POLYSACCHARIDES OF *Aloe arborescens*

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The present paper gives the results of the isolation of a polysaccharide fraction from the leaves of *Aloe arborescens* and its general characteristics. Our knowledge of the polysaccharides of this plant is limited to information on the isolation from the juice of the aloe of a glucuronoglucomannan consisting of residues of mannose (48.8%), glucose (48.8%), and glucuronic acid (2.4%) [1]. It has been shown that this polysaccharide has a favorable effect on the healing of open wounds and burns.

The leaves after treatment with methanol were extracted successively with water and with ammonium oxalate. Two polysaccharide fractions (A and B) were obtained. Fraction A was distinguished by higher contents of ash (8.4% after dialysis) and of methoxy groups (6.5%). Acid hydrolysis gave xylose and a uronic acid. This fraction was not studied in more detail. Fraction B, forming the main polysaccharide fraction of the aloe leaves in amount, was distinguished by a high degree of homogeneity. From the results of gel filtration, the molecular weight of the polysaccharide exceeds 30,000, since its elution volume is equal to the free volume of a column of Bio-Gel P-30 and it is retained on the higher types beginning with P-60. When the polysaccharide was chromatographed on DEAE-celluose and was eluted with solutions of aqueous caustic soda of increasing concentration (0.01→0.3 N NaOH), a single narrow peak was formed. Peaks corresponding to polysaccharides eluted with water and aqueous salt solutions were practically absent. Fraction B did not react with iodine and, consequently, did not contain a glucan of the starch type.

A hydrolyzate of this fraction was shown by paper and gas–liquid chromatography to contain galactose, glucose, mannose, xylose, arabinose, fucose, rhamnose, and galacturonic acid; the high amount of the latter (about 55%) is characteristic for pectin substances.

The polysaccharide of fraction B was cleaved by pectinase, forming galacturonic acid and a number of oligosaccharides. The partial hydrolysis of fraction B gave a galacturonan the complete hydrolysis of which yielded only galacturonic acid and which was hydrolyzed by pectinase. Consequently, in the aloe leaves the main component of the polysaccharide fraction is pectin.

EXPERIMENTAL

Chromatography was performed on FN-3 paper in the following solvent systems (by volume): 1) butan–1–ol–pyridine–water (6:4:3) and 2) ethyl acetate–acetic acid–pyridine–water (5:1:5:3). The monosaccharides were detected with aniline phthalate. Gas–liquid chromatography was performed on a Tsvet-6 instrument (Dzerzhinsk) with a flame-ionization detector. The stationary phase was 3% of HI-EFF-8-BP on Gas-Chrom Q (100-120 mesh) in steel columns (1 m × 0.5 cm). The rate of flow of the carrier gas (argon) was 40 ml/min. The temperature was programmed at 4 degC/min in the range from 170-225°C. The monosaccharides were analyzed in the form of the peracetates of the corresponding aldononitriles [2]. The ion-exchange chromatography of the polysaccharide fractions was performed on DEAE-cellulose by a published method [3]. Bio-Gels P-10, P-30, and P-60 were used for gel filtration. The free volumes

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of the columns were determined with dextran blue. The amounts of uronic acids in the polysaccharides were found by Anderson's method [4]. The complete acid hydrolysis of the polysaccharides (5-10 mg) was performed in a sealed tube with 2 N sulfuric acid at 95°C for 8-10 h. All the solutions were evaporated in vacuum at 30-40°C. The freshly gathered leaves of a three-year *Aloe arborescens* plant were used for the experiments.

Isolation of the Polysaccharides. The comminuted aloe leaves were extracted exhaustively with boiling methanol. Then the air-dry plant material (6 g) was covered with diluted hydrochloric acid (pH 4-5) at 50°C until a permanent acid medium was produced (about 3 h). The residue was washed on the filter with water, and the filtrate was evaporated to small volume and poured into an excess of ethanol. No precipitate whatever of polysaccharides was formed. Then the raw material was treated with water at 100°C three times. The aqueous solution was evaporated and was poured into a fourfold volume of ethanol. The precipitate that deposited was separated off and dissolved in water, and the solution was dialyzed and freeze-dried. This gave polysaccharide fraction A with a yield of 1 g, $[\alpha]_D^{20}$ +99.2° (in water), containing uronic acid (46%), methoxy groups (6.5%), and ash (8.4%). A hydrolyzate of fraction A was shown by paper chromatography in systems 1 and 2 to contain xylose and a hexuronic acid, and by GLC to contain xylose. Then the raw material was extracted with 0.5% aqueous ammonium oxalate at 100°C for 5 h three times. The solution was dialyzed, evaporated, and poured into an excess of ethanol. The precipitate was dissolved in water, and the solution was acidified and was again dialyzed against distilled water, after which it was concentrated by freeze-drying. This gave polysaccharide fraction B with a yield of 2.4 g $[\alpha]_D^{20}$ +171° (in water), containing uronic acid (56%), methoxy groups (0.9%), and ash (less than 1%). The galacturonic acid was identified by its isolation from the mixture through ion-exchange chromatography on Dowex 1×4 (HCO_3^-) followed by the reduction of the methyl ester to galactose with sodium tetrahydroborate.

Enzymatic Hydrolysis of Fraction B. A solution of 100 mg of the polysaccharide in 10 ml of water was treated with 2 mg of pectinase. The mixture was incubated at 37°C for 24 h and was then poured into ethanol, and the filtrate was evaporated and chromatographed on paper in system 3. The hydrolyzate was shown to contain galacturonic acid and a number of oligosaccharides.

Partial Hydrolysis of Fraction B. The polysaccharide (100 mg) was treated with 1 N sulfuric acid (3 ml) at 95°C for 3 h. The precipitated and ethanol-reprecipitated material was hydrolyzed again. The residue was washed on the filter with water. This gave a galacturonan, the complete hydrolysis of which yielded only galacturonic acid. When the galacturonan was treated with pectinase as described above, galacturonic acid was formed.

SUMMARY

An acidic polysaccharide belonging to the class of pectin substances has been isolated from the leaves of *Aloe arborescens*.

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