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A BIOGLYCAN FROM THE GASTROPOD MOLLUSC Rapana thomasiana

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A bioglycan — rapanan — has been isolated from the mantle of the gastropod mollusc Rapana thomasiana, the main carbohydrate chain of which is constructed of α-(1 → 4)-bound D-glucose residues with a small number of side chains attached by α-(1 → 6)-glucosidic bonds to the main chain of the bioglycan.

In recent years, the urgent attention of research workers has been attracted by bioglycans (polysaccharides and glycoconjugates) from marine organisms, which possess a pronounced immunostimulating action [1].

In the present paper we consider the results of a study of a bioglycan isolated with a yield of 4-5% from the mantle of the gastropod mollusc Rapana thomasiana collected in the Azov—Black Sea basin and in the Sea of Japan.

The bioglycan, which was obtained by the aqueous salt extraction of the mantle of Rapana thomasiana after dialysis and lyophilization, has been called rapanan. In a hydrolysate of it only D-glucose was detected and, therefore, rapanan is a D-glucan. The amount of protein in rapanan does not exceed 1% and it is apparently possible to eliminate the protein completely. According to the results of gel filtration on Sephadex G-100 and Sepharose 6B, rapanan is a high-molecular-weight bioglycan with a molecular weight exceeding 2 MD. The high positive angle of rotation, $[\alpha]_D$ +154° (c 0.1; water), indicates the α configuration of the glycosidic bonds.

The action of α- or β-amylase on rapanan gave a mixture of oligosaccharides and partially degraded glucan. The latter was studied with the aid of paramagnetic spectroscopy and ^{13}C nuclear magnetic resonance. In the PMR spectrum, two broadened signals were observed in the region of the resonance of anomeric protons at δ 5.48 and 5.09 ppm with an integral ratio of 8:1, respectively.

In the ^{13}C NMR spectrum there were likewise two signals in the region of anomeric C atoms at δ 100.6 and 99.3 ppm, in the same integral ratio. The ring C-atoms participating in the formation of the glycosidic bond resonated at 78.6 and 68.2 ppm. These results showed, according to literature information [2, 3], that the glucan under investigation had α-(1 → 4)- and α-(1 → 6)-glucosidic bonds in an approximate ratio of 8:1.

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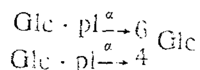
The oligosaccharide fraction obtained on the enzymatic hydrolysis of rapanan was separated with the aid of preparative paper chromatography into two zones corresponding to a disaccharide A, R_{Gal}, 0.80, $[\alpha]_D^{20}$ +60° (c 0.15; water) and a trisaccharide B, R_{Gal} 0.65, $[\alpha]_D^{20}$ +88° (c 0.21; water). The oligosaccharides obtained were subjected to exhaustive methylation followed by complete hydrolysis. Analysis of the hydrolysates in the form of the corresponding acetates of methyl glycosides with the aid of gas-liquid chromatography (GLC) and chromato-mass spectrometry (GLC-MS) enabled 2,3,4,6-tetra- and 2,3,6-tri-O-methylglucoses to be identified in that from disaccharide A, and 2,3,4,6-tetra- and 2,3-dimethylglucoses in that from trisaccharide B. Thus, disaccharide A was maltose, and the branched trisaccharide B probably had the following structure:



These facts indicate the presence of (1 → 4)- and (1 → 6)-glucosidic bonds in rapanan. The result obtained was confirmed by a study of rapanan by the methylation method. In a hydrolysate of permethylated rapanan, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-O-methylglucoses were identified in the form of the corresponding methyl glycoside acetates, the 2,3,6-tri-O-methyl derivative largely predominating in the mixture.

The Smith degradation of rapanan confirmed the results obtained. In a hydrolysate of the polyalcohol obtained on the periodate oxidation of rapanan followed by reduction with tetrahydroborate, glycerol and erythritol, were identified in a ratio of about 1:9. No glucose was found. The formation of erythritol showed the presence of (1 → 4)- and (1 → 6)-bonds between the glucose residues and the glycerol was formed on the oxidation of the terminal residues.

Thus, rapanan from R. thomasiana found in the Sea of Japan and in the Azov—Black Sea basin consists of a high-molecular-weight D-glucan having a main carbohydrate chain of α-(1 → 4)-bound D-glucose residues with a small number of side chains attached to the main chain by α-(1 → 6)-glucosidic bonds.

EXPERIMENTAL

The monosaccharides and oligosaccharides were chromatographed on Filtrak-12 or -15 paper in the solvent system butanol—pyridine—water (6:4:3). The indication of the spots was achieved with the aid of aniline hydrogen phthalate or an alkaline solution of silver nitrate.

Gas-liquid chromatography was performed on a Pye-Unicam 104 chromatograph (United Kingdom) with a flame ionization detector, using a column (0.4 × 150 cm) filled with Gas-Chrom-Q (100-120 mesh) impregnated with 3% of QF-1.

The monosaccharides were analyzed in the form of the corresponding polyol acetates [4] with a rise in the temperature from 125 to 220°C at the rate of 5 deg/min; the rates of flow of the carrier gas (argon) and of air were 60 ml/min. The methylated sugars were analyzed in the form of the peracetates of the corresponding methyl glycosides under the same conditions [5]. Chromato-mass spectrometry was performed on a LKB-9000 instrument (Sweden) using the same column as for GLC.

The total amount of monosaccharides was determined by the phenol/sulfuric acid method [6], and the protein content was established by Lowry's method [7].

PMR and ^{13}C NMR spectra were obtained on a Bruker WM-250 instrument with working frequencies of 250 and 62.9 MHz. The samples were investigated in the form of solutions in D_2O at 80°C, ethanol being used as internal standard. Chemical shifts are given relative to tetramethylsilane. For the PMR spectra, the signal of methanol was taken as δ 3.47 ppm, and in the ^{13}C NMR spectra the chemical shift of methanol was taken as δ 49.6 ppm relative to tetramethylsilane.

Isolation of Rapanan. The mantle was separated from the freshly trapped mollusc and was either treated immediately or was stored in the freeze-dried form. The rapanan was obtained by the aqueous salt extraction of the mantle, dialysis, and lyophilization, with a yield of about 5% on the crude weight of the freshly prepared mantle.

Complete Hydrolysis. Rapanan (7 mg) was heated with 1 N sulfuric acid (3 ml) in a sealed tube at 100°C for 5 h. The hydrolysate was neutralized with barium carbonate and was deionized with KU-2 cation-exchange resin [H^+] and evaporated. The monosaccharides were identified with the aid of PC and GLC.

Action of α- or β-Amylase. A solution of 160 mg of rapanan in 20 ml of distilled water was treated with 700 μg of α- or β-amylase. After the addition of 1 ml of toluene, the resulting mixture was incubated at 37°C for 3 h. Then it was heated in the boiling water bath for 10 min to inactivate the enzyme and was filtered, evaporated to small volume, and poured into ethanol (four volumes). The precipitate that deposited was separated off and freeze-dried.

A high-molecular-weight fraction of degraded glucan was obtained with a yield of 70 mg (about 44%). The supernatant was evaporated to dryness. As a result, a mixture of glucose and oligosaccharides was obtained (yield 85 mg), and this was separated with the aid of preparative PC. The corresponding zones were eluted with water and the eluates were evaporated. This gave disaccharide A (maltose), R_{Gal} 0.8 $[\alpha]_D^{20}$ +60°, yield 22 mg, and trisaccharide B, R_{Gal} 0.65, $[\alpha]_D^{20}$ +88°, yield 19 mg.

Analysis by the Methylation Method. Rapanan (17 mg) and the oligosaccharides (10 mg) were methylated with methyl iodide in the presence of the methylsulfinyl carbanion by Hakomori's method [8]. The completeness of methylation was determined from the absence of an absorption band of a hydroxy group in the IR spectrum. The completely methylated compounds were treated with 1 N hydrogen chloride in absolute methanol in a sealed tube at 100°C for 6 h. The methyl glycosides obtained were acetylated with acetic anhydride in pyridine and were analyzed by GLC in comparison with markers and by chromato-mass spectrometry.

Smith Degradation of Rapanan [9]. Rapanan (220 mg) was kept in 0.1 M sodium metaperiodate solution (33 ml) in the dark at 20°C for 10 days. The polyaldehyde so obtained was treated with sodium tetrahydroborate (200 mg), and after 16 h the solution was acidified with acetic acid to pH 5-6 and was evaporated to dryness with methanol. This gave a polyalcohol which was then oxidized under the same conditions. The final yield of polyalcohol was 100 mg (45%). Of the polyalcohol, 5 mg was hydrolyzed with 1 N sulfuric acid and the products were analyzed by paper and gas-liquid chromatography. Glycerol (10%) and erythritol (90%) were identified in the hydrolysate.

SUMMARY

A high-molecular-weight bioglucan which has been called rapanan has been isolated from the mantle of the gastropod mollusc Rapana thomasiana collected in various regions of the Sea of Japan and the Azov—Black Sea basin.

By means of hydrolysis, enzymatic cleavage, methylation, and Smith degradation it has been shown that rapanan is a D-glucan having a main carbohydrate chain consisting of α-(1 → 4)-bound D-glucose residues with a small number of side chains attached by α-(1 → 6)-glucosidic bonds to the main chain of the bioglucan.

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