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POLYSACCHARIDES FROM THE ROOTS OF THE MARSH MALLOW (*Althaea officinalis* L., var. Rhobusta): STRUCTURAL FEATURES OF AN ACIDIC POLYSACCHARIDE*

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ABSTRACT

An acidic heteropolysaccharide isolated from the mucilage of the roots of the marsh mallow (*Althaea officinalis* L., var. Rhobusta) via its insoluble barium salt contained D-galactose, L-rhamnose, D-glucuronic acid, and D-galacturonic acid in the molar ratios 1.2:1.0:1.0:1.0. It was homogenous on free-boundary electrophoresis and in the analytical ultracentrifuge, and it had $\overline{M}_w$ = 26,700, $\overline{M}_n$ = 23,900. Partial acid hydrolysis and analyses of the methylated and the methylated, carboxyl-reduced polysaccharide indicated that the polymer backbone is composed of (1→4)-linked D-galactopyranuronic acid and (1→2)-linked L-rhamnopyranose units in the ratio of 1:1. Each D-galacturonic unit carries a single β-D-glucopyranuronic residue linked to C-3, and each L-rhamnopyranose unit carries D-galactopyranose residues, mainly as non-reducing terminals linked to C-4. A small number of presumably short chains of (1→4)-linked D-galactopyranose units are also involved in branching.

INTRODUCTION

The roots of *Althaea officinalis* L. are known to contain relatively large amounts of mucilage. Mucilages, as some other plant polysaccharides, may function as reserve carbohydrates, protective colloids, and water reservoirs. Their pharmaceutical effects depend on covering the mucous membranes with a layer that protects against irritations. In this capacity they are used externally as skin creams, and internally in cases of bronchitis and enteritis, or as laxatives.

Lately, several reports have appeared on structural studies of mucilaginous polysaccharides isolated from this plant[1,2] as well as from other related genera and species[3,4]. For example, Tomoda *et al.* described the structural features of a representative polysaccharide, "Althaea mucilage O", from the roots of *A. officinalis L.*[1,2], of "Althaea mucilage R" from the roots of *Althaea rosea*[3], and those of "Abelmoschus mucilage M" from the roots of *Abelmoschus manihot* Medicus[4]. All these mucilages were acidic heteropolysaccharides containg D-galactose, L-rhamnose, D-

* Dedicated to the memory of Hermann O. L. Fischer on the centenary of his birth.
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glucuronic acid, and D-galacturonic acid. The common structural unit of the polysaccharides from the Althaea genus was identified as *O*-β-(D-glucopyranosyluronic acid)-(1→3)-*O*-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose.

We studied the mucilaginous material isolated from *Althaea officinalis* L., var. Rhobusta, systematically in order to broaden our knowledge of the chemical structures of its individual polysaccharide components and throw light on the possible relationship between structures and therapeutic effects. In previous reports we described a highly branched L-arabinan[5] and three virtually linear D-glucans[6] isolated from the roots of this plant. The present work was undertaken to separate and characterize a further component of the mucilage, an acidic carbohydrate polymer.

EXPERIMENTAL

General methods. — Solutions were concentrated under diminished pressure at <40°. Free-boundary electrophoresis was effected with a Zeiss apparatus, on a sample of polysaccharide (10 mg/mL) in 0.05M sodium borate buffer of pH 9.2, for 30 min at 10 V/cm and 6 μA. High voltage paper electrophoresis was performed on Whatman No. 1 paper in 0.1M ammonium formate buffer of pH 3.8 for 3.5 h at 25 V/cm. Optical rotation was measured with a Perkin-Elmer Model 141 polarimeter on a 1% aqueous solution at 20°. The molecular weight ($\overline{M}_w$) of the polysaccharide was estimated by ultracentrifugation at 238,330 × g (MON 110 ultracentrifuge) and extrapolation of the sedimentation coefficient to zero concentration. Six photographs taken at 6-min intervals, beginning at 40 min, showed that the polysaccharide sedimented as a single, symmetrical peak. The partial specific volume was determined pycnometrically for a 1% solution of the polysaccharide. Osmometric pressure measurements were performed in water at 30°, using a Knauer membrane osmometer fitted with a Zweischicht-Membrane (Knauer).

G.l.c. was conducted with a Hewlett-Packard Model 5711 A chromatograph and (A) a column (200 × 0.3 cm) of 3% OV-225 on 80–100 mesh Chromosorb W (AW-DMCS), programmed to hold a temperature of 120° for 4 min, then increase to 170° at 2°/min; or (B) a column (200 × 0.3 cm) of 20% SF-96 on 80–100 mesh Chromosorb W (AW-DMCS), at 110° for 2 min, then increasing to 210° at 4°/min. G.l.c.–m.s. of alditol acetates of methylated saccharides was performed with a JMS-D 100 (JEOL) spectrometer, using a column (200 × 0.3 cm) packed with 100–120 mesh Supelcoport coated with 3% of SP 2340. The inlet helium pressure was 101.3 kPa, temperature programmed from 160 to 240° at 6°/min. The spectra were determined at 23 eV. Infrared spectra were recorded with a Perkin-Elmer spectrometer, model 9836.

Analytical paper chromatography of free saccharides was performed by the descending method on Whatman No. 1 paper with the following systems: S1, 8:2:1 ethyl acetate–pyridine–water; S2, 18:7:8 ethyl acetate–acetic acid–water; and S3, 5:5:1:3 ethyl acetate–acetic acid–formic acid–water. Reducing sugars were detected

by spraying with aniline hydrogen phthalate and heating the papers for 5 min at 105°, and alditols with alkaline silver nitrate reagent. The mobility (R_{Rha}) of the acidic oligosaccharides is expressed relative to that of L-rhamnose. Quantitative determinations of the neutral component sugars (as alditol acetates) were carried out by g.l.c. on column A. The uronic acid content of the original polysaccharide was determined by the carbazole method[7]. Determination of protein content was carried out by the method of Lowry *et al.*[8]. The amino acid composition was established with an Automatic amino analyzer, type 6020 (Mikrotechna, Prague), after hydrolysis of polysaccharide with 6M HCl for 20 h at 100°.

Oligosaccharides were methylated according to Ciucanu *et al.*[9]. The methylated derivatives were purified by t.l.c. before analysis by mass spectrometry. T.l.c. was carried out on silufol (Kavalier, Czechoslovakia) and silica gel G plates in the solvent systems 5:1 benzene–acetone and 20:1 chloroform–methanol. The spots were visualized by spraying the plates with 20% aqueous ammonium sulfate or 3% methanolic sulfuric acid and subsequent heating at 200–300°. Mass spectra were obtained with a JEOL JMS-D 100 spectrometer at an ionizing potential of 70 eV. Samples were evaporated by an in-beam technique. The temperature of the ion source was 250°. The symbols A–J, employed by Kochetkov *et al.*[10], are used to denote the fragment ions, and *a*, *b* and *c* to designate the monosaccharide residues[11]. N.m.r. spectra were recorded at 100 MHz with a JEOL FX-100 instrument for solutions in D_2O with methanol and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) (0.05 p.p.m.) as the internal reference standards.

Isolation of the acidic polysaccharide. — Roots were obtained from plants cultivated at the Center for the Cultivation of Medicinal Plants, Faculty of Medicine, J. E. Purkyně University, Brno (Czechoslovakia). The material (1000 g) was crushed and then macerated in cold water (200 L) for 48 h. The extract was concentrated to 50 L and poured into 96% ethanol (300 L) containing 1% (v/v) of acetic acid. The precipitate was treated with ethanol, suspended in water, and dialyzed against distilled water for 4 days. The water-insoluble portion, which separated, was removed by centrifugation and the supernatant was lyophylized to give polymer mixture A (see Table I). The residual aqueous–ethanolic solution was

TABLE I

WATER-SOLUBLE POLYMER FRACTIONS FROM THE ROOTS OF THE MARSH MALLOW (*Althaea officinalis* L., var. Rhobusta)

Fraction	Composition (molar ratios of monosaccharides)[a]								Protein %
	Gal	Glc	Man	Xyl	Ara	Rha	Fuc	Uronic acids	
A	1.0	1.3	tr.	tr.	0.3	0.1	tr.	0.3	15.8
B	1.0	1.7	0.1	tr.	0.6	0.3	tr.	0.4	13.1
C	1.0	1.3	tr.	0.1	1.5	0.7	tr.	1.4	12.9
D	1.0	3.7	0.1	0.1	5.5	0.6	tr.	2.0	12.0

[a]tr. = trace

made neutral with calcium hydroxide, concentrated to 20 L, poured into 96% ethanol (200 L), and acidified with acetic acid (2 L). Following the procedure already described, polymer mixture B (Table I) was obtained. The second maceration of the roots with cold water and subsequent isolation of polymer mixtures C and D (Table I) were accomplished similarly.

Polymer mixture C (19 g) was dissolved in distilled water (9.5 L) and precipitated with saturated aqueous barium hydroxide (3 L). The precipitate was centrifuged, dissolved in water (3 L), and neutralized with acetic acid. Then the solution was dialyzed against distilled water for 4 days, evaporated to one third of its original volume, and lyophillized. Reprecipitation afforded 6.5 g (34%) of barium salt of the acidic polysaccharide.

Reduction of the acidic polysaccharide. — The polysaccharide (60 mg) was dissolved in water (30 mL), then 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (0.6 g) was added. The pH of the reaction mixture was maintained at 4.75 by titration with 0.1M hydrochloric acid during 2 hours stirring at room temperature, then 2M sodium borodeuteride (8 mL) was added gradually to the reaction mixture during 4 h while the pH was maintained at 7.0 by titration with 4M hydrochloric acid (continued stirring). The solution was dialyzed first against running tapwater and then against distilled water for 4 h, then concentrated to 30 mL. Four more such treatments yielded a carboxyl-reduced product (33 mg).

Methylation analysis of the acidic polysaccharide. — The polysaccharide, both original and carboxyl-reduced, (40 mg) was solubilized in dry dimethyl sulfoxide (5 mL) and methylated with methyl iodide[12] in the presence of methylsulfinyl carbanion. The solution was then poured into water (30 mL), dialyzed for 48 h, and evaporated. The syrupy residue was dissolved in methyl iodide (5 mL), silver oxide[13] (100 mg) was added, and the mixture was stirred and boiled under reflux for 24 h. This procedure was repeated thrice. The fully methylated product (30 mg) was hydrolyzed with 2M trifluoroacetic acid (5 mL) for 24 h at 105°. The hydrolysate of the methylated original polysaccharide was separated on a column of Dowex 1-X8 (acetate form) into neutral and acidic fractions. The neutral partially methylated derivatives were eluted with distilled water, converted into the corresponding alditol acetates, and quantitatively analyzed by g.l.c.-m.s.[14].

Hydrolysis of polysaccharides. — The polysaccharide (5 mg) was treated with 72% sulfuric acid (w/w) (0.6 mL) for 1 h at 5–10°. After dilution with water to an acid concentration of 5%, hydrolysis was performed in a sealed tube for 12 h at 105°. The solution was neutralized with barium carbonate and filtered, and the precipitate was washed with distilled water several times. The excess barium ions were removed by percolating the solution through a column of Dowex 50 W X-8 (H^+).

Partial acid hydrolysis of the acidic polysaccharide. — The polysaccharide (5 g) was hydrolysed with 90% formic acid (500 mL) for 6 h at 105°, then after dilution to an acid concentration of 45%, hydrolysis was continued for 1 h at the same temperature. After removal of the formic acid by successive additions and evaporations of water, the high-molecular portion was precipitated with 96% ethanol (4 volumes).

The ethanol-soluble portion (2 g) was separated on a column of Dowex 1 X-8 (acetate form) ion-exchange resin, to give a neutral (~ 1.4 g) and an acidic portion (520 mg). The former was eluted with water and the latter with 4M acetic acid. The mixture of acidic saccharides was separated on a column (3 × 250 cm) of Sephadex G-25 Fine using acetate buffer (pH 4.2) as eluent. The following two chromatographically and electrophoretically homogeneous oligosaccharides were obtained from the mixture of higher molecular weight oligosaccharides.

O-(α-D-Galactopyranosyluronic acid-(1→2)-L-rhamnopyranose. — This disaccharide (158 mg), $[\alpha]_D$ +94.4°, R_{Rha} 0.51 (system S3), gave D-galacturonic acid and L-rhamnose on acid hydrolysis. M.s. of the permethylated sample gave, *inter alia*, the ions aA_1, aA_2, and aA_3 at *m/z* 233, 201, and 169; abJ_1, and bA_1, and bA_2, at *m/z* 249, 189, and 157, and the ions baB_1 and baF_1^2 at *m/z* 394 and 319. The latter ions together with a low-intensity peak at *m/z* 161 (bB_3) confirmed the linkage to be (1→2). The ^{13}C-n.m.r. spectrum of the original sample showed that the galactopyranosyluronic acid unit has the α-anomeric configuration (C-1 at 97.2 p.p.m.; lit.[15,16] 97.6 p.p.m.).

O-(β-D-Glucopyranosyluronic acid)-(1→3)-O-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose. — This fragment (72 mg), $[\alpha]_D$ +83.8°, R_{Rha} 0.31 (system S3), gave D-galacturonic acid, D-glucuronic acid, and L-rhamnose on acid hydrolysis. M.s. of the permethylated trisaccharide gave, *inter alia*, the following ions: *m/z* 625 ($cbaA_1$), 593 ($cbaA_2$), 597 ($abcE_1$ or $bacE_1$), 451 (baA_1), 407 (bcA_1), 375 (bcA_2), 343 (bcA_3), 233 (aA_1), 201 (aA_2), 169 (aA_3), and 189 (cA_1). These established the nature of the monosaccharide residues and their sequence. The origins of some of these fragments are illustrated in Fig. 1.

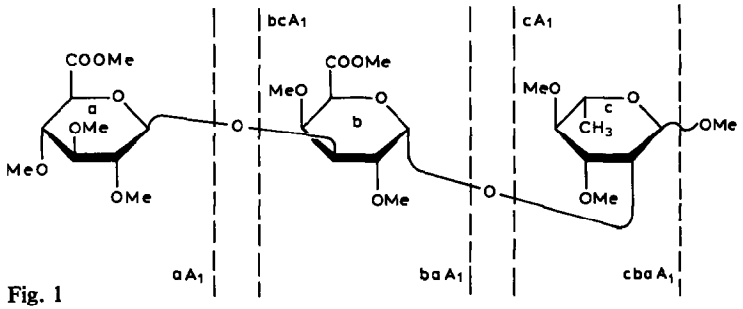


Fig. 1

Some of the other fragments seen in the mass spectrum are regarded as typical of certain types of glycosidic bond. The diagnostic value of these fragments is, however, considerably diminished, as they can be created through different pathways. For example, the ion at *m/z* 467 could be formed by the intitial D cleavage of ring *b*, with the rearrangement of the uronosyl residue *a*, linked to C-3 of ring *b*, to C-1 of the cleaved ring *b* and subsequent destruction of the fragment in the J pathway ($bacJ_1$ ion). This might indicate a (1→3) linkage between rings *a* and *b*. More probably, however, the fragment $abcJ_1$ is formed, which involves the initial D

cleavage of ring *a* with rearrangement of the OMe group from C-3 to C-1, followed by further degradation in a well known way. The presence of the ion at *m/z* 537, most probably the $cabF_1^2$ ion, and the low intensity of the peak at *m/z* 161 (bB_3) might indicate (1→2) linkage between the *b* and *c* rings. Because of these ambiguities in the fragmentation series, a part of the permethylated trisaccharide was reduced with lithium aluminium hydride in ether[17] and hydrolysed, and the partially methylated sugars were converted to the corresponding alditol acetates. G.l.c.–m.s. analysis gave approximately equal proportions of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-D-galactitol, and 1,2,5-tri-*O*-acetyl-3,4,-di-*O*-methyl-L-rhamnitol. This indicates linkages in trisaccharide as shown in Fig. 1. ^{13}C-N.m.r. of the original trimer revealed that the glucopyranosyluronic acid unit has the β-anomeric configuration (C-1 at 103.8 p.p.m.) and the galactopyranosyluronic acid the α configuration (C-1 at 97.0 p.p.m.) (lit.[15,16] 104.3 and 97.6 p.p.m., respectively).

RESULTS AND DISCUSSION

In the framework of a detailed investigation of the mucilages of the roots and leaves of *A. officinalis* L., var. Rhobusta (a hybrid of *A. officinalis* L. and *A. armeniaca* Ten., improved and cultivated in Czechoslovakia), we undertook structural studies of an acidic carbohydrate polymer, the presence of which in all initial, polysaccharide containing fractions isolated from the roots of the plant was indicated by the uronic acid content (Table I).

After maceration of the roots of the plant in cold water, four water-soluble and four water-insoluble polymer fractions were obtained by reprecipitations of the aqueous extracts with ethanol. The water-soluble fractions differed each from the other in the composition of the constituent sugars and in the uronic acid content; the

TABLE II

AMINO ACID COMPOSITION OF THE PROTEIN MOIETY OF POLYMER FRACTION C

Amino acid	*Mole percent*
Aspartic acid	40
Arginine	15
Glutamic acid	8
Glycine	6
Histidine	5
Tyrosine	5
Leucine	4
Alanine	4
Serine	3
Phenylalanine	3
Threonine	2
Isoleucine	2
Lysine	1

protein content of these fractions varied from 12.0 to 15.8% (Table I). No nitrogen-containing compounds other than amino acids were detected in the hydrolysates of these water-soluble fractions. The amino acid composition of the protein moiety in the polymer fraction C is listed in Table II. In the hydrolysates of the water-insoluble fractions D-glucose was found as the main sugar.

The first stage of structural studies focused on fractionation of the macromolecular mixture C. From the latter a highly branched L-arabinan[5] and three low-molecular-weight virtually linear glucans[6] have been isolated and structurally characterized. Repeated fractionations with saturated aqueous barium hydroxide afforded an acidic heteropolysaccharide in 34% yield with regard to the starting polymer mixture. Though aware of the possible consequences, we made use of this particular purification procedure because it was the only one of several separation methods tested that released a homogeneous carbohydrate material suitable for structural studies. The polysaccharide isolated in this way may, in its native state, have been part of a proteoglycan complex. However, upon exposure to alkali the *O*-glycosidic linkages to serine and/or threonine in protein (Table II) were cleaved and so were the *O*-acetyl groups, the presence of which in the original macromolecular mixture was evident from the ^{1}H-n.m.r. spectrum. The i.r. spectrum of the original macromolecular complex C displayed bands at 1735 and 1240 cm^{-1}, characteristic of ester groups. The ester-methoxyl content of the original mixture was 0.84% with regard to the weight of the sample. The presence of methyl ester groups led to some depolymerization, by β-elimination, upon exposure to $Ba(OH)_2$, as indicated by the diminished mucilaginous character of the purified heteropolysaccharide. On ultracentrifugal analysis the polysaccharide gave a single peak and on free-boundary electrophoresis moved as a homogeneous compound ($u = 2.1 \times 10^{-9}$ m^2 V^{-1} s^{-1}). The polysaccharide contained 33.8% of sulfated ash and showed a positive optical rotation $+28.3°$. The molecular weight $\overline{M}_w$, determined by ultracentrifugation, was 26,700 and the number average molecular weight $\overline{M}_n$, determined osmometrically, was 23,900. The $\overline{M}_w/\overline{M}_n$ ratio (1.12) revealed a low degree of polymolecularity. The neutral sugars in the products of acid hydrolysis were identified by paper chromatography as L-rhamnose and D-galactose and the uronic acids, distinguished by high-voltage paper electrophoresis, were D-glucuronic acid and D-galacturonic acid*.

It is generally known that determination of primary sequences of polysaccharides containing uronic acids is often complicated by the unusual stability to acid hydrolysis of glycosidic bonds formed by these residues. Therefore, the carboxyl groups of the D-galacturonic acid and D-glucuronic acid residues of the polysaccharide were reduced with $NaBD_4$ in the presence of carbodiimide[18] to the corresponding 6,6-dideuterio-D-galactosyl and 6,6-dideuterio-D-glucosyl residues, respectively. Reduction was complete after the 5th treatment (no uronic acids were detected). Quantitative analyses by g.l.c. of the alditol acetates of the hydrolysates of the

*The components of the hydrolysate were assumed to be the enantiomers normally found in plant polysaccharides. The absolute configurations were not determined experimentally.

TABLE III

SUGAR ANALYSIS OF THE ORIGINAL AND CARBOXYL-REDUCED POLYSACCHARIDES

Sugar (as alditol acetate)	*Molar ratios*	
	Original	*COOH-reduced*
L-Rhamnose	1.0	1.0
D-Galactose	1.2	2.1
D-Glucose	—	1.0
Hexuronic acids	2.2	—

TABLE IV

PARTIALLY METHYLATED ALDITOL ACETATES OBTAINED FROM THE METHYLATED ORIGINAL AND CARBOXYL-REDUCED POLYSACCHARIDES

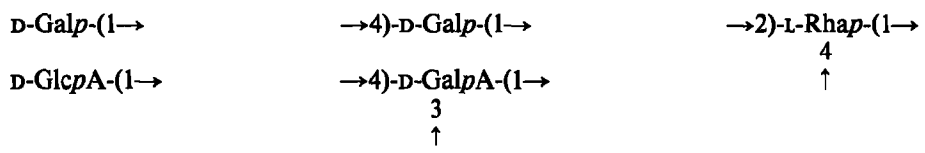
Methylated alditol acetate[a]	*Mole percent*	
	Original	*COOH-reduced*
2,3,4,6-Me_4-Gal	44	23
2,3,4,6-Me_4-Glc	—	25
3-Me-Rha	45	24
2,3,6-Me_3-Gal	11	3
2,3-Me_2-Gal	—	25

[a]2,3,4,6-Me_4-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, etc.

original and carboxyl-reduced polysaccharides showed that the original polysaccharide contained L-rhamnose, D-galactose, and hexuronic acid in the molar ratio 1.0:1.2:2.2, while the carboxyl-reduced polysaccharide was composed of L-rhamnose, D-galactose, and D-glucose in the molar ratio 1.0:2.1:1.0 (Table III).

Permethylation of the polysaccharide was achieved by the Hakomori method[12], followed by three Purdie[13] methylations (no absorption in the i.r. spectrum for the OH group). The products of hydrolysis of the fully methylated original and carboxyl-reduced polysaccharides were analysed by g.l.c–m.s. after conversion to alditol acetates (Table IV). Methyl ethers of the hexuronic acids were removed from the hydrolysis products of the methylated original polysaccharide by treatment with an anion-exchange resin, and the residual products were identified as 2,3,4,6-tetra-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, and 2,3,6-tri-*O*-methyl-D-galactose. They were obtained in the molar ratio of 1.00:1.02:0.24. In the case of the hydrolysis products of the methylated carboxyl-reduced polysaccharide, 6,6-dideuterio-2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, 2,3,6-tri-*O*-methyl-D-galactose, and 6,6-dideuterio-2,6-di-*O*-methyl-D-galactose were identified. The molar ratio was 1.08:1.00:1.04:0.08:1.08. The results suggested that the minimal repeating unit of the heteropolysaccharide was composed of five

kinds of sugar units as shown in Scheme 1.



Scheme 1. Component sugar residues in the minimal repeating unit in the polysaccharide structure.

As seen from the amounts of D-galactose determined in the original and carboxyl-reduced samples (Table III), the polysaccharide lost about one fourth of its D-galactose units in the process of reduction. This phenomenon was observed also by Tomoda *et al.*[1], and could be ascribed to degradation of the polysaccharide, most likely by β-elimination.

The acidic heteropolysaccharide was partially hydrolysed with formic acid and the high-molecular-weight portion was removed by precipitation with ethanol. The acidic portion of the low-molecular-weight products, separated on an anion-exchange resin, was further fractionated on a Sephadex G-25 column. In addition to D-glucuronic and D-galacturonic acids, several oligosaccharides were obtained by elution with acetate buffer. Of these, a dimer and a trimer have been characterized so far. Based on the results of component-sugar analysis, their ^{13}C-n.m.r. spectra, mass spectrometry of their permethylated derivatives, and g.l.c. analyses of the permethylated carboxyl-reduced samples, the oligosaccharides were identified as α-D-Gal*p*A-(1→2)-L-Rha and β-D-Glc*p*A-(1→3)-α-D-Gal*p*A-(1→2)-L-Rha.

From the results presented it can be concluded that the backbone chain in the polysaccharide is composed of (1→2)-linked L-rhamnose and (1→4)-linked D-galacturonic acid units in the ratio of 1:1. The molar ratios of terminal D-galactose and terminal D-glucuronic acid are equal to those of L-rhamnose and D-galacturonic acid units. Consequently, each unit of the backbone chain is a branching point bearing predominantly single nonreducing D-glucuronic acid and D-galactose residues, with occasional side chains composed of multiple D-galactose units. As D-glucuronic acid is attached through a β-(1→3) linkage to D-galacturonic acid, the single D-galactose residues and short chains of (1→4)-linked D-galactose units are bound to C-4 of L-rhamnose.

Despite the different variety of the plant used and different cultivation conditions, the basic structural features of the *Althaea* polysaccharide are similar to those of the mucilage described by Tomoda *et al.*[1]. Some minor differences are evident in the degree of branching. In our case all L-rhamnose units form branching points, as indicated by absence of any di-*O*-methyl derivative of L-rhamnose in the methylation products of the polysaccharide, whereas one third of the L-rhamnose units were unbranched in the material studied by Tomoda *et al.*

REFERENCES

1 M. TOMODA, S. KANEKO, M. EBASHI, AND T. NAGAKURA, *Chem. Pharm. Bull.*, 25 (1977) 1357–1362.
2 M. TOMODA, N. SATOH, AND K. SHIMADA, *Chem. Pharm. Bull.*, 28 (1980) 824–830, and references cited therein.
3 M. TOMODA, K. SHIMADA, AND N. SHIMIZU, *Chem. Pharm. Bull.*, 31 (1983) 2677–2684.
4 M. TOMODA, Y. SUZUKI, AND N. SATOH, *Chem. Pharm. Bull.*, 27 (1979) 1651–1656.
5 P. CAPEK, R. TOMAN, A. KARDOŠOVÁ, AND J. ROSÍK, *Carbohydr. Res.*, 117 (1983) 133–140.
6 P. CAPEK, R. TOMAN, J. ROSÍK, AND A. KARDOŠOVÁ, *Collect. Czech. Chem. Commun.*, 49 (1984) 2674–2679.
7 T. BITTER AND H. MUIR, *Anal. Biochem.*, 4 (1962) 330–334.
8 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
9 I. CIUCANU AND F. KEREK, *Carbohydr. Res.*, 131 (1984) 209–217.
10 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem. Biochem.*, 21 (1966) 39–93.
11 J. LÖNNGREN AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.
12 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
13 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, (1903) 1021–1037.
14 H. BJÖRNDALL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–440.
15 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
16 M. RINAUDO, G. RAVANAT, AND M. VINCENDON, *Macromol. Chem.*, 181 (1980) 1059–1070.
17 M. ABDEL-AKHER AND F. SMITH, *Nature (London)*, 166 (1950) 1037.
18 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.