As can be seen the relative error falls within the limits of accuracy of the method developed.

Determination Procedure. In a Soxhlet apparatus, 1 g of raw material was extracted with 200 ml of 80% ethanol, and the extract was concentrated and transferred quantitatively to a 25-ml measuring flask. The time of extraction was 4 h.

Plates with a fixed layer of silica gel (L 5/40 μ for thin-layer chromatography) divided into two parts were used. On one part was deposited 0.07-0.08 ml of the ethanolic raffinose extract, and on the other 0.07-0.08 ml of a standard solution of raffinose in concentration of 1 mg/ml. Chromatography was carried out with methyl ethyl ketone–acetic acid–water (5:4:1) system. That part of the plate upon which the solution of standard raffinose had been deposited was developed with a mixture of 2% solution of aniline in acetone containing diphenylamine phosphate [3], and after being sprayed the plate was heated to 110°C for 10 min. The time of exposure was 2.5-3 h.

The section of silica gel from the other part of the plate corresponding to the raffinose spot ($R_f \approx 0.35$) was transferred to a Schott No. 3 funnel and was eluted with 5 ml of boiling distilled water. To the eluate were added 4 ml of concentrated sulfuric acid and 6 ml of freshly prepared 0.2% solution of anthrone in 80% sulfuric acid.

The comparison solution was a mixture of 5 ml of distilled water, 4 ml of concentrated sulfuric acid, and 6 ml of the 0.2% solution of anthrone. The two test-tubes containing these mixtures were heated in hot water (80-90°C) for 15 min. The optical densities were measured in a photocolorimeter with a red filter in a cell with a layer thickness of 1 cm.

SUMMARY

A method has been developed for the quantitative determination of raffinose in cottonseed meal. The relative error of a single determination is ±4%.

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LIPOPOLYSACCHARIDES FROM *Mastigocladus laminosus*

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A lipopolysaccharide (LPS) has been isolated by phenol–water extraction from the cells of the blue-green alga *Mastigocladus laminosus*. It has been shown that the LPS contains polysaccharide and lipid components. The polysaccharide component includes a rhamnan fragment constructed of β-1,3- and, possibly, -1,2-bound L-rhamnose residues. The lipid component is constructed of glucosamine, glucose, and fatty acid residues, among which palmitic acid predominates.

Using Westphal's method [1] we have isolated a lipopolysaccharide fraction from air-dry cells of the blue-green alga *Mastigocladus laminosus* collected in thermal springs of Kamchatka. The crude preparation was purified from free phospholipid components by extraction with chloroform–methanol (2:1 by volume), and the accompanying nucleic acids and glucan were eliminated by ultracentrifugation. As a result, a purified lipopolysaccharide (LPS) with a low content of accompanying protein and nucleic acids (not more than 1% and 2%, respectively) was obtained.

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Analysis showed that the LPS obtained included 45% of neutral carbohydrates and 10% of amino sugars (mainly glucosamine); 2-keto-3-deoxyoctonic acid (KDO) and 3,6-dideoxyhexoses were present only in small amounts (0.3% and 0.1%, respectively), and no heptoses were detected. It was shown with the aid of GLC that a hydrolysate of the LPS contained the following neutral monosaccharides: rhamnose, galactose, glucose, mannose, arabinose, and xylose in a percentage ratio of 55:10:14:8:11:2.

The low KDO content led to a stronger bond of the carbohydrate and lipid components, which required more severe conditions of hydrolysis for the separation of the lipid components than is the case for the LPS of Gram-negative bacteria [2]. For the hydrolytic cleavage of the LPS we used treatment with 10% acetic acid. As a result, the lipid component (yield, 12% of the weight of the LPS) and a polysaccharide fraction consisting mainly of a mixture of two polysaccharides were obtained.

The lipid component was separated by centrifugation and was subjected to hydroylsis with subsequent analysis by paper chromatography and gas–liquid chromatography. Glucose and glucosamine were identified as the carbohydrate components, and their presence was confirmed by paper electrophoresis in pyridine–acetate buffer. A study of its fatty acid composition showed that the lipid component of the LPS included residues of myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids, the palmitic acid predominating in the hydrolysate. The composition of the lipid component of the LPS from *M. laminosus* was similar to that of the lipids from the MPSs of other blue-green algae that we have studied previously [3]. At the same time it differed from the lipid obtained from the LPSs of the blue-green algae *Anacystis nidulans* [4], *Cynechocystis* [5], and *Synechococcus* [6] and from lipid A of Gram-negative bacteria by the absence of the β-hydroxy acid residues that play an important role in the manifestation of the endotoxic properties of lipid A and the LPSs.

The polysaccharide component of the LPS after the removal of lipid was separated by gel chromatography on Sephadex G-50 into two polysaccharides: PS-1, issuing immediately after the free volume of the column, and PS-2, which was retained by the gel. By analogy with the LPSs of Gram-negative bacteria, PS-1 may be considered as the polysaccharide of the O-specific side chains, and PS-2 consists of the polysaccharide core.

Complete acid hydrolysis showed that PS-1 consisted mainly of rhamnose residues (more than 60%) and it most probably consists of a rhamnan. Unfortunately, it was impossible to eliminate the other monosaccharides, and in the sample of PS-1 studied, apart from rhamnose, we detected galactose (10%), glucose (10%), mannose (7%), arabinose (6%), and xylose (4%). The rhamnose was isolated in the individual state, and comparison with an authentic sample showed that it belonged to the L series.

The PS-2 also consisted of a heteropolysaccharide, containing residues of rhamnose (29%), mannose (20%), glucose (18%), galactose (16%), xylose (15%), and arabinose (2%). The results obtained indicated that it was impossible with the aid of partial hydrolysis to fragment the polysaccharide component of the LPS into a main carbohydrate chain and lateral O-specific chains.

In view of this, we limited ourselves simply to the study of PS-1, assuming that it reflected the specificity of the initial LPS to a greater extent. Because of the complexity of the monosaccharide composition of PS-1, its ^{13}C NMR spectrum was not easy to interpret unambiguously. Nevertheless, it can be stated that PS-1 contained a fragment consisting only of rhamnose residues (a rhamnan). Furthermore, the ^{13}C NMR spectrum showed the predominant presence of a β-1,3- bond between the rhamnose residues in this fragment (signals with a predominating integral intensity: 101.5 ppm, relating to the anomeric C-1 atom, and 78.7 ppm, showing the presence of a glycosidic bond at the C-3 atom).

To determine the nature of the bonds between the monosaccharide residues in PS-1 more accurately, we used the methylation method. PS-1 was exhaustively methylated by Hakomori's method [7], and the methylated PS obtained was subjected to methanolysis with subsequent analysis of the mixture of products with the aid of gas-liquid chromatography (GLC) and chromato-mass spectrometry [8]. Because of the complexity of the pattern obtained and the smallness of the amounts of individual methylated sugars in the mixture, it was possible reliably to identify only derivatives of 2,4- and 3,4-di- and 2,3,4,-tri-O-methylrhamnoses. These results showed that the rhmanose residues in PS-1 were linked not only by 1,3- but, partially, also by 1,2-glycosidic bonds.

Smith degradation also confirmed the presence of a rhamnan fragment in PS-1. PS-1 was oxidized with periodate (0.66 mole was consumed per anhydro unit), and the resulting polyaldehyde was reduced with tetrahydroborate to the corresponding polyalcohol. Only rhamnose was identified as a monosaccharide component in a hydrolysate of this polyalcohol. The polyalcohol was methylated by Hakomori's method, and in a hydrolysate of the permethylated polyalcohol we identified 2,4-di-O-methylrhamnose, which showed the presence of 1,3-glycosidic bonds between the rhamnose residues in the polyalcohol.

Thus, the polysaccharide moiety of the LPS from *M. laminosus* contained a rhamnan fragment with β-1,3- and, possibly, 1,2-glycosidic bonds between L-rhamnose residues. It was not excluded that the rhamnan plays the role of O-specific polysaccharide in the manifestation of the immunostimulating properties of the LPS under investigation.

EXPERIMENTAL

The total monosaccharide content was determined by the phenol–sulfuric acid method [9] and the amount of amino sugars by the Elson–Morgan method [10] after the preliminary hydrolysis of the bipolymers (4 mg) with 4 N hydrochloric acid (1 ml) at 100°C for 4 h; the amount of proteins was determined by Lowry's method [12], that of nucleic acids according to Spirin [12], and KDO by a method described previously [13]. IR spectra were taken on a UR-20 spectrophotometer and specific rotations were measured on a Perkin-Elmer 141 instrument.

Chromato-mass spectrometry was performed on LKB-900 instrument. The ^{13}C NMR spectrum of PS-1 was obtained on a KhM-250 instrument by V. V. Isakov.

The paper chromatography of the monosaccharides was carried out in the solvent system butanol–pyridine–water (6:4:3, by volume) using Filtrak-3 or -15. The paper electrophoresis of the monosaccharides was performed in pyridine–acetate buffer over 90 min (pH 4.5). The spots were indicated with the aid of aniline hydrogen phthalate, an alkaline solution of silver, or a 0.2% solution of ninhydrin in acetone (for amino sugars).

Gas-liquid chromatography (GLC) was performed on a Pye-Unicam 104 chromatograph with a flame-ionization detector in column 1 (0.4 × 150 cm) containing 3% of QF-I on Gas-Chrom Q (100-120 mesh). Monosaccharides were analyzed with the aid of GLC in the form of the corresponding polyol acetates [14] or aldononitriles [15] on column 1 with a rise in the temperature from 125 to 220°C at the rate of 5 deg/min; the rate of flow of carrier gas (argon and air) was 60 ml/min. Methylated sugars were analyzed with the aid of GLC in the form of the peracetates of the corresponding methyl glycosides by comparison with authentic samples using the same column. Fatty acids were investigated in the form of their methyl esters with the subsequent application of the method of chromato-mass spectrometry under the same conditions [16].

The culture of the blue-green alga *M. laminosus* was collected in thermal springs of Kamchatka. The collected biomass was filtered, and the dry residue was freed from pigments by extraction with ethanol and with acetone and was dried in the air.

Isolation of the LPS. Using Westphal's method [1] as described previously [3], 40 g of biomass was extracted with 45% aqueous phenol. The yield of total fraction amounted to 2.5% of the weight of air-dry cells. The total fraction obtained was centrifuged on an ultracentrifuge at 105,000 × g for 4 h. The precipitate that deposited was separated off and dissolved in water, and the solution was dialyzed and lyophilized. This gave a lipopolysaccharide with a yield of about 1% (on the dry weight of the alga) that contained 45% of neutral carbohydrates, 0.95% of protein, 2% of nucleic acids, and 10% of glucosamine.

Complete Hydrolysis. In a sealed tube, 3-5 mg of the LPS of PS-1 was heated with 1 N sulfuric acid (2 ml) at 100°C for 5 h. The mixture was neutralized with barium carbonate, deionized with KU-2 cation-exchange resin (H^+), and evaporated. The monosaccharides were identified with the aid of paper chromatography and GLC.

Partial Hydrolysis. The LPS (70 mg) was heated with 10% acetic acid (100 ml) at 100°C for 4 h. The lipid that precipitated was separated off by centrifugation, washed with water and with acetone, and dried in the air. The yield of lipid was 7.7 mg (12% of the weight of the LPS).

Hydrolysis of the Lipid. The lipid (4 mg) was hydrolyzed with 4 N hydrochloric acid (4 ml) at 100°C for 6 h. The hydrolysate was extracted with chloroform. The aqueous phase was investigated for its monosaccharide composition. The chloroform extract was evaporated to

dryness. The dried residue was esterified with 2 N hydrogen chloride in absolute methanol at 100°C for 7 h. The fatty acid methyl esters obtained were analyzed with the aid of GLC.

Investigation of the Polysaccharide Fraction. The supernatant solution after the separation of the lipid was freeze-dried. This gave the polysaccharide fraction. The mixture of polysaccharides obtained (56 mg) was chromatographed on a column (67 × 2.2 cm) of Sephadex G-50 with elution by pyridine-acetate buffer, pH 4.2 (4 ml of pyridine—10 ml of acetic acid—986 ml of distilled water). The process of separation was followed by the phenol—sulfuric acid method. The fractions corresponding to the peak on the elution curve were combined and freeze-dried. This gave a polysaccharide issuing immediately after the free volume of the column (PS-1), with a yield of 22 mg (39% of the load on the column), and a polysaccharide retained by the gel (PS-2) with a yield of 15 mg (26%).

Analysis by the Methylation Method. The PS-1 (9 mg) was methylated with methyl iodide in the presence of the methylsulfinyl carbanion by Hakomori's method [7]. The methylated product was purified by dialysis. The completeness of methylation was determined from the absence of an absorption band of hydroxy groups in the IR spectrum. The yield of permethylated PS-1 was 5 mg. It was hydrolyzed with 1 N hydrogen chloride in absolute methanol in a sealed tube at 100°C for 12 h. The resulting methyl glycosides were acetylated with acetic anhydride in pyridine at room temperature for 24 h and the products were investigated by GLC and chromato-mass spectrometry [8].

Periodate Oxidation. The oxidation of 20 mg of PS-1 was carried out with 20 ml of 0.015 M metaperiodate solution at room temperature for 48 h. The consumption of periodate amounted to 0.66 mole per anhydro unit. The resulting polyaldehyde was reduced with sodium tetrahydroborate at 20°C for 16 h. The excess of tetrahydroborate was decomposed with acetic acid, and the residue was evaporated to dryness with methanol. A polyalcohol (12 mg) was obtained, and 5 mg of this was hydrolyzed with 1 N sulfuric acid, after which rhamnose was identified as the sole monosaccharide in the hydrolysate with the aid of GLC.

The polyalcohol (7 mg) was methylated as described above. In a hydrolysate of the permethylated polyalcohol, 2,4-di-O-methylrhamnose was identified with the aid of GLC.

SUMMARY

1. From the blue-green alga *Mastigocladus laminosus* a lipopolysaccharide has been isolated in 1% yield which is distinguished by a low content of 3,6-dideoxyhexose and 2-keto-3-deoxyoctonic acid residues and by the complete absence of heptoses.

2. It has been shown that the LPS includes 12% of a lipid constructed of glucosamine, glucose, and fatty acid residues with a predominance of palmitic acid residues among the latter.

3. It has been found that the polysaccharide components of the LPS consists of a complex heteropolysaccharide composed of rhamnose, galactose, glucose, mannose, arabinose, and xylose residues, the amount of rhamnose substantially exceeding the amount of the other monosaccharides.

4. It has been established that the polysaccharide moiety of the LPS contains a rhamnan fragment constructed of β-1,3- and, possibly, -1,2-bound L-rhamnose residues.

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CARBOHYDRATES OF *Allium*.

IV. GLUCOFRUCTANS OF *Allium longicuspis*

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UDC 547.917

Water-soluble polysaccharides have been isolated from the bulbs of *Allium longicuspis* Rgl. By fractionating the combined water-soluble polysaccharides, homogeneous polysaccharides with molecular weights of 15,500 and 5100 have been isolated. It has been shown by spectral and chemical methods that they consist of glucofructans of a mixed type containing glycosidic bonds of the inulin (2 → 1) and levan (2 → 6) types in a ratio of 2.1:1.

Continuing investigations of the carbohydrates of plants of the family *Alliaceae* [1], we have studied the polysaccharides of *Allium longicuspis* Regel collected in the region of the R. Pskem (Uzbed SSR).

The communited raw material that had been treated with 96% ethanol to eliminate the ballast ethanol-soluble substances was extracted with 82% ethanol. Analysis of the combined ethanolic extracts (8.9% of the absolutely dry raw material) after purification and concentrated by paper chromatography (PC, system 1) showed the presence of glucose, fructose, sucrose, and fructooligosaccharides.

Subsequent extraction of the raw material with water gave water-soluble polysaccharides (WSPSs, 76.6% of the absolutely dry raw material). The isolated WSPSs consisted of a white hygroscopic powder readily soluble in water.

Gel chromatography on Sephadex G-75 showed that the WSPSs consisted of a polydisperse polymer the molecular weight of which ranged from 2000 to 40,000. To obtain a homogeneous fraction the initial WSPSs were fractionated by precipitation from water with ethanol, the results of this procedure being given below:

Fraction	Ethanol added †, ml	Yield, % total	Molecular weight	$[\alpha]_D^{22}$, deg
GF-I	1080	3.6	20,000—40,000	—
GF-II	1000	48.1	15500	−41(H_2O,1.0)
GF-III	1000	10.0	6,000—11,000	—
GF-IV	1900	5.1	5 00	−38(H_2O,1.0)
GF-V	—	15.0	2000—3500	—

On gel chromatography, fractions GF-II and GF-IV proved to homogeneous, and their IR spectra had absorption bands at 835, 870, and 945 cm^{-1} that are characteristic for glucofructans of the mixed type [2, 8].

In the products of the complete acid hydrolysis of GF-II and GF-IV, fructose and traces of glucose were identified by PC.

*Deceased.

†50 g of WSPSs in 500 ml of water.

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