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Note

Studies on anticoagulant-active arabinan sulfates from the green alga, *Codium latum*

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Among the various kinds of sulfated polysaccharides in the marine algal cell wall, blood anticoagulant activities similar to heparin have been reported for some of the sulfated polysaccharides such as fucoidan sulfate from Phaseophyta[1]. Since no heparin analogues (heparinoids) that are inhibitory to thrombin activities have been reported thus far from Chlorophyta, the distribution of antithrombin-active polysaccharides in these algae were surveyed[2]. Although the sugar constituents of water-soluble crude polysaccharides were found to differ from each other, and there were no chemotaxonomical correlations between activities and algal species, a common feature was that all the active compounds showed some sulfate ester content. In the crude, active polysaccharides surveyed, active rhamnan sulfate was found in the hot-water extracts of *Monostroma nitidum*[2]. This product was purified until it exhibited about seven times the activity of standard heparin; however, its lipid-clearing activity was less than that of heparin when injected intravenously into rats[3]. On the other hand, the cold-water extracts from *Codium latum* also showed remarkable activity. The polysaccharide so obtained was characterized as an arabinan sulfate after purification by anion-exchange chromatography, gel-filtration chromatography, and fractional precipitation with dilute potassium chloride solution. Herein, is described the isolation of the purified arabinan sulfate as well as some chemical properties that correlate with its observed activity.

After soaking and suspending the dried algae in ten volumes of distilled water, the mixture was homogenized and kept at room temperature for 1 h. Supernatants from centrifugation were dialyzed with tap water, then with distilled water, giving

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upon lyophilization the crude polysaccharides. Since the crude biomass had been extracted only by cold water, the active polysaccharides seem to be those that are more loosely associated with the cell wall. This is in contrast to those from *Monostroma* that are obtained only by more vigorous procedures such as hot-water extraction after a cold-water extraction. Relative anticoagulant activity, measured as delayed clotting time (fibrin formation from fibrinogen by the inhibition of thrombin[4]) for the crude polysaccharide in physiological saline solution, was determined to be 2.4 × that of standard heparin. This activity was increased to 3.4 × and 5.1 × that of heparin respectively by purification of the polysaccharide by sequential chromatography over DEAE-cellulose (Whatman DE-52) and Toyopearl gel-filtration media. The major sugar components in the hydrolysate of the crude polysaccharide were found to be galactose and arabinose in a ratio of 1:1.9 by measurement of the GLC peak area relative to a standard graph of alditol per-*O*-trifluoroacetate from 2-deoxy-D-*arabino*-hexose (2-deoxy-D-glucose); however, in the course of the purification, the arabinose content was shown to increase relative to that of galactose. Further purification by precipitation of the polysaccharide by addition of solid KCl until 0.2 M concentration to the eluate from the Toyopearl gel filtration procedure, followed by centrifugation, redissolving the polysaccharide in water, dialysis, and lyophilization, gave a product that showed an anticoagulant activity of 9.5 × that of heparin. No activity was detected in the supernatants from the purification. Direct precipitation of the polysaccharide by addition of solid KCl until 0.2 M concentration to a solution of the crude polysaccharide also gave an active polysaccharide.

Gel-filtration chromatography of the active polysaccharide through an analytical column of Toyopearl HW-65 (fine) revealed a sharp, single, symmetrical peak that suggests homogeneity in terms of molecular size. This result, coupled with the fact that the compound migrated toward the cathode in cellulose acetate strip electrophoresis as a single band [using 0.1 M acetate buffer (pH 7.0) with detection using Toluidine Blue], indicates that the compound is a homogeneous, charged polysaccharide. The anomeric resonance (a doublet) in the ^{1}H NMR spectrum at δ 5.240 is in agreement with the assignment as the α-L-configuration (see in the following paragraph the assignment of the sugar configuration). In the IR spectrum, typical absorbances type A (symmetrical ring breathing frequency), types B and C (stretching modes for substituents), and type D (C–H deformation for an H-atom attached to a C-atom that is directly attached to a ring oxygen atom) for a furanose ring system[5] were observed at 920, 880, 843, and 810 cm^{-1}, respectively.

By TLC, HPLC, and GLC identification of the components of the hydrolysate using co-chromatography with authentic samples, the sugar constituent of the purified polysaccharide was determined to be solely arabinose {$[\alpha]_D$ + 190.6° (5 min) → +104.5° (equil.) (*c* 3.0, H_2O)}; however, a considerable amount of galactose along with very small amounts of mannose and glucose were found in the supernatants. The ratios of Gal:Man:Glc were determined to be 25.2:1:2 by GLC analysis of the alditol per-*O*-trifluoroacetates.

Crystalline arabinose diphenylhydrazone obtained from the hydrolyzate of the purified arabinan melted at 184–186°. (Compare the known L-arabinose diphenylhydrazone with mp 184–185° and D-arabinose diphenylhydrazone with mp 189–193°.)[6] A mixture of the arabinose diphenylhydrazone from the polysaccharide with authentic L-arabinose diphenylhydrazone melted at 184–186° (no mp depression), while the mixed mp with D-arabinose diphenylhydrazone was depressed to mp 170–172°. Thus collectively these results show that the arabinose obtained from the *Codium* arabinan is L-arabinose. Quite similar results were obtained for the L-arabinose 2,5-dichlorophenylhydrazone derivatives[6].

The sulfate content was determined by the peak area of the S=O stretching vibration at 1240 cm^{-1} in the IR spectrum and by the rhodizonic acid reagent[7] to be 20.4 and 20.7%, respectively. Elimination of the sulfate ester by selective solvolysis in Me_2SO (ref. 8) was achieved without cleavage of the glycosidic linkages. During the desulfation, correlations between activity and sulfate content was determined at intermediate reaction times. While with completely desulfated arabinan {$[\alpha]_D$ −21.6°} all anticoagulant activity was lost, activity was retained in samples whose sulfate content was ≥ 8%. In the ^{13}C NMR spectrum, the chemical shifts for the desulfated, neutral polysaccharide were assigned as follows: δ 106.15 (C-1), 82.0 (C-4), 81.6 (C-2), 77.5 (C-3), and 66.2 (C-5). These assignments are in agreement with those reported for a synthetic (1 → 5)-α-L-arabinan[9] or one isolated from *Pinus densiflora*[10]. The apparent molecular weight of the desulfated arabinan as determined by gel-filtration, using Shodex P-82 (a pullulan from Showa Denko Co., Ltd.) for calibration, was determined to be 2.75×10^5. This very high molecular weight, coupled with the fact that the desulfated arabinan was less soluble in water than was the sulfated polysaccharide, seems to be due to an increase in intermolecular hydrogen bonding in the desulfated species, which is much as has been proposed for the interaction of the (1 → 3)-β-D-xylan and cellulose in the cell-wall polysaccharide of *Bryopsis maxima*[11].

Detailed structural studies on the uncommon polysaccharides thus obtained, as well as their distribution in the algae of genus *Codium*, are progressing in this laboratory.

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