62. H. H. Sauer, R. D. Bennett, and E. Heftmann, Phytochemistry, 7, 2027 (1968).
63. R. A. Joly, C. M. Svahn, R. D. Bennett, and E. Heftmann, Phytochemistry, 8, 1917 (1969).
64. H. Hikino, T. Kohama, and T. Takemoto, Chem. Pharm. Bull., 17, 415 (1969).
65. H. Hikino, T. Hohama, and T. Takemoto. Phytochemistry, 9, 367 (1970).
66. N. J. de Souza, E. L. Ghisalberti, H. H. Rees, and T. W. Goodwin, Phytochem., 9, 1247 (1970).
67. T. Matsuoka, S. Imai, M. Sakai, and M. Kamada, Annu. Rept. Takeda Res. Lab., 28, 221 (1969).
68. M. N. Galbraith, D. H. S. Horn, E. J. Middleton, and J. A. Thompson, J. Chem. Soc. Chem. Commun., 179 (1970).
69. N. J. de Souza and N. Nes, Science, 162, 363 (1968).
70. M. E. Lowe, D. H. S. Horn, and M. N. Galbraith, Experientia, 24, 518 (1968).
71. A. J. Clark and K. Bloch, J. Biol. Chem., 234, 2578, 2589 (1959).
72. E. Kodicek and H. Z. Levinson, Nature (London), 188, 1023 (1960).
73. R. H. Dadd and T. L. Mittler, Experientia, 22, 823 (1966).
74. P. Ehrhardt, Experientia, 24, 82 (1968).
75. H. Z. Levinson, J. Insect Physiol., 8, 191 (1962).
76. J. A. Svoboda, R. F. N. Hutchins, M. J. Thompson, and W. E. Robbins, Steroids, 14, 469 (1969).
77. G. S. Fraenkel, Science, 129, 1466 (1959).
78. H. Schildknecht, R. Siewerdt, and U. Maschwitz, Angew. Chem., 78, 392 (1966).
79. H. Schildknecht and H. Birringer, Z. Naturforsch., 24b, 1529 (1969).
80. I. L. Novosel'skaya, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 401 (1981).

ASPARAGUS POLYSACCHARIDES.

I. ISOLATION AND CHARACTERIZATION OF POLYSACCHARIDES OF *A. neglectus*: GLUCOMANNANS OF THE ROOTS

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Water-soluble polysaccharides and pectin substances have been isolated from various organs of *A. neglectus* and their quantitative amounts and monosacchardie compositions have been determined. Native acetylated glucomannans (A, B, and C) containing 13, 15, and 20% of acetyl groups and being homogeneous according to the results of gel chromatography have been isolated from the roots. On the basis of the results of periodate oxidation, methylation, and IR spectroscopy it has been established that the neutral polysaccharides of the roots of *A. neglectus* is a mixture of three glucomannans consisting of β-1→4 linear-bound D-gluco- and D-mannopyranose residues.

In Central Asia, the genus *Asparagus* is represented by 13 species [1]. There are reports on the study of the carbohydrates of *Asparagus* [2-6], but the water-soluble polysaccharides (WSPSs) and pectin substances (PecSs) of this genus have been little studied.

We have investigated the amounts of polysaccharides and their monosaccharide compositions in various organs of *A. neglectus* Kar. et Kir collected on May 17, 1977 (Temirlik, KirgSSR) and previously treated with chloroform and ethanol to eliminate low-molecular-weight substances.

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The water-soluble polysaccharides and the pectin substances were isolated from our sample by a method described previously [7]; the results of analysis by PC in system 1 and by GLC (of the acetates of the aldononitrile derivatives) are given in Table 1.

It can be seen from Table 1 that the amount of water-soluble polysaccharides in the roots is greater than that of other polysaccharides, and they also differ from the other polysaccharides in composition. Together with the monosaccharides shown in Table 1, all the polysaccharides were found to contain a uronic acid. The polysaccharides are not glucans of the starch type, as was shown by the negative reaction with iodine, and they do not contain nitrogen.

The water-soluble polysaccharides from the roots were separated on DEAE-cellulose (DE-32, CO_3^{2-} form) into neutral and acid fractions. Elution with water gave 73% of a neutral polysaccharide consisting of glucose and mannose residues (in a ratio of 1:8.4) with traces of arabinose and rhamnose, and elution with 1 M $(NH_4)_2CO_3$ gave 7.3% of an acidic polysaccharide.

The neutral polysaccharide was a white amorphous powder with mp 280-290°C (decomp.), forming a viscous solution in water. The neutral polysaccharide was fractionally precipitated with 96% ethanol, which gave four fractions (Table 2).

Table 2 shows that the neutral polysaccharides consisted of three glucomannans differing from one another in molecular weight and ratio of their polysaccharides.

The IR spectra of the three glucomannans contained absorption bands at 3600-3200 cm^{-1} (–OH), 1730 and 1250 cm^{-1} (ester group), 880 cm^{-1} (β-glycosidic bond), and 815 cm^{-1} (hexapyranose ring) [9]. The negative value of the specific rotation shows the presence of a β-glycosidic bond between the sugars in the fractions, as is confirmed by the results of IR spectroscopy.

The amounts of O-acetyl groups [10] in the polysaccharide fractions A, B, and C were 13, 15, and 20%. These fractions proved to be homogeneous when subjected to gel chromatography on Sephadex G-100 (Fig. 1).

When fraction B was subjected to periodate oxidation [11], the consumption of periodate was 0.6 mole per anhydro unit with the liberation of 0.1 mole of formic acid. After the hydrolysis of the oxidized product, paper chromatography in system 1 showed the presence of erythritol and mannose in a ratio of 16:1. The results of the oxidation of fractions A and C were similar to those for B.

TABLE 1. Amounts of Polysaccharides and Their Monosaccharide Compositions in Various Organs of *A. neglectus*

Plant organ	Type of polysaccharide	Yield* of polysaccharide	Monosaccharide composition; ratio of carbohydrate residues					
			Gal	Glc	Man	Xyl	Ara	Rha
Roots	WSPS	5,0	Tr.	6,1	37,5	Tr.	13,3	1,0
	PecS	0,5	1,4	2,7	2,0	"	4,9	1,0
Rhizomes	WSPS	1,1	2,6	2,3	2,0	"	6,6	1,0
	PecS	0,5	2,03	3,6	1,0	1,4	7,6	1,2
Stems	WSPS	1,8	3,6	1 4	Tr.	1,8	5,1	1,0
	PecS	0,3	6,0	13,8	1,0	4,9	8,4	2,1
Leaves	WSPS	3,1	10,0	4,3	21,5	7,0	15,9	1,0
	PecS	3,2	5,7	1,7	10,3	1,0	5,4	1,7

*Of the weight of the absolutely dry raw material, %.

TABLE 2. Characteristics of the Fractions and the Qualitative Ratio of the Monosaccharides in Them

Fraction	Yield, % on the weight of the NPS	$[\alpha]_D^{27°}$ (c 0,5; water)	η_{rel} (c 0.5; water)	–OAc, %	Ratio of monosaccharides				Molecular weight
					Glc	Man	Ara	Rha	
A	14,0	–40	3,4	13,0	1,0	5,5	Tr.	—	51000
B	38 0	–44	6,8	15,0	1,0	8,5	"	—	46000
C	33,5	–49	3,1	20,0	1,0	14,0	"	—	43000
D	5,0				1,0	14,9	1,0	1,0	

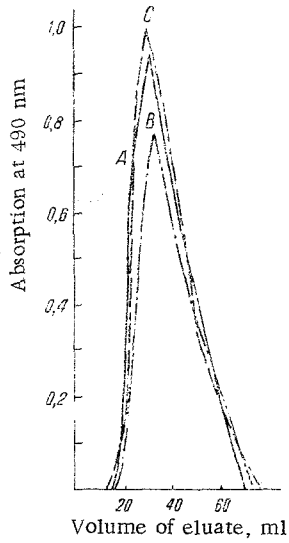

Fig. 1. Gel chromatography of fractions A, B, and C on Sephadex G-100.

Only erythritol was detected in the products of the oxidation of deacetylated A, B, and C. From the fact that after the oxidation of the native acetylated glucomannans a certain amount of mannose was detected while in the products of oxidation of the deacetylated mannans there was none, it follows that some of the monosaccharide residues are substituted by an acetyl group at C-2 or C-3.

Fractions A, B, and C were twice methylated by Hakomori's method [12], and permethylates containing 41.2, 40.3, and 42% of OCH_3, respectively, were obtained. The IR spectra of the permethylates lacked the absorption band of an —OH group, which shows complete methylation. Permethylate B had $[\alpha]_D^{27°}$ −22.5° (c 1.78; acetone). The hydrolysates of all the permethylates were found to contain 2,3,6-tri-O-methylglucose, 2,3,6-tri-O-methylmannose, and traces of a 2,3,4,6-tetra-O-methylhexose, which were identified by TLC in system 3 and by GLC in the form of partially methylated acetates of the polyol derivatives. The ratio of 2,3,6-tri-O-methylglucose and 2,3,6-tri-O-methylmannose in the three permethylates corresponded to the ratio of glucose and mannose in the initial glucomannans, in moles:

Methylated sugars	Glucomannans		
	A	B	C
1,4,5-Tri-OAc-2,3,6-tri-O-Me-glucose	4.1	1.0	1.0
1,4,5-Tri-OAc-2,3,6-tri-O-Me-mannose	22.5	8.2	14.0
1,5-di-OAc-2,3,4,6-tetra-O-Me-hexose	1.0	Tr.	Tr.

The results of GLC and chromato-mass spectrometry of the partially methylated acetates of the polyols correspond to literature information [13].

Thus, on the basis of the results of periodate oxidation, methylation, and IR spectroscopy it has been established that the neutral polysaccharides of the roots of *A. neglectus* are formed by a mixture of three glucomannans consisting of D-gluco- and D-mannopyranose residues linked linearly with one another by β-1 → 4 bonds.

EXPERIMENTAL

For paper chromatography use was made of FN-3, FN-7, and FN-11 papers (GDR) and the following solvent systems: 1) butan-1-ol—pyridine—water (6:4:3); 2) phenol—butan-1-ol—acetic acid—water (20:20:8:40); and 3) methyl ethyl ketone—1% ammonia (30:1). Revealing agents: aniline hydrogen phthalate; 1% permanganate solution; ethanolic solution of urea in 2 N HCl. Gas-liquid chromatography was carried out on a Tsvet-101 instrument with a flame-ionization detector under conditions described previously [7], and chromato-mass spectrometry on a Varian MAT-111 instrument with a column containing 5% of OV-1 (1 meter, steel), temperature regime from 120 to 300°C at 4°C per minute; and IR spectra were obtained on a UR-20 instrument in KBr tablets and paraffin oil.

Isolation of the Polysaccharides. The polysaccharides were isolated from various organs of the plant by a method described previously [7].

Hydrolysis of the Polysaccharides. The hydrolysis of 0.05 g of each sample was carried out with 1 N H_2SO_4 in the boiling water bath for 10 h. The hydrolysates were neutralized with $BaCO_3$ and, after the appropriate working up, were analyzed by PC and GLC.

Chromatography on DEAE-Cellulose. A 4.4 × 24 cm column was charged with 60 g of DEAE-cellulose DE-32 (CO_3^{2-} form) prepared as described by Rakhimov et al. [14], and a 0.5% solution of 3.3 g of water-soluble polysaccharides was introduced. Elution was performed with two liters of water and then with one liter of 1 M $(NH_4)_2CO_3$. The aqueous eluates were evaporated and precipitated with one liter of methanol. The precipitate that deposited (neutral polysaccharide) was centrifuged, washed with ethanol and with acetone, and dried over P_2O_5. The yield of neutral polysaccharides was 2.42 g and of acidic polysaccharides 0.24 g.

Fractional Precipitation with Ethanol. With stirring, 0.35 liter of ethanol was added dropwise to a solution of 2 g of the neutral polysaccharides in 0.2 liter of water. The resulting precipitate A was separated off; yield 0.28 g. Another 0.1 liter of ethanol was added to the supernatant liquid, giving B with a yield of 0.76%. Then another 0.1 liter of ethanol was added to the supernatant liquid, giving fraction C with a yield of 0.67 g. The ethanolic solution was evaporated and precipitated with ethanol, giving fraction D with a yield of 0.07 g.

Gel Chromatography on Sephadex G-100. A 1% solution of the sample was deposited on a column with dimensions of 30 × 2 cm filled with Sephadex G-100, and elution was carried out with 0.3% NaCl solution. The eluates were collected in 3-ml portions and were analyzed by the phenol–sulfuric acid method [11].

Determination of O-Acetyl Groups. The quantitative determination of O-Ac groups was carried out by the method of Dubois et al. [10]. For fractions A, B, and C it amounted to 13, 15, and 20%, respectively.

Deacetylation of the Glucomannans. Fractions A, B, and C, as 100-mg samples, were deacetylated by the method of Dubois et al. [10].

Smith Periodate Oxidation and Degradation. Oxidation was carried out as described previously [14]. The oxidation of fraction B was complete on the 13th day, when the consumption of periodate amounted to 0.6 mole per monosaccharide residue and 0.1 mole of formic acid had been liberated. The hydrolysate was found with the aid of PC in system 1 and by GLC (of the acetates of the corresponding aldononitrile) to contain mannose and erythritol residues in a ratio of 1:16.

Oxidation of the deacetylated fractions was complete after 26 days, the consumption of periodate then amounting to 0.86 mole per monosaccharide residue and 0.1 mole of formic acid having been liberated. The hydrolysates contained no mannose residues.

The methylation of glucomannans A, B, and C was carried out by Hakomori's method [12] twice. The fully methylated glucomannans were obtained with yields of 62, 60, and 67%, respectively; their IR spectra contained no absorption bands of an OH group.

Hydrolysis of the Permethylates. The methylation products were heated with 85% formic acid at 100% for 3 h and then with 0.5 N H_2SO_4 at 100°C for 8 h and they were neutralized with $BaCO_3$ and evaporated. The hydrolysates were reduced with $NaBH_4$ for 18 h and were then treated with KU-2 cation-exchange resin (H^+) and evaporated with the addition of ethanol, and the polyol acetates were obtained by the method of Bouveng et al. [15]. The results of the GLC analysis have been given in the discussion.

SUMMARY

1. The quantitative amounts of polysaccharides in various organs of *A. neglectus* and their monosaccharide compositions have been determined and homogeneous native acetylated glucomannans have been isolated.

2. On the basis of the results of periodate oxidation and methylation, it has been established that the glucomannans of the roots consist of β-1 → 4-linked D-gluco- and D-mannopyranose residues and their molecules have a linear structure.

LITERATURE CITED

1. Flora of the USSR [in Russian], Moscow–Leningrad, Vol. 4 (1935), p. 430.
2. R. Goldberg, Compt. Rend., D269, No. 15, 1419 (1969).
3. R. Goldberg and R. Jancloude, Compt. Rend., D270, No. 5, 702 (1970).
4. N. J. Borras, Phytochemistry, 12, 1331 (1973).
5. A. B. Landge and J. L. Bose, Indian J. Chem., 8, 588 (1970).
6. M. Tomada and N. Sotoh, Chem. Pharm. Bull., 22, 2306 (1974).
7. A. O. Arifkhodzhaev, D. A. Rakhimov, and Z. F. Ismailov, Khim. Prir. Soedin., 246 (1980).
8. M. Zaprometov, Biochemical Methods of Plant Analysis [in Russian], Moscow (1970), p. 296.
9. R. G. Zhbankov, Infrared Spectra and the Structure of Carbohydrates [in Russian] (1972), p. 78.
10. M. Dubois et al., Anal. Chem., 28, 350 (1956).
11. V. I. Sharkov and N. I. Kuibina, The Chemistry of Hemicelluloses [in Russian], Moscow (1972), p. 103.
12. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
13. J. Lönngren and S. Svensson, Adv. Carbohydr. Chem. Biochem., 29, 41 (1974).
14. D. A. Rakhimov, R. K. Rakhmanberdieva, and Z. F. Ismailov, Khim. Prir. Soedin., 555 (1978).
15. H. O. Bouveng, H. Kiessling, B. Lindberg, and J. E. McKay, Acta Chem. Scand., 16, 615 (1965).

DISTRIBUTION OF PHOSPHOLIPIDS IN THE ORGANS OF THE COTTON PLANT IN THE PERIOD OF MASS FRUIT-FORMATION

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The distribution of phosphorus in the vegetative and generative organs of the cotton plant of variety 159-F has been studied. It has been established that the various organs of the cotton plant differ with respect to the qualitative and quantitative compositions of their phospholipids.

The maximum amount of unidentified phospholipids (PLs) in the seeds of the cotton plant of variety 159-F is found at the stage of massive fruit-formation [1, 2]. There are reports on the amount of total phosphorus in different generative and vegetative organs of the cotton plant [3, 4], and some authors [5-9] have determined the amounts of lipid phosphorus in the leaves and in the whole bush. We have studied the distribution of phospholipids in the vegetative and generative organs of the cotton plant of variety 159-F in the period of mass fruit-formation. The yield of extractive substances, the distribution of total phosphorus, and the amount of lipid phosphorus in the various organs of one cotton bush — buds, flowers of the 1st day, flowers of the 2nd day, bolls, leaves, bark of the stem, stem without the bark, and roots — have been determined. The amounts of phosphorus and phospholipids were determined in chloroform–methanolic (2:1), methanolic, and aqueous extracts and in the meal (Tables 1 and 2).

It follows from the figures given in Table 1 that the largest amounts of substances are extracted by chloroform–methanol and by methanol from the leaves and stems. The amounts of extractive substances decrease in the sequence: leaves > stems without bark > bolls > buds > bark > flowers > roots. But if we compare these results with the ratios of the weights of the organ to the weight of the cotton bush, which will show the relative secretion of the extractive substances, we obtain a somewhat different sequence: stems without bark > leaves > buds > flowers > bolls > bark > roots. The amount of phosphorus in the organs changes in

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