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THE PECTIN OF GINSENG. THE STRUCTURE OF THE POLYGALACTURONIDE

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We have previously [1] reported the isolation of a pectin from the roots of Panax ginseng C. A. Mey (ginseng). On partial hydrolysis of the pectin with dilute sulfuric acid, a polygalacturonide is formed. Since methylation of the polygalacturonide takes place with great difficulty, we previously methoxylated it with diazomethane and reduced it with sodium borohydride to the corresponding galactan.

Methylation of the galactan with methyl iodide in dimethyl sulfoxide in the presence of sodium hydride by Hakomori's method [3] gave the fully methylated galactan (OCH_3 41%; no hydroxyl absorption band in the IR spectrum). On paper chromatography, a hydrolysate of the resulting compound revealed the presence of 2, 3, 4, 6-tetra- and 2, 3, 6-tri-O-methyl-D-galactoses and traces of an unidentified compound—probably the methyl ester of 2, 3-di-O-methyl-D-galacturonic acid. On chromatography in a thin layer of silica gel, in addition to the compounds mentioned above, the presence of 1, 2, 3, 5, 6-pentamethyldulcitol was established.

The content of 2, 3, 6-tri-O-methyl-D-galactose was considerably higher than that of the other methylated monosaccharides; the pentamethyldulcitol and tetramethylgalactose were present in approximately equal amounts. No galactose derivatives with a low degree of methylation were found, which shows the absence of branching in the carbohydrate chain.

The mixture of methylated monosaccharides was separated by column chromatography on silica gel. The 2, 3, 6-tri- and 2, 3, 4, 6-tetra-O-methyl derivatives of galactose were isolated in the pure state and were identified by comparison with authentic samples and their derivatives. It follows from this that the galactan has a linear carbohydrate chain with (1 → 4)-glycosidic linkages.

Periodate oxidation of the galactan with subsequent degradation according to Smith led to the formation of threitol and glycerol in the hydrolysate, identical with authentic samples on paper chromatography. The formation of threitol is possible only when (1→) bonds exist between the galactose residues.

Thus, it can be seen from the above information that the polygalacturonide obtained by the partial hydrolysis of ginseng pectin has a linear carbohydrate chain with (1 → 4) linkages between the D-galacturonic acid residues. The high positive rotation of the polygalacturonide shows the α-configuration of the glycosidic linkages.

Experimental

The paper chromatography was carried out on "Whatman 3", "Whatman 3MM", and "Goznak" Leningrad mill papers with the following systems of solvents (by volume): 1) ethyl acetate–pyridine–water–acetic acid (5 : 5 : 3 : 1); and 2) methyl ethyl ketone saturated with ammonia; and for thin-layer chromatography we used type "KSK" silica gel and the solvent system 3) chloroform–methanol (10 : 1).

The spots were revealed with: a) aniline hydrogen phthalate, and b) silver nitrate [4].

The content of uronic acids was determined by a modification of the carbazole method [5]. All solutions were evaporated in vacuum at 30–40° C. Molecular weights were determined viscosimetrically [6].

Isolation of the polyuronide. A solution of 8 g of the pectin in 800 ml of water was treated with 80 ml of 2 N H_2SO_4, and the reaction mixture was kept in the boiling water bath for 3 hr. The solution was cooled and was poured into methanol. The resulting precipitate [2.460 g $[\alpha]_D^{20}$ +240° (in water), OCH_3 5.9%, mol. wt. 9000–16 000] gave only galacturonic acid on hydrolysis.

Reduction of the polygalacturonide. The polygalacturonide (1.9 g) was ground to a powder, and this was moistened with aqueous methanol and treated with a solution of diazomethane in ether. The mixture was kept at room temperature for 12 hr and then the polysaccharide (1.88 g) was filtered off and washed with ether, after which it was dissolved in 75 ml of water and reduced with 1 g of potassium borohydride as described by Aspinall and Ganas-Rodriguez [2].

Methylation of the galactan. The polysaccharide (0.5 g) was methylated with the sulfinyl carbanion in dimethyl sulfoxide by Hakomori's method [3]. The carbanion was obtained by dissolving 0.25 g of sodium hydride in 7.5 ml of dimethyl sulfoxide [7]. The methylation was carried out twice. The yield was 0.15 g. Found, %: OCH_3 41.0. The IR spectrum exhibited no hydroxyl absorption band.

Hydrolysis of the methylated galactan. The methylated polysaccharide was hydrolyzed with 72% and 8% sulfuric acids as described by Garegg and Lindberg [8]. The hydrolysate was chromatographed on paper in system 2 and in a thin layer of silica gel in system 3. The chromatographic results showed the presence in the hydrolysate of 2, 3, 6-tri- and 2, 3, 4, 6-tetra-O-methyl-D-galactoses, 1, 2, 3, 5, 6-pentamethyldulcitol, and an unidentified compound (traces) with a R_f value somewhat lower than that of 2, 3, 6-tri-O-methyl-D-galactose (probably methyl 2, 3-dimethyl-D-galacturonate).

Periodate oxidation of the galactan, reduction of the polyaldehyde, and hydrolysis of the polyalcohol (Smith degradation). The galactan (0.3 g) was oxidized with sodium metaperiodate for 3 days, after which the polyaldehyde was reduced with 0.2 g of potassium borohydride and the polyalcohol was hydrolyzed with 1 N H_2SO_4 for 7 hr. The hydrolysate was chromatographed in system 2 (the spots being revealed with reagent b), and threitol and glycerol were identified by direct comparison.

Separation and identification of the methylated monosaccharides. A mixture of the methylated monosaccharides (170 mg) obtained by the hydrolysis of the methylated galactan was deposited on a column of silica gel (2.1 × 31.7 cm) and was eluted first with chloroform and then with mixtures of chloroform and ethanol with increasing concentrations of ethanol.

Four-milliliter fractions were collected; the separation of the latter was monitored by chromatography in a thin layer of silica gel in system 3. Three combined fractions were obtained: I) a mixture of 1, 2, 3, 5, 6-pentamethyl-dulcitol and 2, 3, 4, 6-tetra-O-methyl-D-galactose (4.0 mg); the 2, 3, 4, 6-tetra-O-methyl-D-galactose was isolated in the pure state by preparative chromatographyon paper in system 2; II) 2, 3, 6-tri-O-methyl-D-galactose (100 mg); and III) 2, 3, 6-tri-O-methyl-D-galactose contaminated with a substance having a slightly lower R_f value (29 mg). The aniline derivative of the 2, 3, 4, 6-tetra-O-methyl-D-galactose was prepared [9]. The melting point of the substance and of a mixed sample was 188° C. The 2, 3, 6-tri-O-methyl-D-galactose was converted into the γ-lactone of 2, 3, 6-tri-O-methyl-D-galactonic acid [10], mp 99.3° C $[\alpha]_D^{20}$ −40° (in water).

Conclusions

The partial hydrolysis of the pectin of Panax ginseng C. A. Mey gives a polygalacturonide which has a linear carbohydrate chain consisting of residues of D-galacturonic acid in the pyranose form linked by α-(1 → 4)-bonds.

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