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Studies on the κ-Polysaccharide of the Red Seaweed *Gigartina Tenella*[*1]

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The polysaccharide extracted from the red seaweed *Gigartina tenella* has been separated into κ- and λ-polysaccharide by potassium chloride. The former polysaccharide is a gelling component precipitated with potassium chloride, while the latter is a non-gelling component insensitive to the precipitant. Complete hydrolysis and methanolysis, together with quantitative determination, have shown that the κ-polysaccharide is composed mainly of D-galactose, 3, 6-anhydro-D-galactose, and an ester sulfate in the molar proportion 1 : 0.98 : 1.17. Very small amounts of L-galactose and D-xylose have also been isolated from the products of hydrolysis. The partial methanolysis of the κ-polysaccharide yielded dimethyl acetal of carrabiose (4-*O*-β-D-galactopyranosyl-3, 6-anhydro-D-galactose) in a good yield. It follows from these results that this disaccharide is a chief repeating unit of the carbohydrate moiety of the polysaccharide, as in the case of κ-carrageenan.

Gigaritina tenella[1] is one of the most common red seaweeds along the coastlines of Japan and Korea. It contains a water-soluble, mucilageous polysaccharide which is utilized as a thickener for wall plasters. However, little has been known of its chemical structure. On the other hand, carrageenan, a polysaccharide extracted from the red seaweed *Chondrus crispus*, consists of at least two distinct components, termed κ- and λ-carrageenan.[2] Both of these components are highly sulfated galactans, and both have been extensively investigated.[3-5] The present paper will report that the polysaccharide of *G. tenella* can also be separated into κ- and λ-polysaccharide, and that the κ-polysaccharide is an analogue of κ-carrageenan in properties, in composition, and in repeating units.

The κ- and λ-polysaccharide were separated by potassium chloride in a manner similar to that used in the case of carrageenan,[6] thus giving their potassium salts in 23% and 13% yields respectively.

In order to examine the sugar components, the κ-polysaccharide was subjected to complete hydrolysis. The hydrolysates were separated into neutral and acidic products using ion exchange resins. The further separation of the neutral products by crystallization, chromatography, and fermentation resulted in the isolation of crystalline D-galactose in a 33% yield, crystalline L-galactose in a 0.6% yield, and syrupy D-xylose in a 0.2% yield. No evidence was obtained to indicate the presence of 6-*O*-methyl-D-galactose, which occurs occasionally in certain red seaweed polysaccharides.[7] The only acidic product detected on paper chromatograms was levulinic acid, which had formed from 3, 6-anhydro-galactose during the acid hydrolysis.[8] The presence of the anhydrosugar was demonstrated by the complete methanolysis of the κ-polysaccharide, followed by the chromatographic separation; this resulted in the isolation of 3, 6-anhydro-D-galactose dimethyl acetal. It has thus been proved that the κ-polysaccharide is composed mainly of D-galactose and 3, 6-anhydro-D-galactose. Although L-galactose and D-xylose were obtained in low yields from the hydrolysates, no conclusion has been reached on whether these sugars were components or contaminants.

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2) D. B. Smith and W. H. Cook, *Arch. Biochem. Biophys.*, **45**, 232 (1953).

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4) D. A. Rees, *J. Chem. Soc.*, **1963**, 1821.

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Quantitative determination showed that the molar ratio of D-galactose, 3, 6-anhydro-D-galactose, and sulfate was 1 : 0.98 : 1.17. This composition is in agreement with the structure recently suggested by Anderson and his associates for κ-carrageenan.⁹⁾

The κ-polysaccharide was then examined by partial methanolysis in order to isolate oligosaccharide fragments. The neutral products of the methanolysis amounted to 71% of the polysaccharide. This figure may reasonably account for the whole polysaccharide sample when sulfate and the potassium contents are subtracted. The neutral products, when separated on a charcoal-Celite column, afforded dimethyl acetal of carrabiose (4-*O*-β-D-galactopyranosyl-3, 6-anhydro-D-galactose) in a 65% yield.

The isolation of this disaccharide derivative shows that β-D-galactopyranosyl residues are linked with the 4 positions of the anhydro-sugar residues in the polysaccharide molecule. The high yield of this compound indicates that the disaccharide is a chief repeating unit of the carbohydrate moiety of the polysaccharide, as in the case of κ-carrageenan.

Experimental

General and Analytical Methods. The solutions were evaporated under reduced pressure at 40°C. Paper chromatograms, using Toyo Filter Paper No. 51, were developed with a 1-butanol - acetic acid - water (4 : 1 : 2 v/v) mixture in the ascending manner and sprayed with (a) an aniline hydrogen phthalate solution¹⁰⁾ in order to locate reducing sugars or (b) *o*-aminophenol in aqueous ethanol acidified with phophosphoric acid¹¹⁾ in order to locate the products of methanolysis. The galactose and 3, 6-anhydro-galactose contents were estimated by the colorimetric method(B) of Yaphe,¹²⁾ using anthrone and resorcinol reagents. The sulfate content was estimated by hydrolysing the polysaccharide with 2 N hydrochloric acid at 100°C for 6 hr and by then determining the liberated sulfuric acid by the gravimetric method in the usual way. The result was calculated as SO_3K. All the analyses were carried out twice; the average values are given below.

Preparation of Polysaccharide. An air-dried seaweed sample (30 g), collected in Korea, was heated with water (2 *l*) in a boiling water bath for 3 hr while being occasionally stirred. The mixture was then centrifuged, and the residue was extracted twice with water (1 *l*). Into the combined centrifugates, potassium chloride was stirred to a final concentration of 0.2 M, after which the mixture was cooled to 10°C. The gelatinous precipitate was separated by centrifugation and washed twice with a 0.2 M potassium chloride solution. The precipitate was then dialysed against running water to complete the dissolution (72 hr). The solution was diluted with water to 2 *l*, after which potassium chloride was again added and the solution was treated in the same manner as has been described above. The final dialysed solution was centrifuged, evaporated to 500 m*l* and poured into ethanol (1.5 *l*). The precipitates were collected by centrifugation, washed successively with ethanol and acetone, and dried in a vacuum at 40°C. The potassium salt of κ-polysaccharide obtained forms colorless powder; yield, 7.0 g; $[\alpha]_D^{24}$ +60.0° (*c* 0.50, water). The following analytical values were obtained: SO_3K, 29.0; galactose (as $C_6H_{10}O_5$), 33.9; 3, 6-anhydro-galactose (as $C_6H_8O_4$), 29.4; protein (Kjieldahl method), 0.6%.

The supernatants and washings from the centrifugation of the crude κ-polysaccharide were combined and evaporated to 1 *l*, dialysed against running water until free from chloride, evaporated to 300 m*l*, and then poured into ethanol (900 m*l*). The resulting precipitate of λ-polysaccharide was isolated in the same manner as κ-polysaccharide, thus forming slightly colored fibrous powders; yield, 4.0 g.

Hydrolysis. The κ polysaccharide (20 g, moisture 16.3%) was heated with N sulfuric acid (300 m*l*) for 20 hr in a boiling water bath. The solution was neutralized with barium carbonate and filtered. The filtrate was deionized by Amberlite IR-120 (600 m*l*) and Amberlite IR-45 (600 m*l*) and evaporated to a syrup (6.1 g). Paper chromatography with the reagent (a) showed the presence of galactose and a trace of xylose. Acidic products, which had been retained by the anion resin, were recovered by elution with 2 N acetic acid. Paper chromatography with the reagent (a) or (b) showed the absence of uronic acid, but with the aniline-xylose reagent,¹³⁾ levulinic acid was detected.

D-Galactose. The crystallization of the syrup obtained above afforded chromatographically-pure galactose (5.6 g). Recrystallization from methanol gave pure D-galactose (4.7 g); mp and mmp 163°C, $[\alpha]_D^5$ +80.0° (final value, *c* 0.73, water).

The evaporation of the filtrate of recrystallization afforded a mixture of D-galactose (0.76 g) and its L-isomer (0.04 g), the proportion of the D- and L-compound being calculated from the optical rotation.

D-Xylose. The filtrate from crude galactose described above was evaporated to a syrup (0.45 g), which was then chromatographed on a cellulose column (3×40 cm) with 1-butanol - water (6 : 1 v/v). The faster-moving component was obtained as a syrup (0.04 g), which gave, on treatment with Breddy and Jones' reagent (1 m*l*),¹⁴⁾ di-*O*-benzylidene-D-xylose dimethyl acetal; mp 208°C, $[\alpha]_D^4$ −6.4° (*c* 0.94, chloroform).¹⁴⁾ The melting point was not depressed on admixture with an authentic sample.

L-Galactose. The fractions following the xylose fractions described above were combined and evaporated to a syrup (0.19 g) consisting of D-galactose (0.13 g) and its L-isomer (0.06 g), the proportion of the D- and L-compounds being calculated from the optical rotation. Thus, the total yields of D- and L-galactose were 33% and 0.6% respectively of the polysaccharide.

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TABLE 1. FRACTIONATION OF THE PARTIAL METHANOLYSATES ON A CHARCOAL-CELITE COLUMN

Fraction	Eluant		Yield	$[\alpha]^{17}_{\mathrm{D}}$ (H_2O)		R_f
	%[a]	l	g	°	c	
I	0 1.5	2 3	0.19	+113.7	0.27	—[b]
II	5.0	4	0.28	—	—	0.67, 0.34
III	7.5 15.0	3 4	0.89	+23.8	0.51	0.34
IV	30.0	3	0.08	+54.5	1.56	0.22, 0.16

a) Concentration of ethanol in water.
b) Methyl D-galactoside is not detectable with the reagent (b).

For isolation, another batch (20 g) of the polysaccharide was hydrolysed; the D-galactose was removed, first by crystallization and then by fermentation with *Saccharomyces sake*. The residual sugars were fractionated on a cellulose column (3×40 cm) with a 1-butanol-water mixture (6 : 1 v/v). The resulting L-galactose (0.03 g) was crystallized from methanol; mp 162°C, $[\alpha]^{19}_{\mathrm{D}}$ $-73.3°$ (c 0.55, water). The melting point was not depressed on admixture with an authentic sample.

Complete Methanolysis. The polysaccharide (0.87 g on a moisture-free basis) was refluxed with 3% methanolic hydrogen chloride (10 ml) for 20 hr. When the resulting solution was treated in the usual way,[15] the neutral, deionized product was obtained as a syrup (0.56 g); $[\alpha]^{14}_{\mathrm{D}}$ $+62.7°$ (c 0.34, water).

3, 6-Anhydro-D-galactose Dimethyl Acetal. The syrup obtained above was fractionated on a cellulose column (3×33 cm) with a 1-butanol-water (6 : 1) mixture in usual way.[15] The anhydro-sugar derivative was obtained as a colorless syrup (0.09 g); R_f 0.68, $[\alpha]^{27}_{\mathrm{D}}$ $+36.2°$ (c 0.61, water).[16] It was hydrolysed with 0.02 N hydrochloric acid to the reducing sugar, which was then converted into the osazone in the usual way; yellow crystals, mp 220°C, $[\alpha]^{29}_{\mathrm{D}}$ $+58.4°$ (final value, c 0.22, pyridine-methanol (2 : 3 v/v)).[16,17] Admixture with an authentic sample showed no depression of the melting point.

Partial Methanolysis. The polysaccharide (4.2 g on a moisture-free basis) was refluxed with 0.5% methanolic hydrogen chloride (100 ml) for 2 hr and then treated in the usual way[18] to give a syrup of the neutral product (3.1 g). A 1.6 g portion of the syrup was chromatographed on a charcoal-Celite column (80 g each, 4.6×34 cm) with water and successively higher concentrations of ethanol.[19] The four fractions shown in Table 1 were obtained. Fraction I, on crystallization from 95% ethanol, afforded methyl α-D-galactoside monohydrate; mp and mmp 109°C, $[\alpha]^{17}_{\mathrm{D}}$ $+175°$ (c 0.35, water). Fraction II was re-chromatographed on a charcoal-Celite column (2.5×22 cm) to give two fractions; IIa (0.31 g), eluted with 5% ethanol (700 ml), $[\alpha]^{17}_{\mathrm{D}}$ $+3.6°$ (c 0.27, water), and IIb (0.15 g), eluted with 7% ethanol (800 ml), $[\alpha]^{17}_{\mathrm{D}}$ $+20°$ (c 0.50, water). IIa, a colorless syrup, was 3, 6-anhydro-D-galactose dimethyl acetal; it was identified in the same manner already described.

Carrabiose Dimethyl Acetal. Fraction III in Table 1 and fraction IIb of the re-chromatography, both colorless amorphous solids, were both carrabiose dimethyl acetal,[18] identified as its hexa-acetate; mp 146—147°C, $[\alpha]^{17}_{\mathrm{D}}$ $-16.7°$ (c 1.08, benzene).[18] The melting point was not depressed on admixture with an authentic sample. Found: C, 50.32; H, 6.25%.

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