SUMMARY

1. It has been established by chemical and spectral methods that the mannan from the bulbs of Ungernia oligostroma has a linear structure containing β-1 → 4-bound D-mannopyranose residues in the chain.

2. It has been established that from the x-radiographic point of view the mannan and its derivatives are amorphous. At the supermolecular level the mannan possesses an amorphous finely disperse structure with loose packing of the particles. In the acetylated mannan a tendency was observed to the formation of characteristic laminar structural elements with a higher degree of ordering of the chains. Such laminae may be fairly thin (down to 0.1-0.3 μm).

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# PRELIMINARY INVESTIGATION OF WATER-SOLUBLE POLYSACCHARIDES FROM GEORGIAN PLANTS

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Water-soluble polysaccharides have been isolated from nine representatives of the Georgian flora belonging to seven families. According to preliminary results two of them contained glucomannans and the others contained acid arabinogalactans. A more detailed study of a polysaccharide from the fruit of the mistletoe Viscum album has permitted it to be assigned to the class of pectin substances.

In the present paper we consider the results of a preliminary study of the polysaccharide compositions of some medicinal plants in the Georgian flora. Water-soluble polysaccharides of plant origin are of interest as potential biostimulators possessing immunomodulating and antitumoral activity [1-3].

The procedure for isolating the polysaccharide, which was the same for all the plants, was as follows. The plants, fixed after collection, were dried, comminuted, and treated with organic solvents to eliminate pigments and substances of low molecular mass. All the yields of polysaccharides were calculated on the defatted material obtained in this way. Then, to extract the polysaccharides, this material was treated successively with several portions of hot water, the extracts obtained were combined or, in some cases, they were studied separately, as shown in Table 1. The polysaccharide preparations from the aqueous solutions were isolated by fractional precipitation with increasing amounts of acetone [4]. Preparations A, B, and C were obtained, being precipitated with 1, 2, and 3

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TABLE 1. Polysaccharide Compositions of Some Higher Plants of Georgia

| Plant | Organ | Number of treatments with hot water | Method of combining the extracts | Fractions | Yield, % | Monosaccharides in the products of acid hydrolysis | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | arabinose | xylose | galactose | glucose | mannose | rhamnose | uronic acids |
| Family Lorantaceae | | | | | | | | | | | | |
| 1. Viscum album L., growing on a pear tree | Fruit | 5 | 1—5 | B | 7,5 | ++ | Tr. | + | | | + | + |
| | | | | C | 8,5 | ++++ | Tr. | ++ | | | + | ++ |
| | | | | D | 17,5 | +++ | Tr. | ++ | | | + | ++ |
| 2. Viscum album L., growing on an oak | Fruit | 5 | 1—5 | B | 14 | ++ | Tr. | + | | | + | + |
| | | | | C | 4 | ++++ | Tr. | ++ | | | + | ++ |
| | | | | D | 19 | +++ | Tr. | ++ | | | + | ++ |
| Family Liliaceae | | | | | | | | | | | | |
| 3. Polygonatum glaberrimum C. Koch. | Rhizomes | 4 | 1 | $B_1$ | 8,6 | | | Tr. | + | ++++ | | Tr. |
| | | | 2—3 | $B_{2-3}$ | 4,1 | ++ | Tr. | +++ | + | +++ | + | ++ |
| | | | 4 | $B_4$ | 0,9 | ++ | Tr. | +++ | | | + | ++ |
| 4. Muscari szovitsianum Baker. | Bulbs | 3 | 1—3 | A | 7,6 | +++ | Tr. | +++ | + | | + | + |
| | | | | B | 0,5 | +++ | Tr. | +++ | + | | + | + |
| | | | | C | 20 | + | | + | +++ | | Tr. | + |
| Family Crassulaceae | | | | | | | | | | | | |
| 5. Sedum caucasicum (Grossh.) Boriss. | Leaves | 4 | 1—4 | A | 10 | + | Tr. | + | +++ | | + | ++ |
| | | | | B | 0,5 | ++ | Tr. | ++ | ++ | | + | ++ |
| | | | | C | 0,2 | ++ | Tr. | ++ | ++ | | + | ++ |
| 6. Sempervivum caucasicum Rupr. ex Boiss. | Leaves | 3 | 1—3 | A | 4,2 | + | | ++ | ++++ | | | +++ |
| Family Boraginaceae | | | | | | | | | | | | |
| 7. Symphytum asperum Lepech* | Roots | 5 | 1-3 | $B_{1-3}$ | 10,2 | +++ | ++ | +++ | +++ | | + | ++ |
| | | | 4—5 | $B_{4-5}$ | 1,2 | +++ | Tr. | +++ | | | + | ++ |
| Family Portulacaceae | | | | | | | | | | | | |
| 8. Portulaca oleracea L. | Stems and leaves | 4 | 1—4 | B | 6 | ++++ | + | ++++ | Tr. | | + | ++ |
| Family Dioscoreaceae | | | | | | | | | | | | |
| 9. Tamus communis L. | Unripe fruit | 3 | 1—3 | A | 27 | | Tr. | + | ++ | ++++ | | Tr. |
| 10. Tamus communis L. | Ripe fruit | 4 | 1—2 | $A_{1-2}$ | 1,1 | ++ | + | +++ | ++ | ++ | + | +++ |
| | | | 3—4 | $B_{3-4}$ | 0,4 | + | + | +++ | ++ | ++ | + | +++ |
| Family Cucurbitaceae | | | | | | | | | | | | |
| 11. Ecbalium elaterium (L.) A. Rich.** | Viscous fruit juice | — | — | A | | Tr. | Tr. | | | | | +++ |
| | | | | B | | + | + | | | | | + |

*The protopectins and glucuronoxylans from the leaves and stems have been studied previously [11, 12].
**From 0.25 liter of juice were obtained 1.2 g of preparation 11A and 0.25 g of preparation 11B.

volumes of acetone, respectively (the absence of any of these fractions from Table 1 means that no precipitation took place from the given extract under the conditions shown). The differences in the monosaccharide compositions of these fractions or preparations from various extracts of a given plant enabled the heterogeneity of the polysaccharides obtained to be estimated. Sometimes a total preparation D was obtained by precipitation with 3 volumes of acetone. In the case of material 11 no defatting and extraction were performed; the polysaccharides were precipitated from the fruit juice after its dilution with water and filtration. The monosaccharide compositions of all the polysaccharide preparations obtained were determined, after acid hydrolysis, by paper chromatography (PC). The yields of the preparations and the results of qualitative analysis of the monosaccharide compositions are given in Table 1.

The majority of the preparations gave arabinose, galactose, and a uronic acid as the main monosaccharides on hydrolysis. On this basis, the corresponding polysaccharides can be assigned to the class of pectin substances. The ratio between the neutral monosaccharides and uronic acids in various samples ranged within wide limits: arabinose and galactose predominated in the hydrolysates of 1C, 2C, and 8B but were detected in only trace amounts in hydrolysate of 11A; the latter was obviously a practically pure homopolyuronan. The hydrolysis of preparations $3B_1$ and 9A gave as the main product mannose, so that these substances were representatives of a different group of polysaccharides. As far as concerns the glucose detected in the hydrolysates, it could have originated at least partially from starch. In actual fact, the treatment of samples 4, 5, 7, and 10 with salivary α-amylase led to the partial elimination of the components consisting of glucose. Amylolysis changed the amount of glucose in $3B_1$ only slightly, and this preparation was most probably a glucomannan (two mannans have been isolated previously from other species of Polygonatum [8-14]).

Considerable changes in the quantitative level and monosaccharide composition of the polysaccharides were detected during the ripening of the fruit of Tamus communis (samples 9 and 10). Preparation 9 did not change on amylolysis, while the treatment of preparation 10 with α-amylase led to the partial elimination of glucose. The first of these samples may be provisionally assigned to the class of glucomannans while the second consists of a complex polysaccharide of the pectin type.

The polysaccharides isolated from Viscum album were characterized in more detail (some of these results have been published in [15]). As can be seen from the figures given below, 1B, 1C, and 1D contained practical identical amounts of uronic acids and differed from one another only by the ratio of neutral sugars:

| Polysaccharide | $[\alpha]_D^{23}$, deg (water) | Rhamnose: arabinose: galactose, molar | Uronic acids, % | N, % |
|---|---|---|---|---|
| 1B | −40 (*c* 0.25) | 1:2:1 | 21.8 | 1.8 |
| 1C | −43 (*c* 0.28) | 1:4.4:1.6 | 19.5 | 1.25 |
| 1D | −41.4 (*c* 0.29) | 1:2.6:1.2 | 20.8 | 2.1 |

The difference in the ratios of rhamnose and uronic acid (1:1 for 1B, and 1:1.5 for 1C) probably explains the difference in their solubilities. The negative value of the optical rotation permits the assumption of the presence of β-D-galactose and α-L-arabinose residues in the polysaccharides. The presence of nitrogen in the mistletoe preparations led to the assumption that they contain protein as an impurity or covalently bound to the polysaccharide. It is known that together with arabinogalactans arabinogalactan-proteins are found in plants [16]. However, we did not concern ourselves with the elucidation of this question.

On the fractionation of 1B and 1C by ion-exchange chromatography on a column containing DEAE-cellulose in the carbonate form [17] we succeeded in separating water-eluted neutral components with yields of 2.9 and 7%, respectively. The first contained arabinose and, in smaller amounts, galactose and xylose and traces of rhamnose, while the second contained arabinose, galactose, and, in smaller amounts, xylose and rhamnose.

In the salt eluates from 1B and 1C extended peaks of polysaccharides containing uronic acids and, as neutral monosaccharides, arabinose and galactose were detected, their ratio in various fractions being approximately constant and close to those for the initial preparations. These results show that 1B and 1C contained mainly arabinogalactans.

To determine the nature of the uronic acid, preparation 1D was subjected to stepwise hydrolysis, first with concentrated and then with dilute sulfuric acid [18]. After the separation of the neutral and acidic monosaccharides on an anion-exchange resin and the reduction of the acids to polyols [19], dulcitol was detected by GLC, which showed the presence of galacturonic acid in the initial polysaccharide. The results of qualitative and quantitative analysis of the monosaccharide compositions of the mistletoe polysaccharides were similar to those recently published by other authors [20]. They permit the water-soluble polysaccharides of the fruit of Viscum album to be assigned to the class of pectin substances.

Thus, the plants investigated by the authors of the present paper can serve as a source of various polysaccharides. A more detailed study of the chemical structure of some of these polysaccharides and the determination of their biological activities will be the subject of our subsequent communications.

## EXPERIMENTAL

Paper chromatography was carried out on Filtrak FN No. 11 paper (GDR) by the descending method in the solvent system butan-1-ol–pyridine–water (96:4:3). The zones of the reducing sugars on the paper were detected with acid aniline phthalate. Photocolorimetric determination was performed on a KFK-2 concentration photoelectric colorimeter. Spectrophotometric determinations were made by SF-4A spectrophotometrometer. The GLC of polyol acetates was performed on a Pye-Unicam 104 chromatograph with a flame-ionization detector (0.6 × 120 cm column with 3% of ECNSS-M, Gas-Chrom Q, 180°C), rate of flow 60 ml/min. Specific rotations were measured on an Al-EPO photoelectronic polarimeter (VNIIÉKIprodmash [All-Union Scientific-Research and Experimental Design Institute of Food Machinery Construction]). Total sugars were determined by the reaction with phenol–$H_2SO_4$ and were calculated as arabinose [21], while uronic acids were determined with the m-hydroxybiphenyl reagent and were calculated as galacturonic acid [22]. Nitrogen was determined by the Kjeldahl method using the Nessler reagent [23].

To determine its monosaccharide compositions 5-10 mg of a polysaccharide was heated in 1 ml of 2 N $H_2SO_4$ at 100°C for 5 h, the hydrolysate was neutralized with $BaCO_3$ and was filtered, deionized with KU-2 cation-exchange resin ($H^+$ form), and investigated by PC. For analysis by the GLC method, the sugars were converted into polyol acetates [24].

Collection and Preliminary Treatment of the Plants. Sample No. 1 was collected in December 1985, No. 2 in December, and Nos. 3 and 9 in July, 1986, and No. 10 in September, 1987, in the Mtskheta region; No. 4 in April, Nos. 5 and 6 in June, and No. 11 in July, 1986, in the environs of Tbilisi; No. 7 in June, 1986, in the Borzhomi region; and No. 8 in July, 1986, in the Lagodekhi region. After collection, the plants were fixed with ethanol, dried in the air, and comminuted and were treated successively in a Soxhlet apparatus with chloroform, methanol, and acetone until colorless extracts had been obtained and were then redried in the air.

Isolation of the Monosaccharides. The material that had been extracted with organic solvents as described above was treated 3-5 times with water at 100°C (liquor ratio 1:50) with stirring and replacement of the solvent every 2-6 h. The extracts were separated by centrifugation and were filtered on a Büchner funnel, concentrated in vacuum at 40°C, and dialyzed against distilled water. The dialysates were filtered again and were concentrated, and an equal volume of acetone was added. The resulting precipitate of fraction A was separated by centrifugation and was washed several times with acetone and dried in vacuum over $P_2O_5$. To the mother solution was added a second and, after the separation of the precipitate of fraction B, a third volume of acetone. The precipitates of fractions B and C were washed and dried as described above. In the case of sample 11, the juice of the fresh fruit was diluted 5- to 6-fold with hot water, filtered through a Büchner funnel, and dialyzed, and the polysaccharides were precipitated as described above. The yields of the fractions for all the samples and their monosaccharide compositions are given in Table 1.

Amylolysis of the Polysaccharides [6]. An aqueous solution of 50 mg of glucose-containing polysaccharide was treated with 1.5 ml of 0.2 M acetate buffer, pH 7.0, 1 ml of a solution of amylase (obtained by diluting saliva with an equal volume of water and centrifuging), and water to 25 ml, and the mixture was incubated at 35°C for 20 h and was then heated at 100°C for 5 min and was cooled. Half the solution was evaporated and the polysaccharides

were precipitated with 10 volumes of ethanol. The precipitate was separated off by centrifugation and the mother solution was evaporated, treated with KU-2 cation-exchange resin ($H^+$), filtered, evaporated, and investigated by PC. The second half was dialyzed and evaporated in vacuum, and the residue was hydrolyzed and studied by PC.

Fractionation of Polysaccharides 1B and 1C on DEAE-Cellulose. A solution of 200 mg of 1B or 1C in 15 ml of water was deposited on a 3 × 28 cm column of DEAE-cellulose (bead polymer, Reanal) in the carbonate form. The column was washed with 1 liter of water and then with 700 ml of $(NH_4)_2CO_3$ solution with a concentration rising linearly from 0 to 0.5 M and with 1 liter of 1 M $(NH_4)_2CO_3$, 10-ml fractions being collected, which were analyzed for the presence of sugars as described above. In the aqueous eluates of 1B and 1C a zone of neutral polysaccharides was detected with yields of 2.9% (composition: arabinose and smaller amounts of galactose, glucose, and xylose with traces of rhamnose) and 7% (arabinose and galactose and smaller amounts of xylose and rhamnose), respectively. In the salt eluates of 1B and 1C extended zones of acid polysaccharides were detected with yields of 58 and 62% (the ratios of arabinose and galactose in the various fractions were close to those in the initial preparations).

Identification of the Uronic Acid. A solution of 11 mg of preparation 1B in 0.1 ml of 72% $H_2SO_4$ was left for 1 h and was then treated with 2.8 ml of water and heated at 100°C for 3 h. After cooling, it was neutralized with $BaCO_3$, the filtrate was evaporated, the residue was dissolved in 1 ml of 1 N $NH_4OH$, 10 mg of $NaBH_4$ was added, and after 16 h the reaction mixture was acidified with AcOH, and the $H_3BO_3$ was eliminated by distillation with methanol. An aqueous solution of the residue was treated with Dowex 1×1 ($AcO^-$), and the resin was separated off and washed with water; it was stirred with 2 ml of 1 N HCl for 30 min, the filtrate was evaporated, the residue was dried in vacuum over KOH and was then dissolved in 1 ml of 0.01 M borate buffer, pH 7.5, and it was reduced with $NaBH_4$ as described above. After acetylation, dulcitol hexaacetate was identified by GLC.

## SUMMARY

Water-soluble polysaccharides have been isolated from nine representatives of the Georgian flora belonging to seven families. According to preliminary results, two of the samples contained glucomannans and the others contained acidic arabinogalactans. A more detailed study of the polysaccharide from the fruit of the mistletoe Viscum album has permitted it to be assigned to the class of pectin substances.

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# CHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF THE LIPOPOLYSACCHARIDES OF Yersinia kristensenii SEROVARS 0:12,25; 0:12,26; and 0:25,35

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A comparative and immunochemical characterization of the lipopolysaccharides of three serovars of Y. kristensenii has been performed and the ratios of the monosaccharides have been established. The results of the immunochemical investigations confirmed the serotyping of the microorganisms.

In recent years, from Yersinia enterocolitica – a microorganism causing an infectious disease of the gastrointestinal tract with a complex chemical pattern – another three types of Yersinia have been isolated on the basis of biochemical characteristics and DNA-DNA-hybridization: Y. kristensenii, Y. frederiksenii, and Y. intermedia [1]. No classification has been developed for these species and there is no information whatever on the structural investigation of the antigens.

In the present paper we give the results of a comparative chemical and immunochemical study of the lipopolysaccharides isolated from Y. kristensenii, serovar 0:12,26 (strain 103), 0:12,25 (strain 490), and 0:25,35 (strain 1647) which have common O-antigens. The lipopolysaccharides were extracted from an acetone powder of the microbial mass with aqueous phenol by Westphal's method [2]. The nucleic acids were separated by precipitation with Cetavlon. Table 1 gives analytical results for the lipopolysaccharides obtained. The smallest yield of lipopolysaccharide was obtained from the serovar 0:25,35, and it was distinguished by a low KDO content.

To establish their monosaccharide compositions, the lipopolysaccharides were subjected to acid hydrolysis, and the hydrolysates, in the form of polyol acetates, were investigated by PC and GLC. An amino acid analyzer was used to determine aminosugars. The ratios of the monosaccharides are given in Table 2. All the lipopolysaccharides had the same qualitative monosaccharide composition, including residues of D-glucose, D-galactose, D-glycero-D-mannoheptose, L-glycero-D-mannoheptose, fucosamine, galactosamine, and glucosamine.

All the lipopolysaccharides possessed serological activity. The results of cross-precipitation in the test system are shown in Fig. 1. All the lipopolysaccharides gave one distinct precipitation band with the homogeneous antiserum, but for the serovar 0:12,25 system another, weaker, precipitation band was observed which formed a spur from the precipitation band for the serovor 0:12,26 system.

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