Structural Characterization of an Acidic Heteropolysaccharide from *Rudbeckia fulgida*, var. *sullivantii* (Boynton et Beadle)

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An acidic heteropolysaccharide composed of L-rhamnose (14.0 %), L-arabinose (17.7 %), D-xylose (3.0 %), D-mannose (2.6 %), D-glucose (5.4 %), D-galactose (38.4 %), and uronic acids (18.9 %) has been isolated from the aerial part of the medicinal plant *Rudbeckia fulgida*, var. *sullivantii*. Compositional and methylation analyses of the native and carboxyl-reduced polymers, ^{13}C NMR spectroscopy measurements as well as the products of partial acid hydrolysis pointed to a branched structure of the polymer with a backbone formed by 3- and 3,6-linked galactose units carrying mainly arabinofuranose (5- and 3-linked) and ramified rhamnogalacturonan side chains. The glucuronic acid and xylose as well as some arabinose and galactose residues occupied terminal positions in the polymer.

The medicinal plant *Rudbeckia fulgida*, var. *sullivantii* (Boynton et Beadle) (*Asteraceae*) has been known for a long time to contain active components, used in the form of extracts for stimulation of non-specific defence mechanisms at infections and chronic inflammations [1, 2]. Recently, it has been found that aqueous ethanol extracts of the aerial tissues of this plant exhibited significant immunostimulating activity [3]. Motivated by this finding, and because of our continuous interest in search for active plant polysaccharides, in our previous work [4] we isolated a water-extractable crude polysaccharide which was proven to possess high antitussive activity [5]. The neutral portion of this mixture was characterized in [4] and now we provide the results on isolation of the acidic portion from the mixture and on structural determination of the dominant acidic heteropolysaccharide.

EXPERIMENTAL

The plant material was obtained from the garden of the Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic and the voucher specimen is deposited at the Herbarium of the Faculty of Pharmacy. Solutions were concentrated under reduced pressure below 40 °C. Free-boundary electrophoresis of 1 % solution of polysaccharide was effected with a Zeiss 35 apparatus, using 0.05 M-sodium tetraborate buffer (pH 9.2) at 150 V cm^{-1} and 6 mA for 30 min. Optical rotation of the polysaccharide (0.5 % in water) was measured at (20 ± 1) °C with a Perkin—Elmer Model 141 polarimeter.

High-performance gel permeation chromatography (HPGPC) was performed at room temperature using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) equipped with two Tessek Separon HEMA-BIO 1000 columns (8 mm × 250 mm) with a 0.1 cm^3 sample loop. The mobile phase used was 0.1 M-$NaNO_3$ at a flow rate of 0.4 cm^3 min^{-1} and the carbohydrate content was monitored by a differential refractometer (RIDK-101). A set of pullulan standards (Shodex Standard, P-82, Macherey—Nagel, Germany) was used for calibration of the columns.

The infrared spectrum of the methylated polysaccharide was recorded with a Nicolet Magna 750 spectrometer. Carbohydrates were determined by the phenol—sulfuric acid assay [6]. Polysaccharides were hydrolyzed with 2 M-TFA at 120 °C for 1 h. Paper chromatography was performed by the descending method on Whatman No. 1 and 3MM papers in the systems S_1 ethyl acetate—pyridine—water (φ_r = 8:2:1), S_2 ethyl acetate—acetic acid—water (φ_r = 18:7:8), and S_3 butan-1-ol—ethanol—water (φ_r = 10:8:7). The saccharides were detected with anilinium hydrogen phthalate. The uronic acid content was determined spectrophotometrically with the 3-hydroxybiphenyl reagent [7]. Quantitative determination of the neutral sugars was carried out in the form of their trifluoroacetates [8] by gas chromatography on a Hewlett—Packard Model 5890 Series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m) at the temperature program of 110—125 (2 °C min^{-1})—165 °C (20 °C min^{-1}) and flow rate of hydrogen 20 cm^3 min^{-1}. Gas chromatography-mass spectrometry of partially methylated alditol acetates [9] was effected on a FINNIGAN MAT SSQ 710 spec-

Table 1. Sugar Composition of DEAE-Sephadex A-50 Fractions of the Crude Polysaccharide from the Aerial Part of *Rudbeckia fulgida*, var. *sullivantii* (Boynton et Beadle)

Fraction	x_i/mole %						
	D-Gal	D-Glc	D-Man	L-Ara	D-Xyl	L-Rha	UA
Crude mucilage	34.0	32.0	2.0	11.0	6.0	15.0	*a*
Water	17.1	47.1	8.6	11.2	3.4	12.6	-
0.1 M-Ammonium carbonate	40.3	11.9	4.0	20.0	5.5	4.0	14.3
0.25 M-Ammonium carbonate	36.0	11.6	1.0	9.8	1.8	22.8	17.0
0.5 M-Ammonium carbonate	12.6	20.2	3.9	5.3	3.4	20.0	34.6
1 M-Ammonium carbonate	7.5	18.7	3.5	4.7	4.3	10.2	51.1

a) Traces.

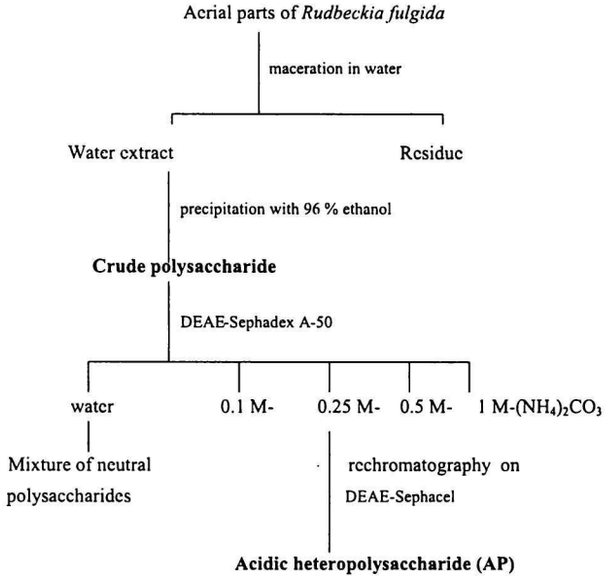

Fig. 1. Isolation scheme of the acidic polysaccharide (AP).

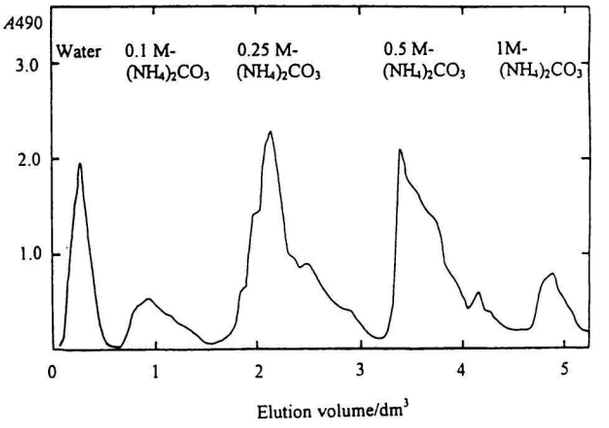

Fig. 2. Elution profile of the crude polysaccharide on DEAE-Sephadex A-50 in carbonate form.

trometer equipped with an SP 2330 column (0.25 mm × 30 m) at 80—240 °C (6 °C min^{-1}), 70 eV, 200 μA, and ion-source temperature 150 °C.

NMR spectra were measured in deuterated water at 25 °C or 60 °C on an FT NMR Bruker AVANCE DPX 300 spectrometer (^1H at 300.13 MHz and ^{13}C at 75.46 MHz) equipped with gradient enhanced spectroscopy kit (GRASP) for generation of *z*-gradients up to 5×10^{-3} T cm^{-1} in 5 mm inverse probe kit. Chemical shifts were referred to external acetone ($\delta = 2.225$ and 31.07 for ^1H and ^{13}C, respectively). The data matrice for HSQC [10] experiment was processed with squared sine function, using Bruker software XWIN-NMR version 1.3.

Isolation of Acidic Polysaccharide

The methanol-pretreated aerial part of the medicinal plant (60 g) was macerated in distilled water (2.5 dm^3) for 48 h at room temperature. The aqueous extract was filtered, concentrated to 600 cm^3, and poured into 96 % ethanol (2.4 dm^3) containing 1 vol. % of acetic acid. The precipitate was collected by centrifugation, washed with aqueous ethanol (70 vol. %), suspended in water, dialyzed, and freeze-dried. The brownish product was obtained in 1.4 % yield, based on dry herb, and contained in addition to carbohydrates also protein (23.9 %) and inorganic material (14.8 %). The crude product (1 g) was dissolved in water (100 cm^3) and loaded on a column (5 cm × 100 cm) of DEAE-Sephadex A-50 in carbonate form and eluted successively with water, 0.1 M-, 0.25 M-, 0.5 M-, and 1 M-ammonium carbonate solutions (Fig. 1). Fractions of 10 cm^3 were collected and analyzed for sugar content with the phenol—sulfuric acid assay. The elution pattern is shown in Fig. 2. Elution with water gave a mixture (0.095 g) of neutral polysaccharides already described [4]. The ammonium carbonate eluates were dialyzed, concentrated, and freeze-dried to give fractions 0.1 mol dm^{-3} (0.034 g), 0.25 mol dm^{-3} (0.156 g), 0.5 mol dm^{-3} (0.127 g), 1 mol dm^{-3} (0.048 g) the sugar composition of which is presented in Table 1. The largest fraction, obtained by elution with 0.25 M-ammonium carbonate, represented 43 % of the acidic portion. This material (0.9 g) was dissolved in distilled water (50 cm^3) and subjected to

rechromatography on a column (6 cm × 10 cm) of DEAE-Sephacel in chloride form by stepwise elution with 0.1 M-, 0.25 M-, 0.5 M-, and 1 M-sodium chloride. The fractions were processed in the same way as above. The dominant polymeric fraction (AP), obtained by elution with 0.25 M-sodium chloride and representing 64 % of the loaded material, was subjected to structural analysis. Its physicochemical data and sugar composition are presented in Table 2.

Table 2. Physicochemical Data and Sugar Composition of the Acidic Polysaccharide AP

Yield/ %	10.0[a]
Relative molecular mass (M_r)	67 000
Optical rotation	−3°
w(Protein)/%	5.9
Monosaccharide composition	x_i/mole %
Galactose	38.4
Glucose	5.4
Mannose	2.6
Arabinose	17.7
Xylose	3.0
Rhamnose	14.0
Uronic acid	18.9

a) Per crude polysaccharide.

Reduction of Acidic Polysaccharide

The acidic polysaccharide AP (45 mg) was dissolved in water (10 cm^3) and 1-cyclohexyl-3-[2-(4-methylmorpholino)ethyl]carbodiimide *p*-toluenesulfonate (0.5 g) was added to the solution [11]. The pH of the reaction mixture was maintained at 4.75 by titration with 0.1 M-hydrochloric acid under stirring. After the reaction had proceeded for 2 h, 2 M-sodium borodeuteride in water was added gradually to the mixture within 2 h, while the pH was maintained at 7.0 by titration with 1 M-hydrochloric acid. A few drops of amyl alcohol were added to prevent foaming. The solution was dialyzed, concentrated, and freeze-dried. The procedure was repeated tree times to yield a carboxyl-reduced polysaccharide AP-R (22.4 mg).

Table 3. Methylation Analysis Data of the Original (AP) and Carboxyl-Reduced (AP-R) Polysaccharides

Sugar derivative	x_i/mole %		Mode of linkage
	AP	AP-R	
2,3,5-Me_3-Ara-ol[a]	6.8 (30.4)[b]	5.2 (30.2)	Ara*f*-(1→
2,3-Me_2-Ara-ol	13.3 (59.4)	10.3 (60.0)	→ 5)-Ara*f*-(1→
2,5-Me_2-Ara-ol	2.3 (10.2)	1.7 (9.8)	→ 3)-Ara*f*-(1→
Total:	22.4	17.2	
2,3,4-Me_3-Xyl-ol	3.8 (100)	3.3 (100)	Xyl*p*-(1→
3,4-Me_2-Rha-ol	9.2 (57.2)	7.4 (57.6)	→ 2)-Rha*p*-(1→
2,4-Me_2-Rha-ol	4.6 (28.6)	4.2 (32.3)	→ 3)-Rha*p*-(1→
2-Me-Rha-ol	1.8 (11.2)	2.8	→ 3,4)-Rha*p*-(1→
4-Me-Rha-ol	Traces	0.6 (4.0)	→ 2,3)-Rha*p*-(1→
3-Me-Rha-ol	0.5 (3.0)	0.8 (6.1)	→ 2,4)-Rha*p*-(1→
Total:	16.1	15.8	
2,3,4,6-Me_4-Glc-ol	1.6 (28.6)	1.3 (7.1)	Glc*p*-(1→
6,6-d_2-2,3,4,6-Me_4-Glc-ol	–	13.1 (71.3)	Glc*p*A-(1→
6,6-d_2-2,4,6-Me_3-Glc-ol	–	0.7 (3.8)	→ 3)-Glc*p*A-(1→
2,3,6-Me_3-Glc-ol	1.9 (33.9)	1.7 (9.2)	→ 4)-Glc*p*-(1→
6,6-d_2-2,3,6-Me_3-Glc-ol	–	0.4 (2.1)	→ 4)-Glc*p*A-(1→
2,3,4-Me_3-Glc-ol	2.1 (37.5)	1.2 (6.5)	→ 6)-Glc*p*-(1→
Total:	5.6	18.4	
3,4,6-Me_3-Man-ol	2.1 (100)	1.5 (100)	→ 2)-Man*p*-(1→
2,3,4,6-Me_4-Gal-ol	10.6 (19.3)	7.1 (16.2)	Gal*p*-(1→
6,6-d_2-2,3,4,6-Me_4-Gal-ol	–	2.4 (5.5)	Gal*p*A-(1→
2,3,6-Me_3-Gal-ol	3.3 (6.6)	1.5 (3.4)	→ 4)-Gal*p*-(1→
6,6-d_2-2,3,6-Me_3-Gal-ol	–	1.9 (4.3)	→ 4)-Gal*p*A-(1→
2,4,6-Me_3-Gal-ol	7.7 (15.3)	5.0 (11.4)	→ 3)-Gal*p*-(1→
2,3,4-Me_3-Gal-ol	3.7 (7.4)	4.6 (10.5)	→ 6)-Gal*p*-(1→
2,6-Me_2-Gal-ol	Traces	1.0 (2.3)	→ 3,4)-Gal*p*-(1→
2,4-Me_2-Gal-ol	24.7 (49.2)	20.3 (46.4)	→ 3,6)-Gal*p*-(1→
Total:	50.0	43.8	

a) 2,3,5-Me_3-Ara-ol = 1,5-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* *b*) Numbers in brackets indicate the fraction (in %) of each linkage with the respective sugar residue.

Methylation Analysis

The dry samples of native (AP) and carboxyl-reduced (AP-R) polysaccharides (5 mg) were solubilized in dry dimethyl sulfoxide (1 cm^3) and methylated by the Hakomori procedure [12]. The methylated products were isolated by partition with dichloromethane, concentrated, dried, and remethylated according to the Purdie method [13]. The permethylated polysaccharides were hydrolyzed with 90 % HCOOH (1 h, 100 °C) and 2 M-TFA (1 h, 120 °C). The partially methylated saccharides were reduced with sodium borodeuteride, acetylated, and analyzed by GLC-MS (Table 3).

Partial Acid Degradation of Acidic Polysaccharide

The polysaccharide AP (460 mg) was treated with 0.5 M-TFA (100 cm^3) at 100 °C for 1 h. TFA was evaporated and the mixture of hydrolytic products was dissolved in water and separated on a column (2.5 cm × 200 cm) of Bio-Gel P-2 by water elution. Fractions of 5 cm^3 were collected and analyzed for the carbohydrate content. The separated fractions were subsequently purified by preparative paper chromatography (S_3).

RESULTS AND DISCUSSION

Water extraction of delipidized aerial parts of *Rudbeckia fulgida* afforded a crude polysaccharide composed of D-galactose (34 %), D-glucose (32 %), D-mannose (2 %), L-arabinose (11 %), D-xylose (6 %), L-rhamnose (15 %), and trace amount of uronic acids [4]. Ion-exchange column chromatography of the polysaccharide mixture, effected by stepwise elution with water and ammonium carbonate solutions (0.1 mol dm^{-3}, 0.25 mol dm^{-3}, 0.5 mol dm^{-3}, and 1 mol dm^{-3}), gave five fractions (Fig. 1) differing in molecular mass, composition of the constitutive saccharides, and in the uronic acid content (Table 1). Of these, the water eluate has already been described [4]. The dominant acidic fraction, eluted with 0.25 M-ammonium carbonate solution, was further fractionated on a different ion-exchanger using sodium chloride eluents to give an acidic polysaccharide (AP) which was shown to be homogeneous by free-boundary electrophoresis and HPGPC (Fig. 3). Its optical rotation was −3° and the number average relative molecular mass (M_N) 67 000 (Table 2). The sugar composition (Table 2) revealed the dominance of D-galactose (38.4 %) and relatively high proportions of L-rhamnose (14.0 %), L-arabinose (17.7 %), and uronic acids (18.9 %).

In order to determine the linkage pattern of the sugar components, the native (AP) and carboxyl-reduced (AP-R) polysaccharides were submitted to methylation analysis. The results are presented in Table 3. The derivatives in the table demonstrate that the ring form of all sugar components, except for arabinose, was pyranoid. The great variety of linkages of the individual sugar components pointed to a branched structure of the polymer. The dominant sugar component of the native polymer, galactose, was found to be involved in six types of linkages. The most abundant derivative, 2,4-di-*O*-methylgalactose (24.7 %), indicated the presence of 3,6-linked units. 2,3,4,6-Tetra-*O*-methyl- (10.6 %), 2,4,6-tri-*O*-methyl- (7.7 %), 2,3,4-tri-*O*-methyl- (3.7 %), and 2,3,6-tri-*O*-methylgalactose (3.3 %) demonstrated terminal as well as 3-, 6-, and 4-linked galactose residues in respective proportions. Arabinose, the second most abundant sugar residue in the polymer was found to occur only in furanoid form. It was detected mainly in 5-linked (13.3 %), nonreducing terminal (6.8 %), and 3-linked (2.3 %) positions. Rhamnose was found to be linked mainly in 2- (9.2 %) and 3- (4.6 %) positions and low proportion (2.3 %) occurred as branching point. Minor portions of glucose occupied nonreducing terminal (1.6 %), 4- (1.9 %), and 6-linked (2.1 %) positions. Xylose was shown to form nonreducing end (3.8 %) and mannose was substituted in 2-position (2.1 %).

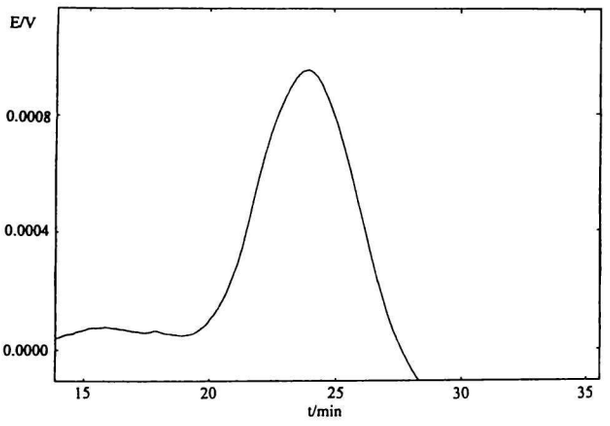

Fig. 3. HPGPC record of the acidic polysaccharide (AP).

Methylation analysis of the carboxyl-reduced polysaccharide afforded, in addition to the above-mentioned derivatives, 6,6-d_2-2,3,4,6-tetra-*O*-methyl- (13.1 %), 6,6-d_2-2,4,6-tri-*O*-methyl- (0.7 %), and 6,6-d_2-2,3,6-tri-*O*-methylglucose (0.4 %), derived from terminal and 3- and 4-linked glucuronic acid, respectively, as well as 6,6-d_2-2,3,4,6-tetra-*O*-methyl- (2.4 %) and 6,6-d_2-2,3,6-tri-*O*-methylgalactose (1.9 %), originating from terminal and 4-linked galacturonic acid. The mole proportions were established from the mass spectra on the basis of the ion intensities at m/z 205 and 207, and 233 and 235, respectively, derived from nondeuterated and deuterated C-6 atoms in 2,3,4,6-tetra-*O*-methyl- and 2,3,6-tri-*O*-methylgalactose/glucose derivatives, respectively.

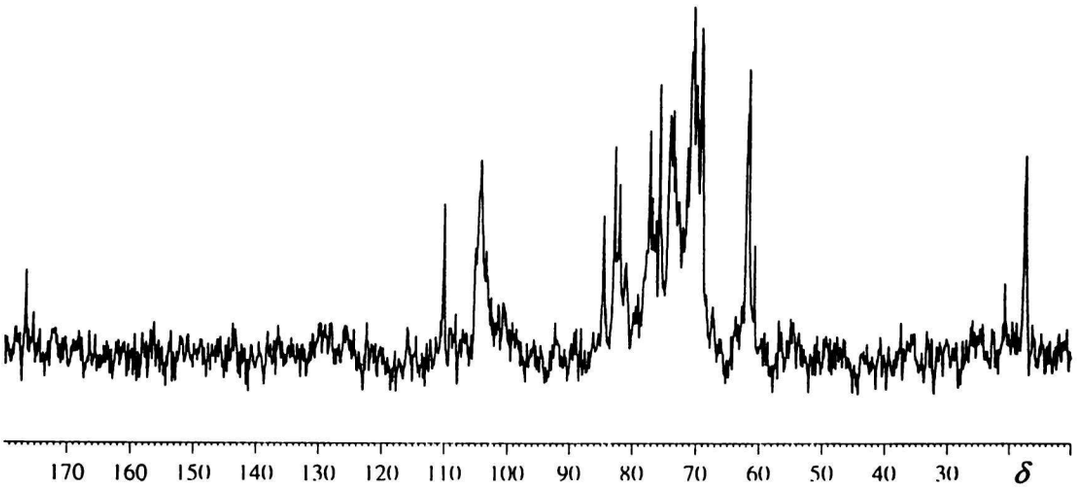

Fig. 4. ^{13}C NMR spectrum of the polysaccharide AP.

The structural reporter signals in the ^{13}C NMR spectrum of the polysaccharide (Fig. 4) supported the results of compositional and linkage analyses presented above. The occurrence of the anomeric signals at δ = 110.18 and 108.35, attributed to carbons of terminal and internal α-L-arabinofuranosyl residues [14, 15], is in good agreement with the respective 2,3,5-tri- and 2,3-di-*O*-methyl derivatives of arabinofuranose, identified in the methylation products. The dominant, not very well resolved signals in the region of δ = 103.5—105.0 could be assigned to C-1 of variously linked (terminal, internal, and branched) β-D-galactose residues, to β-linked D-glucuronic acid and D-xylose residues [15—18] in accordance with the results in Table 3. The other anomeric signals at δ = 101.50—102.50 arose from resonances of C-1 atoms of internal and branched L-rhamnopyranose residues [16, 19]. The anomeric signals of less abundant sugar components (D-glucose, D-mannose, and D-galacturonic acid) were overlapped by C-1 resonances of the dominant ones. The signals reporting about the substitution sites of galactose were observed at δ = 83.01 due to resonance of the ring C-3 atom in 3- and 3,6-linked residues, while C-6 substitution was reflected in the signal at δ = 69.81. The signals occurring in the region of δ = 60.9—63.4 arose from the carbon resonances of hexopyranose units, and free CH_2OH of arabinose, repectively. The split signal in high magnetic field at δ = 17.6, 17.8, and 18.1 arose from the resonance of C-6 of variously linked rhamnopyranosyl residues [16], in agreement with the results of methylation analysis (Table 3). The two signals in the lowest magnetic field at δ = 176.52 and 175.86 due to resonances of carboxyl carbons confirmed the presence of D-glucuronic and D-galacturonic acids, respectively [17]. As the ^{13}C NMR spectrum of the polysaccharide was crowded, reflecting a highly branched structure, unambiguous assignment of all structural reporter signals was not possible.

In the two-dimensional ^1H and ^{13}C NMR (HSQC) spectrum (Fig. 5) of the polysaccharide the cross peaks of H-1 (C-1) atoms of terminal and internal α-L-arabinofuranosyl residues could be well recognized at δ = 5.23 (110.23) and 5.12 (108.35), but of the other cross peaks in the anomeric region only the two intensive ones at δ = 4.50 (104.80) and δ = 4.48 (103.20) belonging to variously linked galactose residues were recognizable. The further well resolved cross peak at δ = 1.28 (17.50) apparently belonged to H-6 (C-6) of L-rhamnose units (not shown in the figure).

The mixture of hydrolytic products obtained on partial acid hydrolysis of the native polysaccharide yielded after separation by size-exclusion chromatography five fractions: a polymeric residue (1F), mixtures of oligosaccharides (2F, 3F, and 4F) and a mixture of monosaccharides (5F). The fractions of oligomers were subsequently purified by preparative paper chromatography to give oligomers $2F_1$, $3F_1$, and $4F_1$. Compositional analysis of the purified oligomers revealed the presence of D-glucuronic acid and D-galactose in $2F_1$ and $3F_1$ and D-galactose in $4F_1$ in equimolar ratio. The hydrolytic products of the borohydride-reduced oligomers proved the reducing end position of D-galactose in all cases. Structure determination of the obtained oligomers was based on interpretation of their ^1H and ^{13}C NMR spectra in comparison with the literature data. Occurrence of 3 signals in the anomeric region of all ^{13}C NMR spectra indicated a disaccharide structure (resonances of C-1 of nonreducing moiety and C-1α and C-1β for the reducing end).

The ^1H and ^{13}C spectra of $2F_1$, composed of D-glucuronic acid and D-galactose, showed chemical shifts for H-1 (C-1) at δ = 5.21 (93.25) and at δ = 4.58 (97.16) for the reducing α and β D-galactose moiety, respectively. The anomeric signal observed at δ = 4.47 (103.93) arose from the resonance of C-1 of the D-glucuronic acid moiety and indicated, together with

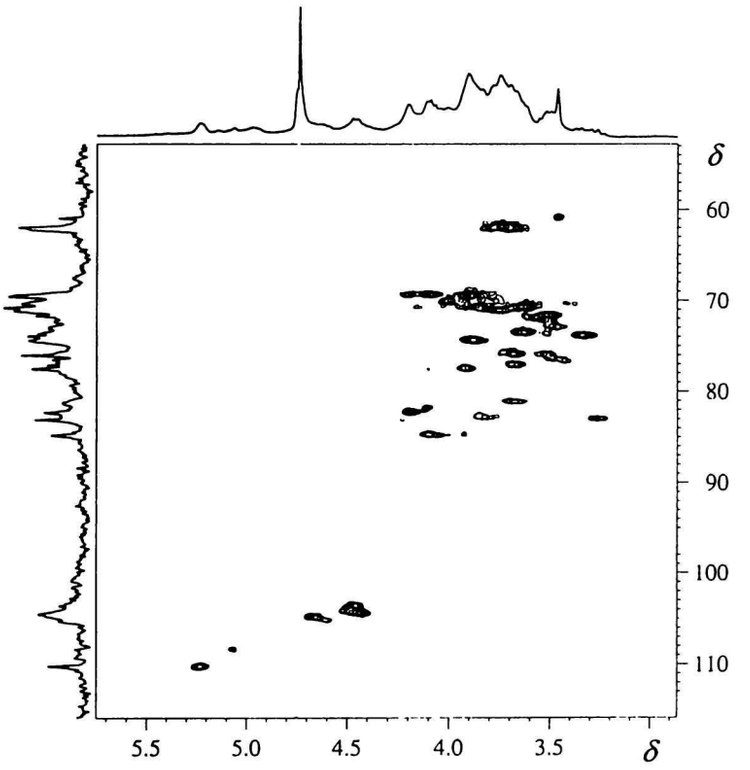

Fig. 5. HSQC spectrum of the polysaccharide AP.

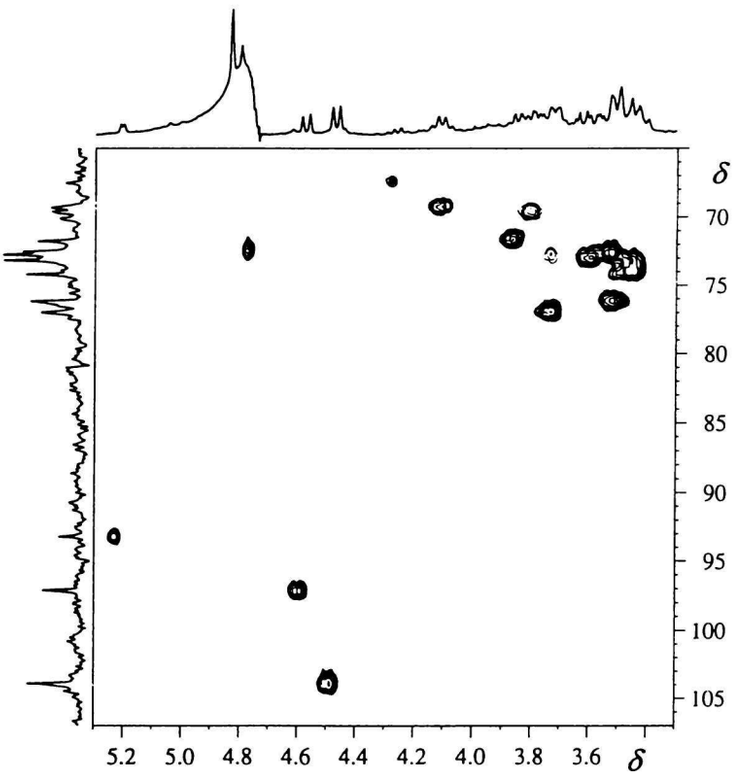

Fig. 6. HSQC spectrum of $2F_1$.

the coupling constant $J_{1,2} = 7.3$ Hz, the β-type of the glycosidic bond. The signal at $\delta = 70.10$ and the absence of the resonance due to the free CH_2OH group evidenced the C-6 substitution of the galactose moi-

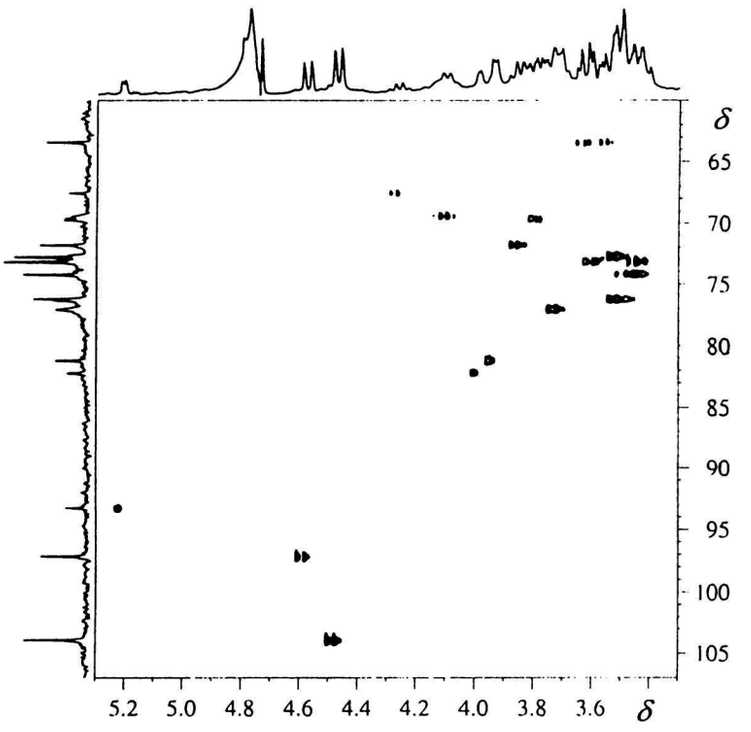

Fig. 7. HSQC spectrum of $3F_1$.

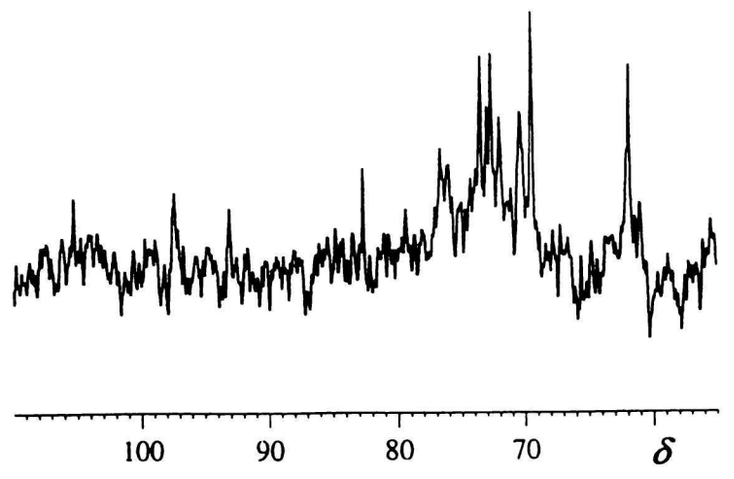

Fig. 8. ^{13}C NMR spectrum of $4F_1$.

ety (Fig. 6). Thus, the disaccharide has a structure of an aldobiouronic acid, *i.e.* β-D-glucuronopyranosyl-(1→6)-D-galactopyranose [16, 17].

The compound $3F_1$ showed the anomeric ^1H and ^{13}C signals H-1 (C-1) at $\delta = 5.21$ (93.24) and $\delta = 4.58$ (97.15) for reducing α- and β-galactose residues, while the doublet at $\delta = 4.48$ ($J_{1,2} = 7.5$ Hz) and the signal in the ^{13}C NMR spectrum at $\delta = 103.92$ reflected nonreducing terminal position of D-glucuronic acid in the disaccharide and β-type of the glycosidic bond. The cross peaks (H-3 (C-3)) in the HSQC spectrum occurring at $\delta = 3.98$ (82.20) and 3.94 (81.16) confirmed the C-3 substitution of the galactose moiety (Fig. 7). Consequently, the compound $3F_1$ is an aldobiouronic acid β-D-glucuronopyranosyl-(1→3)-D-galactopyranose [16, 17].

The ^{13}C NMR spectrum of $4F_1$, composed of D-galactose, showed the anomeric signals at $\delta = 93.08$ and $\delta = 97.38$, reflecting the resonance of C-1α and C-1β of the reducing moiety, and a signal at $\delta = 105.28$ due to the resonance of C-1 of the nonreducing galactose residue involved in a β-glycosidic linkage. The two signals in the region of skeleton carbons at $\delta = 82.67$ and $\delta = 79.40$ indicated the substitution of the C-3 atom of both anomeric forms of the reducing moiety in the disaccharide (Fig. 8). Thus, the disaccharide is a β-D-galactopyranosyl-(1→3)-D-galactopyranose [16].

The high proportion of 1,3,6-linked D-galactose

residues in AP is a feature very similar to branched arabino-3,6-galactans (type II) widely distributed in plant kingdom. The 1,3-linked β-D-galactose backbone of this type of polysaccharide is heavily branched in position O-6 and may carry a considerable proportion of different complex arrangements of neutral saccharide and uronic acid residues in the periphery of the chain [20]. In our case, the attachment of L-arabinofuranosyl units, mainly 1,5-linked, to O-6 positions of the 1,3-galactopyranan core was confirmed by complete liberation of L-arabinose on partial acid hydrolysis.

The products of partial acid hydrolysis $2F_1$ and $3F_1$ proved the direct attachment of D-glucuronic acid as nonreducing residue to O-6 of the 1,3-galactopyranan core and to O-3 of galactose units occurring most probably in side chains. With regard to mild conditions of partial acid hydrolysis, we assume that also the disaccharide $4F_1$, β-D-Gal*p*-(1→3)-D-Gal*p*, was split from side chains.

The occurrence of the other dominant saccharide component L-rhamnose in mixtures of lower and higher oligomers obtained on partial acid hydrolysis of AP (linkage analysis not presented due to ambiguity) suggests that this sugar originates probably from side rhamnogalacturonan chains, generally recognized in plant branched arabinogalactans (type II) [20]. The other, minority constituents of AP, detected in the partial acid hydrolyzate almost as early as arabinose, are evidently internal (glucose, mannose) and terminal (xylose) residues of side chains.

It can be concluded that the acidic heteropolysaccharide has a highly branched structure with 1,3-galactopyranan core carrying at O-6 mainly 1,5- and 1,3-linked arabinofuranose, 1,3-linked galactose, and rhamnogalacturonan chains. The uronic acids and xylose as well as some arabinose and galactose units occupy terminal positions. Branched polysaccharides with similar structural features were found in *Echinacea* plants [21].

Currently, much attention is paid to investigation of natural sources of drugs, ensuring minimum side effects. Medicinal plants are a source of many active polysaccharides which are still not sufficiently characterized. We showed in our previous work [4] that the crude polysaccharide from *Rudbeckia fulgida* possessed antitussive activity noticeably higher than the comparative synthetic drugs used in clinical practice to treat coughing. Isolation and structural characterization of the present acidic polysaccharide enlarged the knowledge on the higly active crude polysaccharide. It was a necessary step in view of future acceptance of this natural product as a cough-treatment drug.

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