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POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. VIII. STRUCTURAL INVESTIGATION OF A GLUCOGALACTAN FROM THE ROOTS OF *Allochrusa gypsophiloides*

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It has been established that the glucogalactan is a branched polysaccharide, that its main chain consists of α-1→6-bound galactopyranose residues, C-2 atoms of the galactopyranose residues serve as the branching points, and the reducing ends are both galactopyranose and glucopyranose residues.

We have previously isolated from the roots of *Allochrusa gypsophiloides* Rgl. a neutral polysaccharide (NPS) [1] that is polydisperse according to the results of gel chromatography on Sephadexes. To obtain a homogeneous fraction, the NPS was fractionated on a column of Sephadex G-50, the emergence of the polysaccharide being monitored by the phenol/sulfuric acid method [2]. Three fractions (I-III) were obtained, with yields of 1.5% for I, 93.0% for II, and 3.6% for III. Fraction II was the main one quantitatively, and it was accumulated by column separation.

According to gel chromatography on Sephadex G-50, fraction II was homogeneous, and in a hydrolysate of it *D*-glucose and *D*-galactose were detected by PC and GLC in a ratio of 1:5; consequently, this fraction was a glucogalactan.

The glucogalactan formed a white amorphous powder readily soluble in water, $[\alpha]_D^{26}$ +176° (*c* 1.0; water); it did not give a positive reaction with iodine for the presence of starch, and nitrogen was absent. Its molecular mass, determined by gel chromatography on Sephadex G-50 from a calibration curve based on known dextran standards, was 2000. Its IR spectrum contained absorption bands at 860, 920, 980, 1080, 1160, 1360, 1415, 1650, 2375, 2940, and 3400 cm^{-1}, and there were no signals corresponding to methyl, acetyl, and sulfate groups.

On periodate oxidation, 1 mole of anhydro unit consumed 1.5 mole of $NaIO_4$, with the formation of 0.54 mole of HCOOH. Only glycerol was detected by PC and GLC in the products of Smith degradation [3], which showed the presence of 1→2 and 1→6 bonds between the hexose residues.

The Hakomori methylation [4] of the glucogalactan gave a permethylate the IR spectrum of which lacked the absorption bands of hydroxy groups. After formolysis and hydrolysis we identified in the cleavage products by TLC, GLC with authentic specimens, and by chromato-mass spectrometry (of the polyol acetate derivatives), products given in Table 1.

The finding of 2,3,4,6-tetra-O-methylgalactopyranose and 2,3,4,6-tetra-O-methylglucopyranose showed that the polymer chain had galactopyranose and glucopyranose residues at its nonreducing end. The presence of 3,4,6-tri- and 2,3,4-tri-O-Me-galactopyranoses was evidence in favor of 1→2 and 1→6 bonds of the *D*-galactopyranose residues in the polysaccharide.

The presence of 3,4-di-O-Me-galactopyranose showed that the main chain of the GG consisted of *D*-galactopyranose residues linked by 1→6 bonds, with branching at C-2 of galactopyranose.

The high positive specific rotation of the glucogalactan and the absorption bands at 860 and 920 cm^{-1} in the IR spectrum indicated the presence of α-glycosidic bonds between the monosaccharide residues. This hypothesis was confirmed by the oxidation of the completely acetylated polysaccharide with chromium trioxide. The oxidation products included free glucose and galactose residues; i.e., the polysaccharide was not oxidized. It is known that under these conditions only monosaccharide residues bound by β-glycosidic bonds undergo oxidation [5].

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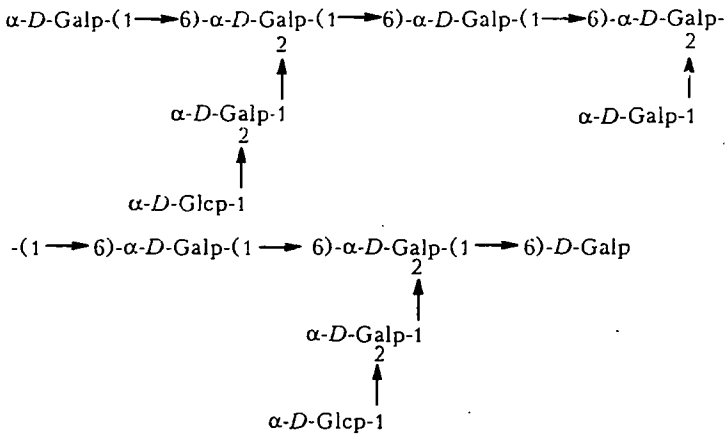
TABLE 1. Results of Analysis of the Methylation of the *Allochrusa gypsophiloides* Glucogalactan

Sugar component	Time, min	Molar ratio	Main mass-spectral fragments, *m/z*	Bonds
2,3,4,6-Tetra-O-Me-Galp	10.83	2	43, 45, 71, 87, 101, 117, 129, 145, 161, 205	Galp-(1→
2,3,4,6-Tetra-O-Me-Glcp	12.09	2	43, 45, 71, 87, 101, 117, 129, 145, 161, 205	Glcp-(1→
3,4,6-Tri-O-Me-Galp	12.67	2	43, 87, 99, 101, 129, 161, 189	→2)-Galp-(1→
2,3,4-Tri-O-Me-Galp	13.25	3	43, 87, 99, 101, 117, 129, 161, 189	→6)-Galp-(1→
3,4-Di-O-Me-Galp	14.30	3	43, 87, 99, 129, 189	→2,6)-Galp-(1→

To confirm the chemical results, the glucogalactan was studied by ^{13}C NMR spectroscopy. The spectrum was recorded in the range of resonance from 50 to 105 ppm that is characteristic for many polysaccharides.* The spectrum contained intense signals at (ppm) 100.2 (C-1), 69.4 (C-2), 70.4 (C-3), 70.5 (C-4), 72.2 (C-5), and 62.4 (C-6) relating to carbon atoms of galactopyranose residues [6] and less intense resonance lines at 99.9 (C-1), 72.5 (C-2), 74.3 (C-3), 70.8 (C-4), 73.3 (C-5) and 61.8 (C-6), which are characteristic for the carbon atoms of glucopyranose residues. Peaks at 62.4 and 63.2 ppm related to the C-6 atoms of unsubstituted hexopyranoses.

The chemical shifts of 100.2 and 99.9 ppm showed that the galactopyranose and glucopyranose residues had the α-configuration [6], while the substituted C-6 atoms of galactopyranoses resonated at 66.7 and 68.2 ppm, and a chemical shift of 92.1 ppm was due to the resonance of the reducing C-1 α-galactopyranose atom. The regions of resonance at 102.3 and 103.0 ppm were characteristic of the C-1 atom of a galactopyranose present at a branch-point, while the signals of the substituted C-2 atoms of galactopyranose residues appeared at 81.9 and 86.6 ppm.

We have found no information in the literature on ^{13}C NMR spectroscopy actually for glucogalactan polysaccharides or galactooligosaccharides with the α-1→2 and α-1→6 types of linkage. It may be assumed that the glucogalactan is a branched polysaccharide. It main chain consists of α-(1→6)-bound galactopyranose residues with branching at the C-2 atoms of some of these residues, the reducing ends being galacto- and glucopyranose residues. A possible structure for the glucogalactan is suggested:



Structure of the glucogalactan of *Allochrusa gypsophiloides*.

EXPERIMENTAL

TLC was conducted on Silufol UV-254 plates and on type LS-5/40 μm silica gel under the conditions of [7]. For PC we used Filtrak-1,3,11,12 paper and the solvent systems and revealing agents given in [7].

*The spectra were recorded in IOKh RAN im. M. D. Zelinskogo [M. D. Zelinskii Institute of Organic Chemistry of the Russian Academy of Sciences], and we thank A. S. Shashkov for the help provided.

Gas–liquid chromatography was performed on a Chrom-5 chromatograph with a flame-ionization detector under the conditions given in [7]. Acetates of aldononitriles and of polyols were obtained by the method of Lance and Jones [8]. Specific rotations of the substances were measured on a Zeiss polarimeter in a tube 1 dm long with a capacity of 10 ml.

The substances were reduced with an excess of sodium tetrahydroborate, as in [9].

The IR spectrum of the sample was taken on a UR-20 instrument in tablets with KBr and petrolatum. Chromato-mass spectra of samples were obtained on a Varian MAT III Gnom instrument, with a 0.3 × 120 cm column containing 10% of OV-1 on Chromosorb. The ^{13}C NMR spectra of the glucogalactan were recorded on a Bruker WR-60 instrument with a working frequency for carbon of 15.08 MHz under the conditions of [9].

Fractionation of the NPS by Gel Chromatography. A column with dimensions of 3 × 60 cm was filled with Sephadex G-50 (45 g) and was then washed with distilled water, after which 0.5 g of the NPS in 5 ml of water was deposited on it and was eluted with water, 3-ml fractions being collected every 15 min. The emergence of the polysaccharides was monitored by the phenol/sulfuric acid method. This gave fractions I-III of polysaccharides with yields of 0.75, 4.65, and 1.8 g, respectively. A solution of 0.01 g of fraction II in 1 ml of 0.3% sodium chloride was deposited on a column (2 × 40 cm) of Sephadex G-50. Determinations with authentic specimens of dextrans showed that fraction II was homogeneous and had a molecular mass of 2000.

The complete acid hydrolysis of the glucogalactan was carried out under the conditions of [1].

Periodate Oxidation and Smith Degradation. The glucogalactan (0.05 g) was oxidized with a 0.05 M solution of sodium periodate under the conditions of [7]. After 8 days the consumption of sodium periodate was 1.5 moles and it did not undergo further change; 0.54 mole of formic acid was formed. After the appropriate work-up, only glycerol was detected by PC and GLC in a hydrolysate of the oxidation product.

Methylation of the Glucogalactan. The glucogalactan (0.1 g) was methylated twice by Hakomori's method [4]. The fully methylated product (its IR spectrum lacked the absorption band of OH groups) was obtained with a yield of 0.9 g [sic]; $O-CH_3$ 42.3%, $[\alpha]_D^{25}$ +112° (*c* 0.9, acetone).

Formolysis and Hydrolysis of the Permethylate. The permethylate of the glucogalactan (0.05 g) was subjected to formolysis and hydrolysis as in [7]. In the hydrolysis products, TLC and GLC showed the presence of 2,3,4,6-tetra-O-Me-*D*-galactose, 2,3,4,6-tetra-O-Me-*D*-glucose, 3,4,6-tri-O-Me-*D*-galactose, 2,3,4-tri-O-Me-*D*-galactose, and 3,4-di-O-Me-*D*-galactose in a ratio of 2:2:2:3:3, respectively. Derivatives of partially methylated polyol acetates were obtained and were studied by chromato-mass spectrometry.

Acetylation of the Glucogalactan. The compound (0.1 g) was acetylated under the conditions of [7] and the peracetate was obtained with a yield of 0.136 g. The completeness of acetylation was checked by IR spectroscopy.

Oxidation of the Peracetate of the Glucogalactan with Chromium Trioxide. The peracetate of the glucogalactan (0.1 g) was oxidized with chromium trioxide as described in [7]. A hydrolysate of the oxidation product was shown by PC and GLC to contain free glucopyranose and galactopyranose residues in a ratio of 1:5.

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