POLYSACCHARIDES FROM THE STEMS OF *Althea nudiflora*

N. E. Zaitseva and I. S. Kozhina

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From the heart of the stems of the hollyhock *Althea nudiflora* (Lindl.) Boiss., growing on the territory of Southern Kazakhstan we have isolated a polysaccharide (yield 5%) containing residues of glucuronic and galacturonic acids (totalling 42%), and also of glucose, rhamnose, galactose, arabinose, xylose (traces) and mannose (traces). From the bark of the stems of this plant we have isolated a polysaccharide (yield 1.8%) consisting of a branched polymer composed of residues of rhamnose, glucose, and arabinose (22:17:2) and also of glucuronic and galacturonic acids (totalling 30%). In the products of the methylation of this polysaccharide previously reduced at the COOH groups we have established the presence of 2,3,4-tri-O-methylrhamnose, 3,4-di-O-methylrhamnose, 2,3,4,6-tetra-O-methylglucose, 2,6-di-O-methylglucose, and a mixture of 2,3,6-tri-O-methylglucose and 2,3,6-tri-O-methylgalactose (1:10:14:5:7).

One of us has previously [1] shown that various polysaccharides are present in the bark and heart of the stems of *Althea lenkoranica* Iljin, each of these saccharides containing uronic acids. Continuing a study of the polysaccharides from the stems of some species of the hollyhocks of the flora of the USSR, we have isolated from the stems of the hollyhock *Althea nudiflora* (Lindl.) Boiss. growing on the territory of Southern Kazakhstan two polysaccharides: one from the heart and the other from the bark of the stems.

The polysaccharide from the heart of the stems, with a yield of 5% (on the weight of the air-dry plant material), contained 42% of uronic acids. On dissolution it gave a viscous solution: the relative viscosity of a 0.6% solution of the polysaccharide η_{rel} = 1.72 (in borate buffer solution, pH 9.21). By chromatographic analysis on paper (systems 1 and 2) a hydrolysate of the polysaccharide was found to contain glucose, rhamnose, galactose, and arabinose, and traces of xylose and mannose, in addition to glucuronic and galacturonic acids. However, electrophoresis showed that the polysaccharide isolated was not homogeneous in its chemical composition. It apparently contained a glucan as impurity, since, judging from the PC of hydrolysates, samples isolated in different ways contained different amounts of glucose.

We studied the polysaccharide from the bark of the stems of this hollyhock (yield 1.8%) in more detail. It contained 30% of uronic acids and gave a viscous solution (relative viscosity of a 0.6% solution of the polysaccharide in borate buffer, pH 9.21, η_{rel} = 2.27). The polysaccharide was homogeneous according to paper electrophoresis and ultracentrifugation.

In a hydrolysate of the polysaccharide from the bark (PC in systems 1 and 2) we identified glucose, rhamnose and traces of arabinose, and also glucuronic and galacturonic acids.

GLC analysis (column VI) of the corresponding polyol acetates showed that the ratio of glucose, rhamnose, and arabinose was 17:22:2.

In the IR spectrum of the polysaccharide, strong absorption bands were observed at 1600 cm^{-1} (COO^- groups), 1245, 1375, and 1720 cm^{-1} (acetyl groups), and 3300 to 3400 cm^{-1} (broad band of the absorption of OH groups), and also at 850 and 890 cm^{-1} (α- and β-glycosidic bonds, respectively).

To determine the types of bonds in the polysaccharide we reduced it with $NaBH_4$ after preliminary deionization with the aid of KU-2 resin [H^+] and etherification/esterification with ethylene oxide. The neutral polysaccharide was methylated by Hakomori's method [2]. The completely methylated polysaccharide was subjected to methanolysis; part of the reaction

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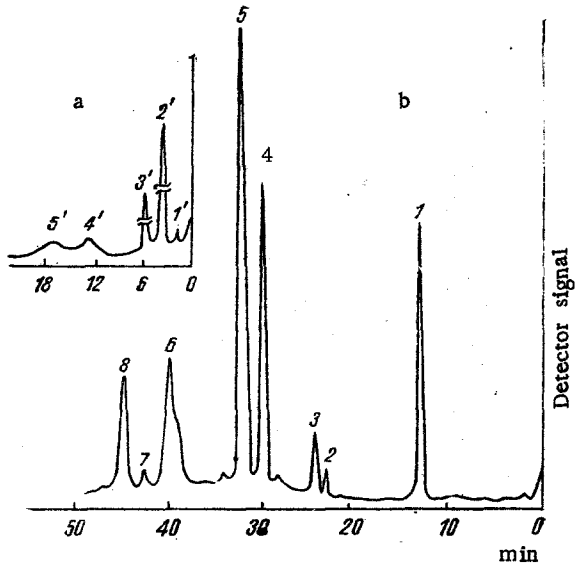

Fig. 1. Gas–liquid chromatogram of the methyl glycosides of the partially methylated carbohydrates (separation conditions II) (a) and of the acetates of the partially methylated polyols (separation conditions IV) (b): 1') Methyl 2,3,4-tri-O-methylrhamnoside; 2') methyl 3,4-di-O-methylrhamnoside; 2', 3') methyl 2,3,4,6-tetra-O-methylglucoside; 4', 5') methyl 2,3,6-tri-O-methylglucoside + methyl 2,3,6-tri-O-methylgalactoside; 1) presumably an O-acetyltetra-O-methyl-6-deoxyhexitol; 2) a 1,5-di-O-acetyl-2,3,4-tri-O-methyl-6-deoxyhexitol; 3) not identified; 4) a 1,2,5-tri-O-acetyl-3,4-di-O-methyl-6-deoxyhexitol; 5) a 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol; 6) a 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylhexitol; 7) not identified; 8) a 1,3,4,5-tetra-O-acetyl-2,6-di-O-methylhexitol.

products was separated by GLC (columns I and II), and the bulk was hydrolyzed with a solution of H_2SO_4. The composition of the products of the hydrolysis of the methylated polysaccharide was determined by PC (systems 3, 4, and 5) and GLC (acetates of the partially methylated polyols) on columns III, IV, and V.

To identify the acetates of the partially methylated polyols we used mass spectrometry [3]. Below we give the chromatographic mobilities of the partially methylated monosaccharides:

Partially methylated monosaccharides	R_g* in systems:		
	3	4	5
2,3,4-Tri-O-methylrhamnose	1.01	1.03	1.00
2,3,4,6-Tetra-O-methylglucose	1.00	1.00	1.00
3,4-Di-O-methylrhamnose	0.90	0.89	0.92
2,3,6-Tri-O-methylglucose + 2,3,6-tri-O-methylgalactose	0.80	0.79	0.85
2,6-Di-O-methylglucose	0.62	0.65	0.69

The results obtained on GLC analysis of the methyl glycosides of the partially methylated carbohydrates (Fig. 1a) and also on analysis of the partially methylated monosaccharides with the aid of PC corresponded to those obtained on the basis of a mass-spectrometric analysis of the acetates of the partially methylated polyols (GLC, Fig. 1b).

*Relative to 2,3,4,6-tetra-O-methylglucose.

Thus, in the molecule of the polysaccharide from the bark of the stems of the hollyhock *A. nudiflora*, after its reduction at the COOH group, the following types of bonds were detected:

Sugar residue	Positions of the O-methyl groups	Glycosidic bond	Quantitative ratio of the sugar residues, moles
Rhamnose	2,3,4-	terminal	1
	3,4-	2	10
Glucose	2,3,4,6-	terminal	14
	2,3,6-*	4	7
	2,6-	3,4	5

EXPERIMENTAL

For paper chromatographic analysis we used FN-2, FN-4, and FN-12 papers (GDR) and the following systems: 1) ethyl acetate—pyridine—water (8:2:1); 2) butan-1-ol—pyridine—water (6:4:3; two or three times); 3) water-saturated butan-1-ol; 4) butan-1-ol—acetic acid—water (4:1:5; upper layer); 5) butan-1-ol—ethanol—water (40:11:19). Aniline hydrogen phthalate and dimethylaniline were used as chromogenic agents.

IR spectra were recorded in paraffin oil on a UR-10 instrument and using films of the substance on a Perkin-Elmer 577 instrument.

Gas—liquid chromatography (GLC) of the methyl glycosides from the methylated hydrocarbons was performed on a Pye Unicam series 104 instrument using the following columns: I) 15% of NPGA, 150 × 0.4 cm, temperature 150°C; V_{Ar} = 30 ml/min; II) 15% of BDS, 100 × 0.4 cm, temperature 175°C, V_{Ar} = 40 ml/min. The acetates of the partially methylated polyols were separated on the following columns (150 × 0.4 cm): III) 3% of QF-1 with programming of the temperature over 110-220°C/4°C, V_{Ar} = 20 ml/min; IV) 3% of OV-225, temperature 130-220°C/2°C, V_{Ar} = 30 ml/min; and V) 15% of NPGS, temperature 125-225°C/6°C, V_{Ar} = 60 ml/min.

The full polyol acetates were analyzed on column VI — 3% of OV-225, 150 × 0.4 cm, temperature 190°C, V_{Ar} = 40 ml/min. Chromaton (0.16-0.20 mm) was used as inert support.

The mass spectra of the acetates of the partially methylated polyols were recorded in chloroform on LKB-2091 and LKB-9000s instruments (separation of the mixture on column IV).

The isolation and purification of the polysaccharide, the establishment of its homogeneity, and the determination of the uronic acids were carried out as described by Kozhina et al. [4].

The determination of the relative viscosity of the polysaccharide, its etherification/esterification, reduction, and Hakomori methylation, and the preparation of the acetates of the partially methylated polyols, and also the determination of the quantitative ratio of neutral monosaccharides in the polysaccharide molecule were performed under the conditions that we have used previously [5].

The plant material was collected and determined by N. A. Trukhaleva, and the mass spectra of the acetates of the partially methylated polyols were obtained by S. A. Zabelinskii and I. G. Zenkevich. The IR spectra of the polysaccharide in the form of a film were taken and interpreted by M. P. Filippov.

SUMMARY

1. From the heart of the stems of the hollyhock *Althea nudiflora* growing on the territory of Southern Kazakhstan we have isolated a polysaccharide (yield 5%) containing residues of glucuronic and galacturonic acids (totalling 42%) and also of glucose, rhamnose, galactose, and arabinose, together with traces of xylose and mannose.

2. From the bark of the stems of this plant we have isolated a polysaccharide (yield 1.8%) consisting of a branched polymer composed of residues of rhamnose, glucose, and arabinose (22:17:2) and also of glucuronic and galacturonic acids (totalling 30%).

*Apparently with 2,3,6-tri-O-methylgalactose as an impurity.

3. The presence of 2,3,4-tri-O-methylrhamnose, 3,4,di-O-methylrhamnose, 2,3,4,6-tetra-O-methylglucose, and 2,6-di-O-methylglucose and of a mixture of 2,3,6-tri-O-methylglucose and 2,3,6-tri-O-methylgalactose (1:10:14:5:7) in the products of the methylation of the polysaccharide from the bark of *A. nudiflora* after its reduction at the carboxy groups has been established.

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PREPARATION OF TRITIUM-LABELLED OLEIC ACID AND PROSTAGLANDINS E_2 AND $F_{1\alpha}$. A STUDY OF THE PROCESS OF INCLUDING TRITIUM IN MOLECULES OF UNSATURATED FATTY ACIDS AND PROSTAGLANDINS

V. P. Shevchenko, N. F. Myasoedov,
V. V. Bezuglov, and L. D. Bergel'son

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[^{3}H]Prostaglandin E_2 and [^{3}H]prostaglandin $F_{1\alpha}$ have been obtained with the aid of heterogeneous catalytic isotope exchange with gaseous tritium in solution. The distribution of the tritium in the labelled unsaturated fatty acids and prostaglandins has been studied.

We have previously shown the possibility of obtaining tritium-labelled saturated and unsaturated fatty acids by heterogeneous isotope exchange with gaseous tritium in the presence of transition metals [1, 2]. The process of introducing tritium into the molecules of natural compounds by this method is performed under mild conditions and does not require the preliminary protection of functional groups. By using this method we have obtained tritium-labelled eicosa-8,11,13-trienoic and arachidonic acids, which retained their biological activities, as was shown by the enzymatic conversion of these labelled acids into tritium-containing prostaglandins E_1 and E_2, respectively [2].

Isotope exchange between gaseous tritium and the hydrogen of unsaturated fatty acids is accompanied by the addition of hydrogen and of tritium at the double bonds. By suitable selection of the catalyst and of the reaction time it is possible to suppress the addition reaction to a considerable degree. Table 1 gives information characterizing the capacity of three batches of catalysts (A, B, and C) for effecting isotope exchange and also for adding hydrogen and tritium at the double bond of methyl oleate. For the subsequent investigations we used catalyst C, which gave the highest yield of labelled substance of the initial structure with a high specific activity. By isotope exchange on this catalyst we obtained tritium-labelled prostaglandin E_2 (PGE_2) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$).

On further investigation of the processes taking place during isotope exchange, we established that as well as addition reactions at least two other processes lowering the yield of desired product occurred: cis-trans isomerization and the migration of the double bond along the chain. In order to study these processes, the substances after isotope exchange with 80% tritium, were first purified by preparative TLC and were then separated on silica gel impregnated with silver nitrate. It has been shown previously [3] that under

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