POLYSACCHARIDES OF Eremurus.

XXVI. STUDY OF THE STRUCTURE OF THE PECTIN FROM THE LEAVES OF Eremurus regelii

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The partial acid hydrolysis of the homogeneous fraction C of the pectin from Eremurus regelii Vved. leaves has given a galacturonan. Its structure has been established on the basis of the results of periodate–nitric acid oxidation, methylation, and ^{13}C NMR spectroscopy.

We have previously isolated homogeneous fractions of pectins (A, B, and C) from the leaves of Eremurus regelii [1]. The homogeneity of the fractions was confirmed by gel chromatography and ultracentrifugation. Below we give the molecular masses of the homogeneous fractions [I) – by gel chromatography [2]; II) – by ultracentrifugation [3]]:

Fraction	Molecular mass	
	I	II
A	79500	78000
B	64600	66000
C	63100	62500

We have now studied the structure of the homogeneous fraction C of the pectin, since its yield amounted to 40.5%. This fraction was subjected to partial acid hydrolysis. This gave a galacturonan with a yield of 75%. A hydrolysate of the galacturonan was shown by paper chromatography (PC) to contain D-galacturonic acid. D-Galacturonic acid was also detected by PC among the products of enzymatic hydrolysis. The molecular mass of the galacturonan determined by ultracentrifugation was 37,000, and the degree of polymerization 228.

The IR spectrum of the galacturonan had the following absorption bands (cm^{-1}): 3400, 2930, 1750, 1630, 1410, 1340, 1230, 1110, 1020, 950, 890, and 830 [4]. Bands of the absorption of the stretching vibrations of a carboxylic methyl ester lay in the 1750 cm^{-1} region; bands of ionized carboxyl at 1630 and 1410 cm^{-1}; bands at 1110 and 1120 cm^{-1} related to the vibrations of a pyranose ring; an absorption band at 950 cm^{-1} reflected the out-of-plane deformation vibrations of methyl and methylene groups; an absorption band at 890 cm^{-1} belonged to the $1 \rightarrow 4$ type of glycosidic bonds; and absorption at 830 cm^{-1} was characteristic for pectins having the α-configuration of this bond.

The high positive specific rotation of the galacturonan, $[\alpha]_D^{20}$ + 220° (c 0.2; 0.01 N NaOH) likewise confirmed that the glycosidic bonds between the D-galacturonic acid residues in the pyranose form had the α-configuration. To confirm the order of the bond, the galacturonan was oxidized successively with periodic and nitric acids [5].

Oxalic and tartaric acids were detected by PC in the oxidation products of the galacturonan. The formation of tartaric acid showed that α-diol groupings at the second and third carbon atoms had undergone oxidation. This is possible only in the case of a $1 \rightarrow 4$ bond between the D-galacturonic acid residues.

To increase its degree of methylation, the galacturonan was esterified with diazomethane and was then reduced with sodium tetrahydroborate [6]. The reduced galacturonan was methylated by Hakomori's [7] and Purdie's [8] methods, and a permethylate was obtained. Completeness of methylation was checked by IR spectroscopy from the absence of absorption bands of hydroxy groups. The permethylate was subjected to formolysis and hydrolysis, and

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in the products 2,3,6-tri-O-methyl-D-galactose and 2,4,6-tetra-O-methyl-D-galactose were identified by thin-layer chromatography (TLC) in comparison with markers. The formation of 2,3,6-tri-O-methyl-D-galactose as the main product showed the presence of 1 → 4 bonds in the galacturonan.

In the ^{13}C NMR spectrum of the galacturonan signals were observed with the following chemical shifts (ppm): C-1 (100.8), C-2 (69.2), C-3 (69.6), C-4 (79.2), C-5 (71.7), and C-6 (173.6). The values of these chemical shifts show that galacturonan consisted of a chain composed of α-(1→4)-bound D-galacturonic acid residues [9, 10].

Thus, on the basis of an analysis of the results of periodate–nitric acid oxidation, methylation, and ^{13}C NMR spectroscopy the galacturonan has been shown to be a linear polymer with the following structure:

$$\text{GalUA-}\alpha\text{-1}-[\rightarrow 4\text{-GalUA-}\alpha\text{-1}\rightarrow 4\text{-GalUA-}\alpha\text{-1}-]_{113}\rightarrow 4\text{-GalUA}$$

EXPERIMENTAL

Solutions were concentrated in a rotary evaporator at 40 ± 5°C. Thin-layer chromatography was conducted on Silufol UV-254 plates and on type KSK silica gel, LS-5/40 mm, in the following solvent systems: 1) benzene–acetone (1:1), (1:2), and (4:1); and 2) chloroform–methanol (9:1).

For paper chromatography we used Filtrak FN-11 paper and the following solvent systems: 3) butan-1-ol–pyridine–water (6:4:3); 4) butan-1-ol–acetic acid–water (4:1:2), and 5) ethyl acetate–formic acid–water–acetic acid (18:1:4:3).

The substances were detected by spraying with the following reagents: 1) acid aniline phthalate; 2) o-toluidine salicylate; and 3) aniline–diphenylamine–phosphoric acid. The specific rotations of the substances were determined on a Zeiss polarimeter in a tube 1 dm long with a volume of 10 ml. The IR spectra of the samples were taken on a UR-20 instrument in tablets with KBr and in petrolatum. The ^{13}C NMR spectra of the galacturonan were taken on a Bruker WR-60 instrument with a working frequency for carbon of 15.08 MHz, using complete suppression of proton interactions. A 3% solution in D_2O was prepared with the internal standard methanol, the chemical shift of the signal of which relative to TMS was taken as 50.15 ppm.

Ultracentrifugation was carried out on a MOM 3170 instrument (50,000 rpm, temperature 20°C, c 1.0water, 0.6% NaOH), exposure time 30 min. The gel chromatography of the homogeneous fractions A, B, and C was conducted on a column (1.9 × 45 cm) of Sephadex G-100, and they were eluted with water and were analyzed by the phenol/sulfuric acid method [11]. The column was calibrated by the passage of dextrans with molecular masses of 40,000 and 80,000. Molecular masses were determined from a calibration curve of the dependence of the molecular mass on the elution volume [2].

Production of a Galacturonan from the Homogeneous Fraction C of the Pectin. A solution of 1 g of the homogeneous fraction C in 50 ml of water was treated with 50 ml of 4 N H_2SO_4 and the mixture was heated in the water bath at 95°C for 4 h. The precipitate that had deposited was centrifuged off. It was washed with 1% H_2SO_4 solution and then with 80% methanol and with pure methanol until the medium was neutral.

The yield of galacturonan was 0.75 g. D-Galacturonic acid was detected by PC (systems 3 and 5, spray reagent 1) in the products of its complete acid hydrolysis (2 N H_2SO_4, 95°C, 48 h).

Enzymatic Hydrolysis. The galacturonan (0.1 g) in 10 ml of water was treated with 0.2 g of pectinase (Fluka, Sweden). Enzymatic hydrolysis was conducted at pH 4 and a temperature of 37°C (48 h). The products of enzymatic hydrolysis were studied by the PC method in systems 3 and 5 with revealing agent 1.

Periodate–Acetic Acid Oxidation of the Galacturonan. A suspension of 1 g of the galacturonan in 15 ml of a 5% solution of dry hydrogen chloride in absolute methanol was boiled on the water bath with stirring for 8 h. The solid matter was separated off, washed with methanol, and dried. The substance obtained was dissolved in the minimum amount of water and the solution was centrifuged. The addition of a fivefold volume of ethanol to the clear solution led to the formation of a precipitate. This was separated off, washed with ethanol and with acetone, and dried over P_2O_5 in vacuum.

The yield was 0.68 g of a substance with $[\alpha]_D^{20}$ + 190° (c 0.1; H_2O), and a solution of this in 17.4 ml of water was treated with 4.25 g of crystalline periodic acid in 23.6 ml of water. Oxidation was conducted in the dark at 20°C with stirring for 60 h. The solution was deionized with AN-8 anion-exchange resin (HCO_3^-). It was then evaporated to dryness, giving an oily polyaldehyde. The latter was dissolved in 7 ml of conc. nitric acid (d = 1.19) and the solution was heated at 95°C for 3 h. The oxidate was evaporated to dryness. The products of the oxidation of the polyaldehyde were hydrolyzed with 1 N H_2SO_4 at 95°C for 8 h. The sulfuric acid was neutralized quantitatively with a 0.2 N solution of barium hydroxide. The resulting solution was concentrated to a syrup, and oxalic and tartaric acids were detected by PC (systems 3 and 4, spray reagent 1).

A 30% solution of potassium hydroxide to give pH 8 and 0.6 ml of glacial acetic acid were added to the residue of the hydrolysate (1 ml). The resulting solution was left at 40°C for 20 h. The crystals of potassium hydrogen tartrate were filtered off and were washed with 50% ethanol and with acetone. The potassium hydrogen tartrate was dissolved in water and the solution was treated with KU-2 cation-exchange resin (H^+). The solution of the acid was evaporated to dryness and, after recrystallization from a mixture of diethyl ether and ethanol (1:1), the melting point of the tartaric acid was 168-170°C.

Esterification. The galacturonan (2 g) was wetted with 80% methanol and esterification was carried out with an ethereal solution of diazomethane at +4°C for 18 h. This gave 1.9 g of esterified galacturonan.

Reduction. The esterified galacturonan (1.9 g) was dissolved in 100 ml of water and reduced with sodium tetrahydroborate (1 g). The solution was treated with KU-4 cation-exchange resin (H^+) and evaporated with the addition of methanol. The esterification and reduction of the galacturonan were performed ten times. As a result, 0.4 g of reduced galacturonan containing 12% of D-galacturonic acid was obtained.

Methylation. The reduced galacturonan (0.4 g) was methylated by Hakomori's and Purdie's methods (3 times). The yield of permethylate was 0.06 g. The galacturonan permethylate was a syrupy product soluble in tetrahydrofuran, acetone, and chloroform, and insoluble in water $[\alpha]_D^{20}$ + 18° (c 1.5; $CHCl_3$).

Formolysis and Hydrolysis. The galacturonan permethylate (0.15 g) was subjected to formolysis in 1 ml of 90% formic acid at 80°C for 1 h, the mixture was concentrated with the addition of methanol, and the residue was hydrolyzed with 1 N H_2SO_4 at 95°C for 20 h. The hydrolysate was investigated by TLC (systems 1 and 2, revealing agents 2 and 3).

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