; OCH_3 5.41; ash 8.8.

Fractionation of the pectin with Cetavlon. A solution of 0.73 g of the pectin in 73 ml of distilled water was treated with 75 ml of a 1% solution of Cetavlon. The precipitate was separated off by centrifuging, washed with 0.1% Cetavlon solution, and dissolved in a 2 N solution of sodium chloride with the addition of a small amount of ethanol. The resulting solution was poured into ethanol (3 volumes); the precipitate (acid fraction) was separated off, washed with ethanol, and dried in the air. Yield 0.55–0.65 g.

Found, %: anhydrogalacturonic acid 69.5; OCH_3 4.56; ash 13.8.

After the separation of the acid fraction, the solution was washed with a small amount of chloroform, evaporated to small volume and poured into ethanol. The precipitate which deposited (neutral fraction) was separated off, washed with alcohol, and dried in air. (Yield 0.06 g).

Found, %: OCH_3 2.2; ash 12.5.

Separation of the acid fraction of the pectin on DEAE-cellulose. A solution of 0.3 g of the polysaccharide in 15 ml of water was transferred to a column of DEAE-cellulose (Cl^--form, 3.6 × 40 cm) and eluted using gradient elution changing from water to a 1.5 M solution of potassium chloride and then from water to a 0.3 M solution of caustic soda. Twenty-milliliter fractions were collected (see Fig. 1). The fractions corresponding to the first and second peaks, respectively, were combined, dialyzed against distilled water, and evaporated. The yield of the first polysaccharide was 30–40 mg and of the second 70–75 mg. Galacturonic acid, galactose, arabinose, and xylose were identified in the hydrolyzates of both polysaccharides by paper chromatography, the relative amount of galacturonic acid in the hydrolyzate of the second polysaccharide being considerably greater than in the hydrolyzate of the first polysaccharide.

Separation of the acid fraction of the pectin on DEAE-Sephadex A-50. A solution of 0.3 g of the polysaccharide in 11 ml of a 0.01 M solution of tris buffer (pH 8.0) was transferred to a column of DEAE-Sephadex A-50 (3.7 × 60 cm) charged in the same buffer. The column was eluted successively with a solution of tris buffer (0.4 *l*) and sodium chloride: 0.3 M–0.4 *l*; 0.6 M–0.5 *l*, and 1 M– 1 *l*. Fractions of 20 ml were collected (see Fig. 2). The fractions corresponding to each peak were combined, dialyzed against distilled water, and evaporated. This gave three polysaccharide components. The yield of the second was about 150 mg, that of the first 15 mg, and that of the third 15–20 mg. The polysaccharides were hydrolyzed as described in A. Paper chromatography showed the presence of galacturonic acid, galactose, arabinose, and xylose in the first and second polysaccharide residues, the content of neutral monosaccharides in the second polysaccharide being very small. Hydrolysis of the third polysaccharide gave galactose, arabinose, and xylose.

The analyses were carried out by our colleagues L. I. Glebko, Zh. I. Ul'kina, and Z. S. Suppes of the Institute of Biologically Active Substances, Siberian Division AS USSR.

Summary

1. A polysaccharide fraction consisting of about 40% by weight of the roots has been isolated from the roots of Panax ginseng C. A. Mey and characterized.

2. It has been established that the polysaccharides of ginseng contain a glucan of the starch type and a pectin.

REFERENCES

1. S. Shibato, O. Tanaka, K. Soma, Y. Iida, I. Ando and H. Nakamura, Tetrah. Lett., 207, 1965.
2. G. B. Elyakov, L. I. Strigina and N. K. Kochetkov, KhPS, 149, 1965.
3. N. I. Uvarova (et al.), KhPS, 82, 1965.
4. Yu. S. Ovodov (et al.), KhPS, 3, 1965.
5. L. A. Elyakova (et al.), KhPS, 32, 1965.
6. K. Takiura and J. Nakagama, J. Pharm. Soc. Japan, **83**, 305, 1963.
7. Z. Kertesz. The Pectic Substances, N. Y.–L., 7, 1951.
8. E. V. Sapozhnikova, The Pectic Substances of Fruits [in Russian], Moscow, 12–15, 1965.
9. S. S. Bhattacharjee and T. E. Timell, Can. J. Chem., **43**, 758, 1965.
10. M. Gee and M. McCready, Anal. Chem., **29**, 257, 1957.
11. H. Neukom, H. Deuel, W. J. Heri and W. Klindig, Helv. Chim. Acta, **43**, 64, 1960.
12. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Anal. Chem., **28**, 350, 1956.
13. D. M. W. Anderson and S. Garbutt, Anal. Chim. Acta, **29**, 31, 1963.
14. S. Peat, J. R. Turvey and J. M. Evans, J. Chem. Soc., 3341, 1959.

4 January 1966

Institute of Biologically Active Substances,
Far Eastern Branch, Siberian Division AS USSR, Vladivostok