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PECTINIC SUBSTANCES FROM *Aloe arborescens*

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Pectinic substances were isolated in 3.82% yield of the fresh material mass from leaves of Aloe arborescens *Mill. (Asphodelaceae). Fractionation over DEAE-cellulose produced the dominant polymer APS-3 of molecular weight 71.1 kDa,* $[\alpha]_D^{20}$ *+224.4°. Physicochemical methods established that APS-3 was a partially methoxylated and acetylated rhamnopolygalacturonan consisting of α-(1→4)-bonded galacturonic acid and α-(1→2)-bonded rhamnopyranose in the main chain. Side substituents of single galacto- and glucopyranose units were located on the C-3 atom of galacturonic acid.*

Key words: *Aloe arborescens* Mill., pectinic substances, rhamnopolygalacturonan, ^{13}C NMR.

We continued the investigation of *Aloe arborescens* Mill. [1, 2] by isolating and studying the structure of pectinic substances of this species. Pectin containing uronic acids (56%) and galactose, glucose, mannose, xylose, arabinose, fucose, and rhamnose was previously obtained from *A. arborescens* in 40% yield of the raw material mass but its structure was not investigated [3].

Pectinic substances were isolated from *A. arborescens* leaves by extraction with an oxalate mixture after removing alcohol- and water-soluble components. The resulting extract was dialyzed and precipitated with acetone to isolate the APS fraction in 3.82% yield of the fresh material mass (37.09% of air-dried raw material mass). The content of carbohydrates was 98.4%; of protein and ash, less than 0.3%. The APS solutions had a positive specific rotation $[\alpha]_D^{20}$ +157.1° (Table 1). The IR spectrum exhibited bands characteristic of pectinic substances with a pyranose ring (764.2, 837.2, 1097.9, 1330.1 cm^{-1}), an α-bond (852.6), and carboxylic group (1605.7) [4]. The study of the APS monosaccharide composition showed that the fraction contained 78.4% galacturonic acid and neutral carbohydrates including galactose, arabinose, glucose, rhamnose, xylose, and mannose in a 70:57:57:25:5:1 ratio (Table 2). The contents of free (K_f) and esterified (K_e) carboxylic groups were 13.83 and 1.94%, respectively. Therefore, the isolated pectin was low-esterified (degree of esterification λ = 12.30%). An ester band in the IR spectrum (1235.0, 1742.4) was consistent with the presence of acetyls, the content of which according to chemical analysis (hydroxylamine method) was 12.92% (by IR spectroscopy, 12.54%).

Gel chromatography showed that the APS was heterogeneous. Therefore, the total complex was separated using chromatography over DEAE-cellulose, which produced six fractions: APS-1 (water eluent), APS-2 (0.1 M NaCl), APS-3 (0.3 M NaCl), APS-4 (0.5 M NaCl), APS-5 (1 M NaCl), and APS-6 (0.1 M NaOH). Tables 1 and 2 list the physicochemical properties and the monosaccharide composition of the fractions.

APS-1 (6.3% of the APS mass) was a homogeneous polymer with MW 91.3 kDa and contained galactose, arabinose, and glucose in a 1.3:1.1:1.0 ratio in addition to galacturonic acid, rhamnose, and xylose in trace quantities.

The dominant fraction APS-3, which represented over 70% of the APS mass, was investigated further.

APS-3 was a homogeneous polymer with MW 71.1 kDa and contained 97.4% galacturonic acid and neutral carbohydrates rhamnose, glucose, and galactose in a 1.4:1.3:1.0 ratio, $[\alpha]_D^{20}$ +224.4°, K_f 17.21%, K_e 2.90%, λ 14.42%, K_{Ac} 15.24% (hydroxylamine method) and 15.43% (IR spectroscopy).

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TABLE 1. Physicochemical Properties of APS, APS-1–APS-6, and APS-3′

Fraction	Yield, % of APS mass	$[\alpha]_D^{20}$, ° (c 1.0, 1% NaOH)	MW, kDa	K_f,%	K_e,%	λ, %	K_{Ac},%*
APS	–	+157.1	–	13.83	1.94	12.30	12.92/12.54
APS-1	6.3	+53.7	91.3	–	–	–	–
APS-2	13.5	+144.1	83.3, 52.4	6.19	0.63	9.24	12.28/12.15
APS-3	73.1	+224.2	71.1	17.21	2.90	14.42	15.24/15.43
APS-4	2.5	+177.0	62.7	8.06	0.29	3.47	–/11.32
APS-5	0.2	–	–	–	–	–	–
APS-6	0.4	–	–	–	–	–	–
APS-3′	–	+231.4	64.6	25.11	0	0	0/0

"–", not determined; *chemical analysis (hydroxylamine method)/IR spectroscopy.

TABLE 2. Monosaccharide Composition of APS, APS-1–APS-6, and APS-3′

Fraction	Monosaccharide composition, mol %						
	Ara	Gal	Glc	Man	Rha	Xyl	GalUA
APS	5.7	7.0	5.7	0.1	2.5	0.5	78.4
APS-1	30.7	37.2	28.7	–	1.0	0.2	2.1
APS-2	16.2	21.0	16.1	1.2	8.2	2.5	34.7
APS-3	–	0.9	0.7	–	1.0	–	97.4
APS-4	26.8	21.4	2.2	–	12.1	–	37.4
APS-5	14.7	31.1	8.8	1.7	12.4	–	31.2
APS-6	19.4	14.8	9.1	2.9	7.3	2.8	43.6
APS-3′	–	0.4	0.1	–	1.1	–	98.3

Alkaline hydrolysis of APS-3 produced component APS-3′ that was free of methoxyls and acetyls. This was confirmed by IR spectroscopy (Table 1). APS-3′ contained galacturonic acid (98.3%), rhamnose, galactose, and glucose (11:4:1 ratio).

Periodate oxidation of APS-3′ consumed 0.98 mol of IO_4^- per anhydro unit and released less than 0.001 mol of HCOOH. The type of bonding in APS-3′ was determined by using nitric-acid oxidation of the polyaldehyde obtained after periodate oxidation of the permethylate. The principal hydrolysis products were oxalic and tartaric acids, which was possible with a 1→4 bond. Enzymatic hydrolysis by α-pectinase decomposed APS-3′ to give galacturonic acid as the principal product and several oligosaccharides, which was also possible for a 1→4 bond. This was also confirmed by the presence in the IR spectrum of a band at 893 cm^{-1} that was characteristic of a 1→4 bond.

The α-configuration for galacturonic acid, rhamnose, galactose, and glucose was proved by oxidation of acetylated APS-3′ by CrO_3 because the hydrolysate contained these monosaccharides.

^{13}C NMR spectroscopy of polysaccharide APS-3 exhibited a resonance for an anomeric C atom at 102.35 ppm that was indicative of the α-configuration of the galacturonic acid (Table 3). Atom C-6 gave two resonances at weak field, the stronger one at 178.17 ppm assigned to the C atom of a free carboxylic group and the weaker one at 174.21, to a methoxylated carboxylic group [5]. Strong-field resonances at 52.91 and 21.87 confirmed that APS-3 contained methoxyls and acetyls, respectively. The positions of the other resonances agreed with those for spectra of α-(1→4)-D-galactopyranosyluronate [6]. Resonances corresponding to C atoms of the other monosaccharides were not examined in this instance because of their low signal strength.

The ^{13}C NMR spectrum of APS-3′ lacked resonances for methoxylated carboxylic group, methoxyls, and acetyls. The positions of the other resonances were analogous to those for APS-3.

TABLE 3. ^{13}C NMR Data for APS-3 and APS-3′

Monosaccharide unit	C-1	C-2	C-3	C-4	C-5	C-6	$COO\underline{C}H_3$	$CO\underline{C}H_3$
				APS-3				
→4-GalUA*p*-α-1→	102.35	71.11	72.20	81.19	74.55	178.17 174.41	52.91	21.87
				APS-3′				
→4-GalUA*p*-α-1→	102.24	71.11	72.08	81.11	74.63	178.21		

The structural position of the rhamnose in APS-3′ was determined after it was methylated and reduced with $NaBH_4$ followed by methylation of the reduced product with methyliodide by the Ciucanu—Kerek method. Formolysis produced in the hydrolysate of the permethylate 2,3,6-tri-*O*-Me-D-Gal*p* (97.4%) and traces of 2,6-di-*O*-Me-D-Gal*p*, 2,3,4,6-tetra-*O*-Me-D-Gal*p*, 3,4,6-tri-*O*-Me-L-Rha*p*, and 2,3,4,6-tetra-*O*-Me-D-Glc*p* (total 2.5%). The presence of 2,3,6-tri-*O*-Me-D-Gal*p* as the main hydrolysis product indicated that APS-3′ was a (1→4)-bonded polygalacturonan and that the main polymer chain contained (1→4)-bonded rhamnose. The presence of disubstituted galactopyranose was explained by the presence of branching points at C-3 of galacturonic acid consisting of single galactose and glucose units.

Thus, the investigation showed that the dominant polymer APS-3 of the pectinic substances from *A. arborescens* was a partially methoxylated and acetylated high-molecular-weight rhamnopolygalacturonan consisting of α-(1→4)-bonded galacturonic acid and α-(1→2)-bonded rhamnopyranose in the main chain and side chains on galacturonic acid at C-3 of single galacto- and glucopyranose units.

EXPERIMENTAL

Leaves of *A. arborescens* were collected in 2008 at the greenhouse of the Siberian Institute of Plant Physiology and Biochemistry, SB, RAS (Irkutsk) from 3-year-old plants. HPTLC was performed on Sorbfil PTSKh-AF-V plates (Sorbpolimer) using solvent systems *i*-PrOH:$CHCl_3$:H_2O (7:4:1, two-fold elution to 4 and 8 cm, 1); CH_2Cl_2:AcOH:MeOH:H_2O (10:5:3:2, 2); and $(CH_3)_2CO$:NH_3(25%):EtOH:$CHCl_3$:H_2O (60:22:10:6:2, three-fold elution to 6 cm, 3). The detectors were *p*-diphenylaminophthalanilic acid:H_3PO_4 (1) and methyl red:bromphenol blue (2).

Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. Potentiometric studies used a pH-410 pH-meter (Akvilon). IR spectra were recorded in films on KRS-5 plates on a Spectrum 100 IR-Fourier spectrometer (Perkin—Elmer) at 4000–650 cm^{-1}. ^{13}C NMR spectra were recorded in DMSO-d_6 solutions (1%) on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. GC/MS analysis of methylated carbohydrates was carried out on an Agilent GC-MS with a mass-selective detector (No. 5973) with a diffusion pump using a PH-Innowax 30 m/250 μm/0.50 μm capillary column. The temperature gradient was 150–250°C; heating rate 2°/min; carrier gas He at flow rate 1 mL/min.

Isolation of Pectinic Substances from *A. arborescens*. Fresh leaves (500 g) were ground and extracted with ethanol (95%) and water until exhausted (phenol:H_2SO_4 method). The remaining raw material was extracted with a mixture of ammonium oxalate and oxalic acid solutions (0.5%, 1:1, 50:1 ratio, 5×). The combined extract was dialyzed through a nitrocellulose membrane against distilled water. The non-dialyzed remainder was precipitated with acetone (1:5). The resulting precipitate (APS) was centrifuged and dried by solvent exchange. Yield of APS, 19.11 g.

APS. $[\alpha]_D^{20}$ +157.1° (*c* 1.0, 1% NaOH). IR spectrum (ν, cm^{-1}): 3354.1, 2942.0, 2143.7, 1742.4, 1605.7, 1413.3, 1330.1, 1235.0, 1137.6, 1097.9, 1019.5, 956.3, 913.0, 890.2, 852.6, 837.2, 764.2, 741.8.

Ion-exchange Chromatography of APS over DEAE-cellulose. APS (15 g) was dissolved in water (200 mL). The resulting solution was placed on a DEAE-cellulose column (HCO_3^--form, 6 × 30 cm) and eluted successively with water, NaCl (0.1, 0.3, 0.5, and 1 M), and NaOH (0.1 M). The eluates were dialyzed through a nitrocellulose membrane against distilled water. The non-dialyzed remainder was precipitated with acetone (1:4). The resulting precipitates were centrifuged

and dried by solvent exchange to afford six fractions of APS: APS-1 (H_2O, 0.94 g), APS-2 (0.1 M NaCl, 2.03 g), APS-3 (0.3 M NaCl, 10.96 g), APS-4 (0.5 M NaCl, 0.37 g), APS-5 (1 M NaCl, 0.022 g), and APS-6 (0.1 M NaOH, 0.057 g).

APS-3. $[\alpha]_D^{20}$ +224.2° (*c* 1.0, 1% NaOH). MW 71.1 kDa. IR spectrum (ν, cm^{-1}): 3399.8, 2943.3, 2159.5, 1741.7, 1606.1, 1415.9, 1332.9, 1237.6, 1144.4, 1101.8, 1020.7, 958.4, 916.7, 893.4, 851.2, 834.6, 770.8, 741.6.

The carbohydrate content was determined by the anthrone method and calculated as galacturonic acid [7]; protein, by the Bradford method [9]; ash content, by a gravimetric method after ashing; galacturonic acid (K_{GalUA}), by reaction with 3,5-dimethylphenol [9]; free (K_f) and esterified (K_e) carboxylic groups, by potentiometric titration [10]; content of acetyls (K_{Ac}), by a hydroxylamine method [11] and IR spectroscopy [12].

Total hydrolysis of polysaccharides was performed in TFA (2 M) at 110°C for 6 h, after which the hydrolysate was concentrated in vacuo in the presence of MeOH and analyzed by HPTLC (system 1, detector 1). The quantitative monosaccharide composition was determined by a densitometric method.

Enzymatic hydrolysis was carried out using α-pectinase (5000 PSU/g, Fluka) [13]. The hydrolysate was analyzed by HPTLC (system 2, detector 1).

Gel chromatography was performed over Sephadex G-200 (3 × 80 cm, Pharmacia) with NaCl eluent (0.5%), flow rate 0.1 mL/min; yield detection by phenol:H_2SO_4 [14]. The standards were dextrans with molecular weights 2000, 80, 50, and 10 kDa (Pharmacia). APS-3′ was chromatographed by dissolving a portion of the compound beforehand in NH_3 solution (10%).

Saponification of APS-3. Compound (5 g) was dissolved in water (200 mL), treated with NaOH (8 M, 20 mL), left with constant stirring on a magnetic stirrer at 200°C for 1 h, and treated with conc. HCl until the pH was 5.0. The resulting precipitate (APS-3′) was centrifuged and dried by solvent exchange. Yield of APS-3′, 4.63 g (92.6%).

APS-3′. $[\alpha]_D^{20}$ +231.4° (*c* 1.0, 1% NaOH). MW 64.6 kDa. IR spectrum (ν, cm^{-1}): 3400.2, 2945.7, 2151.4, 1739.9, 1415.9, 1333.3, 1142.9, 1102.5, 1019.3, 957.2, 916.7, 893.0, 850.4, 835.0, 770.5, 741.4.

Periodate oxidation and Smith degradation were carried out as before [15]. The consumption of periodate was calculated from the decrease of absorption at 223 nm [16]; quantitative determination of HCOOH, by titration with NaOH (0.01 M).

Periodate—nitric-acid oxidation of APS-3′ was carried out as before [17, 18] to afford methoxylated polygalacturonan with $[\alpha]_D^{20}$ +212.4° (*c* 0.5, H_2O) and polyaldehyde $[\alpha]_D^{20}$ –89.1° (*c* 0.78, H_2O). HPTLC (system 3, detector 2) of the oxidation products of the polyaldehyde detected oxalic and tartaric acids.

Acetylation of APS-3′. Finely ground powdered APS-3′ (150 mg) was suspended in freshly distilled pyridine (10 mL) in an ultrasonic bath (Sapfir, Akvilon, operating frequency 35 kHz) at 35°C for 30 min, treated with acetic anhydride (50 mL), left at 40°C for 48 h, and treated with HCl (100 mL, 5%). The resulting precipitate was centrifuged, washed with ethanol (95%), dissolved in acetone (10 mL), and centrifuged. The supernatant was treated with diethylether (50 mL). The resulting precipitate was centrifuged and dried. Yield of APS-3′ acetate, 132 mg; $[\alpha]_D^{20}$ +224.8° (*c* 1.0, acetone); K_{Ac}, 31.12%. Oxidation by CrO_3 of APS-3′ acetate was performed as before [19].

Methylation of APS-3′. A suspention of finely ground compound (0.5 g) in MeOH (10 mL) was esterified by diazomethane at +2°C for 24 h. The esterified product was dissolved in water (50 mL) and reduced by $NaBH_4$ (1 g). After 10 h the solution was de-ionized with cation-exchanger KU-2-8 (H^+-form). The eluate was concentrated in vacuo in the presence of MeOH to afford reduced product (0.38 g) containing galacturonic acid (6.7%). Methylation of reduced APS-3′ was performed as before [20]. Yield of permethylate, 267 mg.

Formolysis and Hydrolysis. A weighed portion of the permethylate (50 mg) was heated at 80°C with HCOOH (90%, 10 mL) for 1 h. The HCOOH was removed in vacuo in the presence of MeOH. The solid was treated with H_2SO_4 (5 mL, 1 M) and heated at 120°C for 6 h. The hydrolysate was neutralized with anion-exchanger ASD-4-5p (CO_3^{2-}-form), concentrated, and studied by GC/MS.

REFERENCES

1. D. N. Olennikov, A. V. Rokhin, and I. N. Zilfikarov, *Khim. Prir. Soedin.*, 578 (2008).
2. D. N. Olennikov, T. A. Ibragimov, V. A. Chelombit′ko, A. V. Nazarova, A. V. Rokhin, and I. N. Zilfikarov, *Khim. Prir. Soedin.*, 407 (2009).

3. R. G. Ovodova, V. F. Lapchik, and Yu. S. Ovodov, *Khim. Prir. Soedin.*, 3 (1973).
4. J. Copikova, A. Synytsyn, M. Cerna, J. Kaasova, and M. Novotna, *Czech. J. Food Sci.*, **19**, 51 (2001).
5. P. Odonmazig, D. Bagda, A. Ebringerova, and J. Alfoldi, *Carbohydr. Res.*, **226**, 353 (1992).
6. R. G. Ovodova, V. V. Golovchenko, A. S. Shashkov, S. V. Popov, and Yu. S. Ovodov, *Bioorg. Khim.*, **26**, 743 (2000).
7. D. N. Olennikov, L. M. Tankhaeva, and A. B. Samuelsen, *Khim. Prir. Soedin.*, 378 (2005).
8. M. M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
9. A. T. Usov, M. I. Bilan, and N. G. Klochkova, *Bot. Mar.*, **35**, 43 (1995).
10. N. Sh. Kaisheva, S. N. Shcherbak, V. A. Kompantsev, N. I. Krikova, and L. I. Ivanova, *Zh. Anal. Khim.*, **49**, 1158 (1994).
11. E. Tomasch and J. Mayer, *Acta Pol. Pharm.*, **17**, 139 (1960).
12. T. Heinze, T. Liebert, and A. Koschella, *Esterification of Polysaccharides*, Berlin, Heidelberg, 2006.
13. R. G. Ovodova, V. V. Golovchenko, S. V. Popov, A. S. Shashkov, and Yu. S. Ovodov, *Bioorg. Khim.*, **26**, 61 (2000).
14. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
15. A. I. Usov, S. V. Yarotskii, and M. L. Esteves, *Bioorg. Khim.*, **4**, 66 (1978).
16. A. Gutierrez, A. Prieto, and A. T. Martinez, *Carbohydr. Res.*, **281**, 143 (1996).
17. A. G. Gorin, *Khim. Prir. Soedin.*, 80 (1967).
18. M. Kh. Malikova, *Khim. Prir. Soedin.*, 320 (1994).
19. J. Hoffman, B. Lindberg, and S. Svenson, *Acta Chem. Scand.*, **26**, 661 (1972).
20. I. Ciucanu and F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).