Water-Extractable Polysaccharide Complex of *Rudbeckia fulgida*, var. *sullivantii* (Boynton et Beadle) Possesses Antitussive Activity

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Received 5 November 1996

Water extraction of the methanol-pretreated aerial parts of the medicinal plant *Rudbeckia fulgida*, var. *sullivantii* (Boynton et Beadle) afforded a polysaccharide complex, the carbohydrate moiety of which consisted of D-Gal (34 %), D-Glc (32 %), D-Man (2 %), L-Ara (11 %), D-Xyl (6 %), and L-Rha (15 %). Successive employment of ion-exchange and size-exclusion chromatography of the complex afforded one homogeneous component which was proved to be a low-molecular (1→6)-α-D-glucan. The other two fractions were heterogeneous by HPGPC and differed in mole representation of the sugar components and linkages. The polysaccharide complex was tested for antitussive activity on mechanically induced cough in nonanaesthetized cats. It was found that the complex significantly reduced the number of cough efforts in the irritated laryngopharyngeal and tracheobronchial mucous membranes of the airways without a negative influence on expectoration. Its cough-suppressing activity (46.5 %) was higher than that of the comparative drugs used in clinical practice, *i.e.* of dropropizine (28.4 %) and prenoxdiazine (24.8 %).

As a part of our on-going program directed towards isolation of new, active polysaccharides of plant origin, we devoted attention to water-extractable polysaccharides of the aerial tissues of *Rudbeckia fulgida,* var. *sullivantii* (Boynton et Beadle). The choice of this medicinal plant was motivated by the recent finding [1] that its aqueous—ethanol extracts exhibited significant immunostimulating activity, indicated by increased phagocyte and metabolic activity of peritoneal macrophages and increased bactericidal activity of the activated macrophages on the *Escherichia coli* cells. This herb has not been investigated for the polysaccharide content thus far, though it belongs to the same family as the *Echinacea* genus, which has been known for a long time to contain active components, used in the form of extracts for stimulation of nonspecific defence mechanisms at infections and chronic inflammations [2, 3]. Moreover, crude heteroglycans [4, 5] and structurally defined 4-*O*-methyl-D-glucuronoarabinoxylan [6], contained in *Echinacea purpurea*, were reported to possess immunostimulating activity.

In our previous works we found that crude mucilages isolated from the medicinal plants *Althaea officinalis* L. [7] and *Malva mauritiana* L. [8] exhibited antitussive activity on mechanically induced cough in cats. Considering the fact that treatment of catarrhs of the respiratory system by conventional antitussives, such as codeine and codeine-like compounds, induces drug dependence, decreased expectoration, hypotension, and obstipation [9], search for further active natural products, potential substitutes for some synthetic drugs, seemed to be reasonable.

The present communication provides results on the isolation of the water-extractable polysaccharide complex from the aerial parts of the title herb, characterization of its carbohydrate moiety by compositional and linkage analyses, and results of the tests for antitussive activity in comparison to drugs used in clinical practice.

EXPERIMENTAL

Material and Methods

The medicinal plant was purchased from the Faculty of Pharmacy, Comenius University, Bratislava. Concentrations were performed under reduced pressure at bath temperature not exceeding 45 °C. Optical rotations (1 cm^3 cells) were measured at (20 ±

1)°C with a Perkin—Elmer Model 141 polarimeter. Free-boundary electrophoresis of polysaccharide solution (10 mg cm^{-3}) was performed in 0.05 M sodium tetraborate buffer (pH 9.3) with a Zeiss 35 apparatus at 150 V and 8 mA for 30 min. The number average molecular mass $\overline{M}_r$ was determined osmometrically at 30 °C, using a Knauer Pressure Osmometer. Infrared spectra of the methylated products were recorded with a Nicolet Magna 750 spectrometer. HPGPC was performed using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) equipped with two Tessek Separon HEMA BIO-100 exclusion columns (8 mm × 250 mm) and aqueous 0.1 M-$NaNO_3$ as solvent (0.4 cm^3 min^{-1}). The eluate was monitored by RI (carbohydrates) and UV (proteins) detectors.

Descending paper chromatography was performed on Whatman No. 1 paper in the solvent systems S_1 ethyl acetate—pyridine–water (φ_r = 8 2 1) and S_2 ethyl acetate—acetic acid—water (φ_r = 18 7 8), the sugars being detected with anilinium hydrogen phthalate. TLC was effected on Silica Gel 60 plates (Merck, Darmstadt, Germany) with propan-1-ol—methanol—water (φ_r = 2 1 1). After spraying the plates with 20 % ammonium sulfate, the spots were visualized by charring. Carbohydrates were determined by the phenol—sulfuric acid assay [10] and the uronic acid content by the method of *Blumenkratz* [11]. Determination of proteins was effected by the method of *Lowry et al.* [12], using bovine serum albumin as standard. The amino acid composition was determined with an Automatic Amino Analyzer T 339 (Mikrotechna, Prague, Czech Republic) after hydrolysis of the polymers with 6 M-HCl for 20 h at 100 °C.

Gas chromatography of alditol trifluoroacetates was conducted on a Hewlett—Packard 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}

GC-MS analyses of partially methylated alditol acetates were effected on a FINNIGAN MAT SSQ 710 spectrometer 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. Chemical ionization was performed at the same conditions using protonated pyridine as reagent gas [13]. Proton-decoupled FT ^{13}C NMR spectra were recorded with a Bruker AM-300 spectrometer at 75 MHz for solutions in D_2O at 25 °C. Chemical shifts were referenced to internal MeOH (δ = 50.15).

Polysaccharides were hydrolyzed with 2 M-TFA at 120 °C for 2 h or with 5 mM-H_2SO_4 at 100 °C for 1 h.

Methylation analysis was performed on 50 mg samples applying the method of *Ciucanu* and *Kerek* [14]. The polysaccharide sample was dissolved in dimethyl sulfoxide (5 cm^3) under stirring at ambient temperature. The solution was then treated with pulverized NaOH (200 mg) and methyl iodide (1.3 cm^3) for 1 h. The methylated product was isolated by partition with $CHCl_3$ (13 cm^3) and treated further according to the Purdie method [15] to give a fully methylated product, as evidenced by the absence of IR absorption for hydroxyl. The product was converted to partially methylated alditol acetates by hydrolysis, reduction with $NaBD_4$, and acetylation, and subjected to linkage analysis by GC-MS. The disaccharide (7 mg) was methylated with methyl iodide (2 cm^3) and sodium hydride (20 mg) in *N*,*N*-dimethylformamide (2 cm^3).

The glucan (20 mg) was oxidized with 15 mM sodium periodate (20 cm^3) at 4 °C in the dark. The periodate consumption was monitored spectrophotometrically [16] on aliquots at 223 nm. The reaction was complete after 20 h.

Isolation and Fractionation of the Polysaccharide Complex

The air-dried, methanol-pretreated aerial parts of the herb (60 g) were macerated in cold water (2.5 dm^3) for 48 h at room temperature. The extract was filtered, centrifuged, concentrated to 600 cm^3, and poured into ethanol (2.4 dm^3). The precipitate was collected by centrifugation, washed with aqueous ethanol (70 vol. %), suspended in water, exhaustively dialyzed, and freeze-dried. The brownish product (PC) 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 polysaccharide complex (1 g) was loaded onto a column (5 cm × 100 cm) of DEAE-Sephadex A-50 (carbonate form) and irrigated successively with water, 0.25 M and 0.5 M ammonium carbonate solutions, and finally with 1 M-NaCl. The fractions were dialyzed and freeze-dried. The strongly coloured 0.5 M carbonate and 1 M-NaCl eluates were not investigated further. The yield of the water eluate (WF) was 95 mg and that of the carbonate fraction (CF) 150 mg.

The yellowish WF (200 mg) was further separated on a column (4 cm × 150 cm) of Sephadex G-75. Assay for total carbohydrates in the water eluate revealed two fractions (WFI: 130 mg and WFII: 40 mg) of different sugar composition. WFII was homogeneous upon free-boundary electrophoresis, gave a symmetrical narrow band on HPGPC, and its only sugar component was D-glucose. The fractions WFI and CF showed on HPGPC molecular heterogeneity and on hydrolysis yielded all the sugar components of the native complex, but in different mole ratios. The fractions were subjected to linkage analysis.

Antitussive Activity Tests

Besides the polysaccharide complex (PC), isolated herein, commercial products generally used in clinical practice, *i.e.* non-narcotic prenoxdiazine (P) and

dropropizine (D) as well as the narcotic codeine (C) were tested for comparative purposes.

Adult nonanaesthetized cats of both sexes weighing 1500—2500 g (8 in each set) were used for the experiments. After several days quarantine, a tracheal cannula was implanted into the animals. This served for mechanical stimulation of airways as well as for recording of the side tracheal pressure on a Biograph 13-03 electromanometer. The mucous membranes of the laryngopharyngeal (LP) and tracheobronchial (TB) areas were stimulated consecutively five times with a 0.3 mm diameter nylon fibre. The cough parameters, *i.e.* the number of efforts (NE), intensity of cough attack in expirium (IA^+) and inspirium (IA^-), cough frequency (NE min^{-1}), and intensity of maximum cough efforts in expirium (IME^+) and inspirium (IME^-) were evaluated on the basis of the pressure values recorded during the experiment. The values of cough parameters obtained prior to administration of the compound represented the normal value (N). All compounds tested were administered perorally in aqueous solutions. The effect of compounds, reflected in pressure change, was monitored in time intervals of 0.5 h, 1 h, 2 h, and 5 h after administration. Statistical evaluation of the results was effected by the method of *Wilcoxon* [17].

Table 1. Characteristic Data of the Polysaccharide Complex

Yield/%	1.4[a]
w(Protein)/%	23.9
w(Ash)/%	14.8
Sugar composition of the carbohydrate moiety (x_i/mole %)	
Rhamnose	15.0
Arabinose	11.2
Xylose	6.1
Glucose	32.3
Galactose	33.4
Mannose	2.0
Uronic acid	traces
Amino acid composition of the protein moiety (x_i/mole %)	
Aspartic acid	16.8
Threonine	4.7
Serine	5.5
Glutamic acid	21.3
Proline	5.8
Glycine	9.3
Alanine	8.6
Valine	5.0
Isoleucine	3.2
Leucine	4.8
Tyrosine	3.4
Phenylalanine	2.9
Histidine	1.9
Lysine	4.0
Arginine	2.7

a) Per mass of the dry herb.

RESULTS AND DISCUSSION

From the delipidized aerial parts of the title herb a polysaccharide complex was obtained by water extraction, followed by precipitation with ethanol. The nondialyzable, freeze-dried product contained besides carbohydrates also protein, inorganic material, and dyes. Characteristic data of the complex are summarized in Table 1. The carbohydrate moiety consisted of six neutral aldoses, of which galactose, glucose, rhamnose, and arabinose dominated. The protein moiety was rich in glutamic and aspartic acids, glycine, and alanine. Ion-exchange chromatography was employed to achieve a gross separation of the complex to fractions eluted by water (WF) and 0.25 M ammonium carbonate solution (CF). The protein content of WF was 7 % and of CF only 2 %. Both fractions showed molecular heterogeneity on HPGPC. The elution pattern of carbohydrate in WF coincided with that of protein, suggesting that protein might be an integrated part of the polysaccharide fraction. WF was submitted to further separation by size-exclusion chromatography to give WFI and WFII. The sugar composition of all three fractions is presented in Table 2. WFII, eluted at higher elution volume, was a homogeneous polymer readily soluble in water, showed a positive optical rotation (+ 145°), had a number average molecular mass 6 200 (DP = 38), and on hydrolysis yielded only D-glucose. Partial hydrolysis afforded beside the monomer a disaccharide, which was identified on the basis of its optical rotation + 97° and the methanolysis products of the methylated compound, *i.e.* methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, being in the mole ratio 1:1, as isomaltose. The hydrolytic products of the fully methylated polymer, converted to the corresponding alditol acetates, were identified by GC-MS as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-

Table 2. Sugar Composition of WFI, WFII, and CF

Fraction	w_i/mole %						
	L-Rha	L-Ara	D-Glc	D-Xyl	D-Gal	D-Man	D-GalA
WFI	15.4	14.9	17.8	10.4	37.8	3.6	
WFII			100.0				
CF	26.1	11.2	13.3	2.0	41.1	1.2	5.1

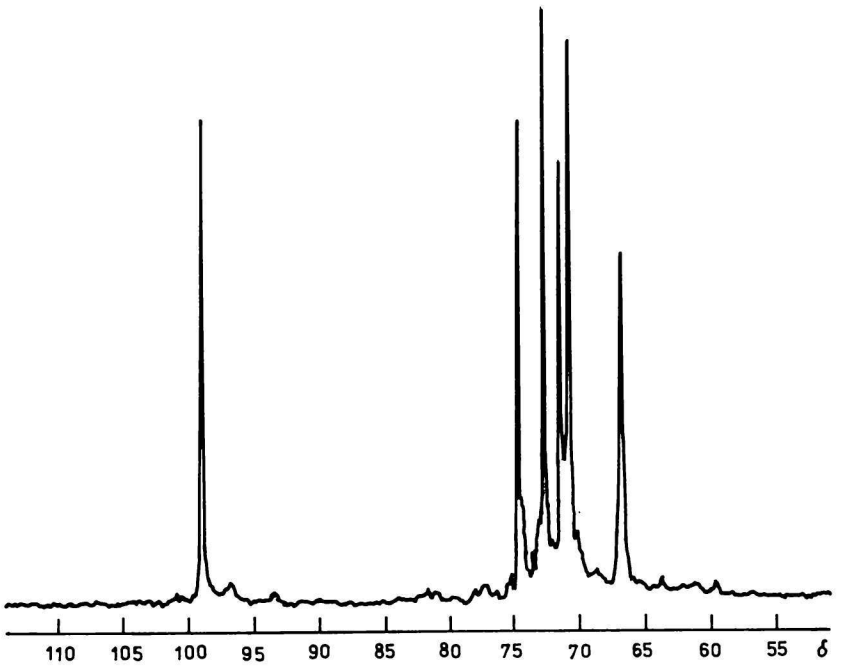

Fig. 1. ^{13}C NMR spectrum of the (1—6)-α-D-glucan.

D-glucitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, indicating a linear glucan chain with (1→6) linkages. The amount of the tetra-*O*-methyl derivative (2.7 %) well corresponded to one nonreducing end in a linear hexosyl polymer of DP = 38. The periodate consumption within 20 h oxidation of the polysaccharide was 1.9 mol per glucose unit, which is very close to the theoretical 2 mol per a hexose unit involved in a (1→6) linkage. The ^{13}C NMR spectrum of the glucan (Fig. 1), showing the signal of C-1 at $\delta = 98.9$ and that of C-6 at $\delta = 66.7$ is another evidence for the (1→6)-α-D-glucan structure. Glucans of similar structures were isolated from the leaves of *Althaea officinalis* L. [18] and *Plantago lanceolata* L., var. *libor* [19].

The fractions WFI and CF were constituted by the same sugar components, except of the galacturonic acid in CF, but in different proportions (Table 2). They afforded on mild acid treatment arabinose, and WFI also a minute amount of galactose, suggesting the furanose form of both sugar units occupying terminal positions. The fractions were submitted to methylation analysis, the results of which (Table 3) pointed to a wide range of linkages of all components. The mole representation of the individual sugars was in good agreement with that obtained in compositional analysis (Table 2), except of the trace amounts of fucose (below 1 %), detected in both fractions as terminal, 1,3- (WFI), and 1,2-linked (CF) pyranosyl units (Table 3).

The amount of 2,3,5-tri-*O*-methylarabinitol, predominating in CF, shows that arabinofuranose occupies mainly terminal position. A small portion of a pyranose form was detected in the end position as well. Apart from being end groups, Ara*f* is also linked in the chain through O-3 and O-5, the portion of 1,3-linked Ara*f* being much higher in WFI than in CF. Trace amounts of 1,2-linked and branched units were also detected.

The galactose derivatives, dominant in both fractions, evidenced the pyranose form, although in WFI also terminal furanose units were observed in accordance with the early appearance of Gal in the mild hydrolysis products. The prevalence of the 2,3,4-tri-*O*-, 2,4,6-tri-*O*-, and 2,4-di-*O*-methyl derivatives in both fractions, more pronounced in CF, together with the above discussed types and amounts of the arabinose derivatives indicated the presence of arabinosyl-substituted branched 3,6-galactan polysaccharide (formerly Type II), while the 2,3,6-tri-*O*- and 2-*O*-methyl derivatives, though detected in low proportions, suggested that also the arabinosyl-substituted 3,4-galactan polysaccharide (formerly Type I) was present. Both types are widely distributed in plant kingdom and are typical of variety of plant material [20]. The relatively high abundance of the 3,4,6-tri-*O*-methyl derivative in WFI (19 %) is rather striking, when considering the rare occurrence of 1,2-linked galactosyl units in plant material. Galactoglucomannan isolated from suspension-cultured cells of *Nicotiana tabacum* [21] was reported to contain 1,2-linked D-Gal*p* units in side chains attached to the mannosyl backbone. Some xyloglucans, structurally related to cellulose, were also found to carry side chains containing galactopyranose units substituted at C-2, *e.g.* α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p* [22].

Rhamnose, the second most abundant sugar component in CF and the third one in WFI, was found to occur in pyranose form and involved in 7 types of

Table 3. Methylation Analysis Data of Fractions WFI and CF

Derivative[a]	w_i/mole %		Linkage indicated
	WFI	CF	
2,3,4,6-Me_4-Gal	2.8 (7.3)[b]	6.3 (14.8)	Gal*p*-(1→
2,3,5,6-Me_4-Gal	1.8 (4.7)		Gal*f*-(1→
2,3,4-Me_3-Gal	7.9 (20.4)	6.7 (15.8)	→6)-Gal*p*-(1→
2,3,6-Me_3-Gal	2.3 (6.0)	1.9 (4.4)	→4)-Gal*p*-(1→
2,4,6-Me_3-Gal	3.6 (9.4)	8.6 (20.2)	→3)-Gal*p*-(1→
3,4,6-Me_3-Gal	7.2 (18.9)	1.4 (3.3)	→2)-Gal*p*-(1→
2,4-Me_2-Gal	9.7 (25.5)	16.8 (39.5)	→3,6)-Gal*p*-(1→
3,6-Me_2-Gal	1.8 (4.7)		→2,4)-Gal*p*-(1→
2-Me-Gal	0.8 (2.1)	0.7 (1.6)	→3,4,6)-Gal*p*-(1→
Total:	37.9	42.4	
2,3,4,6-Me_4-Glc	6.8 (40.9)	4.5 (32.1)	Glc*p*-(1→
2,3,4-Me_3-Glc	2.3 (13.8)	4.5 (32.1)	→6)-Glc*p*-(1→
2,3,6-Me_3-Glc	0.9 (5.4)	1.8 (12.8)	→4)-Glc*p*-(1→
2,4,6-Me_3-Glc	4.9 (29.5)	0.3 (2.1)	→3)-Glc*p*-(1→
3,4,6-Me_3-Glc	0.5 (3.0)	1.5 (10.7)	→2)-Glc*p*-(1→
2,6-Me_2-Glc	0.3 (1.8)	0.6 (4.2)	→3,4)-Glc*p*-(1→
3,4-Me_2-Glc	0.2 (1.2)	0.2 (1.4)	→2,6)-Glc*p*-(1→
2-Me-Glc	0.7 (4.2)	0.6 (4.2)	→3,4,6)-Glc*p*-(1→
Total:	16.6	14.0	
2,3,4-Me_3-Rha	3.0 (19.1)	0.5 (1.8)	Rha*p*-(1→
2,3-Me_2-Rha	3.7 (23.7)	3.6 (13.8)	→4)-Rha*p*-(1→
2,4-Me_2-Rha	4.7 (30.1)	12.3 (47.3)	→3)-Rha*p*-(1→
3,4-Me_2-Rha	2.0 (12.8)	3.7 (14.2)	→2)-Rha*p*-(1→
2-Me-Rha	0.5 (3.2)	3.7 (14.2)	→3,4)-Rha*p*-(1→
3-Me-Rha	0.3 (1.9)	1.5 (5.0)	→2,4)-Rha*p*-(1→
4-Me-Rha	1.4 (8.9)	0.9 (3.4)	→2,3)-Rha*p*-(1→
Total:	15.6	26.0	
2,3,4-Me_3-Ara	1.3 (9.1)	0.7 (5.3)	Ara*p*-(1→
2,3,5-Me_3-Ara	4.1 (28.8)	6.8 (51.9)	Ara*f*-(1→
2,3-Me_2-Ara	1.9 (13.3)	2.3 (17.5)	→5)-Ara*f*-(1→
2,5-Me_2-Ara	6.2 (43.6)	2.3 (17.5)	→3)-Ara*f*-(1→
3,5-Me_2-Ara	0.5 (3.5)	0.8 (6.1)	→2)-Ara*f*-(1→
2-Me-Ara	0.2 (1.4)	0.2 (1.5)	→3,5)-Ara*f*-(1→
Total:	14.2	13.1	
2,3,4-Me_3-Xyl	4.2 (38.5)	1.3 (50)	Xyl*p*-(1→
2,3-Me_2-Xyl	2.7 (24.7)	1.3 (50)	→4)-Xyl*p*-(1→
3-Me-Xyl	4.0 (36.6)		→2,4)-Xyl*p*-(1→
Total:	10.9	2.6	
2,3,4,6-Me_4-Man	0.5 (12.8)	0.3 (27.2)	Man*p*-(1→
2,3,4-Me_3-Man	1.1 (28.2)	0.2 (18.1)	→6)-Man*p*-(1→
2,3,6-Me_3-Man	0.9 (23.0)	0.4 (36.3)	→4)-Man*p*-(1→
2,6-Me_2-Man	0.3 (7.6)	0.2 (18.1)	→3,4)-Man*p*-(1→
4,6-Me_2-Man	1.1 (28.2)		→2,3)-Man*p*-(1→
Total:	3.9	1.1	
2,3,4-Me_3-Fuc	0.3 (37.5)	0.2 (25.0)	Fuc*p*-(1→
2,4-Me_2-Fuc	0.5 (62.5)		→3)-Fuc*p*-(1→
3,4-Me_2-Fuc		0.6 (75)	→2)-Fuc*p*-(1→
Total:	0.8	0.8	

a) 2,3,4,6-Me_4-Gal = 2,3,4,6-tetra-*O*-methylgalactose, *etc.*, determined as partially methylated alditol acetates.
b) Numbers in brackets indicate percentage of each linkage in the respective sugar residue.

linkages in both fractions, though in different proportions. The highest difference was observed between the portions of the terminally-linked Rha*p*. In WFI it was 19.1 % and in CF only 1.9 %. The 1,3-linked chain units dominated in both fractions. In CF they represented as much as 47.3 %. The 1,4-linked residues were more abundant in WFI, while the 1,2-linked ones were approximately equal in both fractions. The difference in the 1,3,4-branched units was also noticeable, being 11 % in favour of CF. On the other hand, in WFI 8.9 % of 1,2,3-linked Rha*p* were found, whereas in CF only 3.4 %. In water-extractable polysaccharides of

plant origin Rha has been found to be located along the galactan chain either as a simple nonreducing substituent, or terminating side arabinofuranosyl chains, particularly in arabinogalactans of the Type II [23, 24]. More often it is found as one of the constituents of the inner chain of various acid rhamnogalacturonoglycans [20]. We assume that some of the terminal Rha*p* identified in WFI are integral part of the present arabinogalactans. The occurrence of D-galacturonic acid in CF (5.1 %) suggests that the variously linked chain and branched Rha*p* units may originate from either type (I and II) of rhamnogalacturonoglycans [25].

The derivatives of glucose indicated pyranose form and involvement in almost all of the linkages possible. Terminal units dominated in WFI (40.9 %), whereas in CF their amount was somewhat less (32.1 %) and equal to that of the 1,6-linked residues. Marked difference was observed in the 1,3-linked units. In WFI their amount was almost 30 %, while in CF only 2 %. On the other hand, more 1,6-, 1,4-, and 1,2-linked residues were found in CF. The portions of branched residues were small in both fractions. The ^{13}C NMR spectra of both fractions (to be discussed later) revealed an anomeric signal at $\delta = 98.8$, identical with that observed in the spectrum of the 1,6-α-D-glucan fraction (WFII). With regard to this fact and the relatively high portion of the 1,6-linked residues, it is probable that these fractions comprise a similar type of polymer. The 1,3-linked units, observed in WFI in high amount, may originate from nonendospermic D-glucans found in cell walls of leaves and stems of many grasses [20, 26].

The xylose derivatives detected in WFI evidenced terminal, 1,4-, and 1,2,4-linked xylopyranose units. Such derivatives suggest the presence of a discrete xylan fragment, though not all end units are its integral parts, as indicated by the amount of the terminal groups. As in the nonendospermic xylans the xylosyl residues carry on O-2 mainly D-glucuronic acid (or its 4-*O*-methyl derivative) [26], it is probable that uronic acid escaped detection in the initial fraction due to its low content.

The mannose derivatives, present in very low amount (3.9 %) in WFI, indicated besides terminal mainly 1,6-, 1,4-, and 1,2,3-linked Man*p* units. In CF these derivatives were detected in minute amounts or not at all. As mannose is not known to be a part of arabinogalactans or arabinogalactan-protein complexes, it might originate from some contaminating mannoproteins [27].

Attempts were made to support the results of methylation analysis by ^{13}C NMR spectral measurements. However, the spectra of both fractions were crowded and unambiguous assignment of signals was impossible. Nevertheless, the anomeric region revealed in both cases about 10 more or less intensive signals, showing the complexity of the fractions. Some of them could be assigned on the basis of comparison with the literature data [28—31]. The prominent low-field signals at $\delta = 110.3$ and $\delta = 108.5$ were consistent with the presence of furanosyl forms of terminal and 1,3- and 1,5-linked α-L-Ara*f* residues. The signals at $\delta = 104.3$—105.4 were not completely resolved, but they most probably comprised the resonances of C-1 of β-D-Gal*p* (terminal, chain units, branching points). The signals corresponding to α-L-Ara*f* residues were much sharper than those for β-D-Gal*p*, indicating that they had an enhanced mobility, consistent with the location at the periphery of the molecule structure. The low-intensity signal appearing in the spectrum of WFI at $\delta = 103.6$ probably arised from the resonance of C-1 of β-D-Glc*p* residues and the not well resolved signals at $\delta = 101.5$—101.7, present in both spectra, were due to C-1 of α-L-Rha*p* units. The signal at $\delta = 100.5$, observed in the spectrum of CF, and those occurring in both spectra at $\delta = 98.8$ were due to the resonance of C-1 of α-D-Glc*p* units. The spectrum of WFI showed a signal at $\delta = 102.5$, ascribed to C-1 of β-D-Xyl*p* residues. The two distinct signals at $\delta = 97.5$ and 96.6 were due to C-1 of reducing β-D-Gal*p* and β-D-Glc*p*, respectively, the former being less intensive in CF It can be concluded that the results of the ^{13}C NMR measurements are in accordance with the methylation analysis data.

The polysaccharide complex (PC) was tested for antitussive activity on mechanically induced cough in cats. According to *Korpáš* and *Nosáľová* [32], cats are the most suitable animals for cough modelling and testing of various preparations for their effect on the cough reflex. The cats were not anaesthetized in order to eliminate the possibility of influencing the results

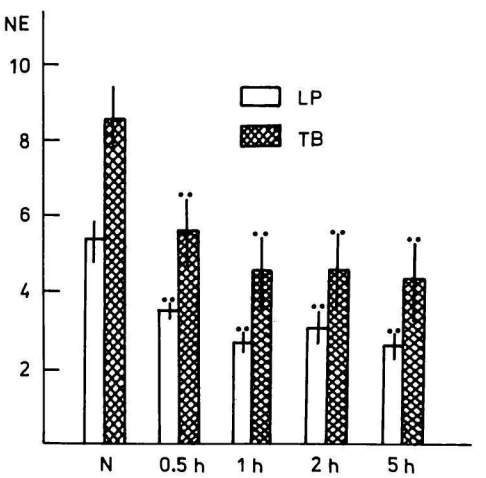

Fig. 2. Effect of the polysaccharide complex (PC), administered perorally in the dose 50 mg kg^{-1}, on number of efforts (NE) from laryngopharyngeal (LP) and tracheobronchial (TB) areas. N = normal value; the column represents the mean value; the range denotes standard error of means; 5 % significance is marked with one dot, 1 % with two dots.

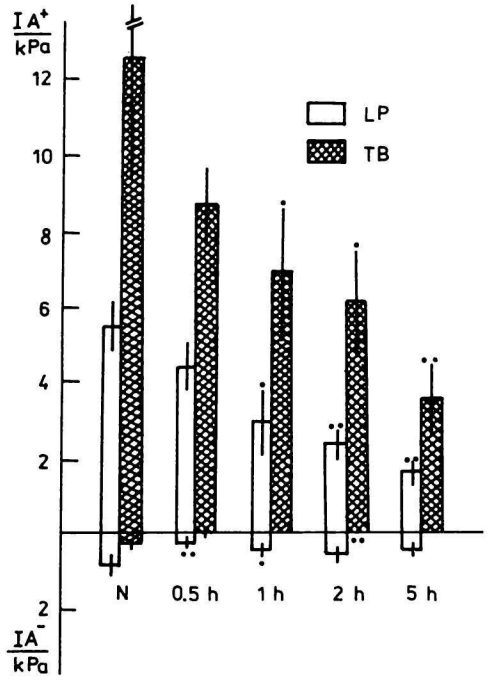

Fig. 3. Effect of PC, administered perorally in the dose 50 mg kg^{-1}, on intensity of cough attacks in expirium (IA^+) and inspirium (IA^-). For other symbols see Fig. 2.

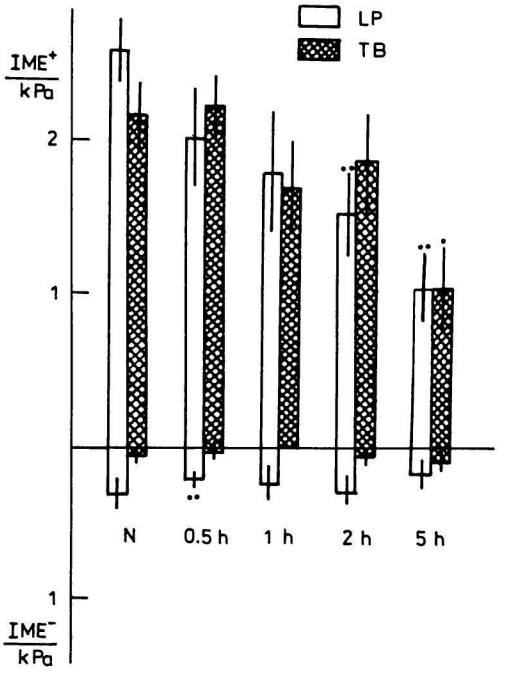

Fig. 5. Changes of maximum expiratory (IME^+) and inspiratory (IME^-) cough effort after peroral administration of PC in the dose 50 mg kg^{-1} For other symbols see Fig. 2.

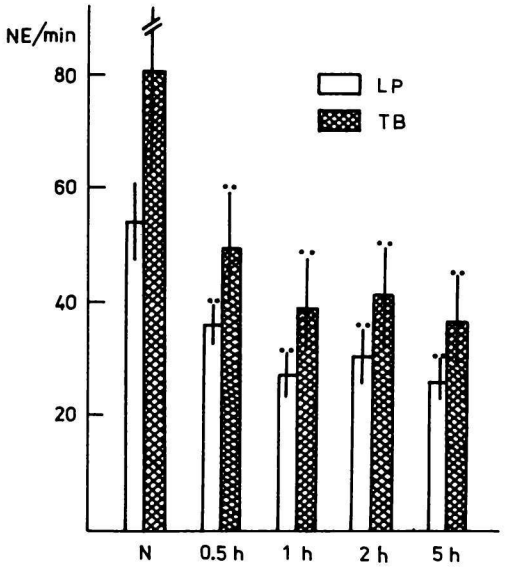

Fig. 4. Changes of cough frequency (NE min^{-1}) after peroral administration of PC in the dose 50 mg kg^{-1} For other symbols see Fig. 2.

by the anaesthetics. Mechanical induction of coughing was preferred to chemical or electrical stimulation because this impulse simulates in the most suitable way the natural conditions, when coughing is induced by foreign solids. Moreover, it is a point stimulation, the intensity of irritation is constant, and the possibility of adaptation of the receptors to this kind of irritation is improbable [33]. The results of tests are illustrated in Figs. 2—5. Application of PC resulted in a high suppressive effect on the number of cough efforts (Fig. 2), cough frequency (Fig. 4), and intensity of cough attack in expirium and inspirium (Fig. 3) both from laryngopharyngeal and tracheobronchial mucous membranes of the airways. However, the intensity of maximum cough effort in expirium from the TB area (Fig. 5), the parameter characterizing expectoration, increased, but not significantly, 30 min after administration of the compound. This finding is important because it indicates that the tested compound suppressed the cough reflex, but promoted expectoration [34]. In order to recognize the importance of the observed antitussive activity of PC, comparative experiments were performed with some drugs generally used in clinical practice. The drugs were tested for their ability to suppress the number of efforts and intensity of cough attacks in expirium and inspirium, the parameters most important from the clinical point of view. From the results presented in Table 4 it is evident that though the antitussive activity of PC was lower than that of the most often used narcotic codeine, it was noticeably higher than that of the non-narcotic dropropizine and prenoxdiazine, drugs approved experimentally and clinically. Finally, it should be mentioned that undesirable side effects were not observed either during or after the experiments with PC.

On the basis of the results obtained it may be con-

Table 4. Antitussive Activity of the Polysaccharide Complex and Comparative Drugs

Compound	Dose[a]	Activity
	mg kg^{-1}	%
Polysaccharide complex	50	46.5
Prenoxdiazine	30	24.8
Dropropizine	100	28.4
Codeine	10	61.8

a) The doses of the individual comparative drugs represent the amounts which in the earlier tests exhibited the highest antitussive activity.

cluded that the carbohydrate moiety of the polysaccharide complex comprises, in addition to the linear, low-molecular (1→6)-α-D-glucan, both types (I and II) of branched arabinogalactans, a (1→4)-β-D-xylan, rhamnogalacturonans, and β-D-glucans. As the aerial parts of the herb contained both the stem and leaf tissues, the complexity of the isolated polysaccharides is understandable. Further investigation will be necessary to obtain homogeneous populations available for detailed structure determinations. All the more that the high antitussive activity of the complex makes this natural product a promising substitute for some synthetic drugs used in treatment of coughing.

Acknowledgements. This work was supported by the Grant No. 2/1240 of the Slovak Scientific Grant Agency (VEGA).

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Translated by A. Kardošová