IR Studies on Carrageenan of *Ahnfeltia concinna*, a Marine Red Alga

GERTRUDES A. SANTOS and MAXWELL S. DOTY ˣ

Abstract □ The principal polysaccharide of *Ahnfeltia concinna* gave IR absorptions at 1240, 930, 845–850, and 805 cm^{-1}, all typical of a carrageenan. The polysaccharide was separated into soluble and insoluble fractions with potassium chloride.

Keyphrases □ Carrageenan—isolation from *Ahnfeltia concinna*, IR identification □ *Ahnfeltia concinna*—isolation of carrageenan, IR identification □ IR spectrophotometry—identification, carrageenan isolated from *Ahnfeltia concinna* □ Alga—IR identification of carrageenan isolated from *Ahnfeltia concinna*

Carrageenan is a red seaweed polysaccharide extensively used as a food additive, especially in dairy products, and as a suspending agent in the cosmetic and pharmaceutical industries. Carrageenan is prepared from different species of *Chondrus, Gigartina, Eucheuma, Hypnea,* and *Iridaea.* These genera and the genus *Ahnfeltia* belong to the order Gigartinales. Although *Ahnfeltia plicata* has long been been known as the major source of agar in Russia, *Ahnfeltia durvilliae* of Peru was reported to contain carrageenan (1).

There are three main groups of carrageenans: the κ-, λ-, and ι-carrageenan groups. The three types differ only in their 3,6-anhydrogalactose content and in their content and position of ester sulfate groups in the molecule. Scheme I shows the idealized structure of κ-, ι-, and λ-carrageenans proposed previously (2). The μ- and ν-carrageenans are the precursors of κ- and ι-carrageenans, respectively.

From the report of Lawson *et al.* (3), *A. durvilliae* contains a "deviant" ι-carrageenan. Methylation studies showed that the polysaccharide consists of β-D-galactopyranose 4-sulfate (45%), β-D-galactopyranose (5%), 3,6-anhydro-α-D-galactopyranose (24%), 3,6-anhydro-α-D-galactopyranose 2-sulfate (12%), 4-linked α-D-galactopyranose 2,6-disulfate (7%), and 4-linked α-D-galactopyranose (7%) (4).

A. concinna is a marine red alga widely distributed in the Hawaiian Islands. It forms a yellow-brown to dark red-brown band in the intertidal region on basalt rocks. In a preliminary report on the polysaccharide of this alga, the name ahnfeltan was given to the principal carbohydrate (5). The purpose of this investigation was to show whether the polysaccharide of *A. concinna* is definitely a carrageenan or at least not an agar.

EXPERIMENTAL

The sample used was collected from north of Makapuu Point on the island of Oahu, Hawaii. The seaweed was quite clean and devoid of epiphytes. The fresh live seaweed gave as much as 27% clean dry weed.

Extraction of Polysaccharide—Clean dry weed (20 g) was heated in water (700 ml) adjusted to pH 9 by addition of 2% sodium hydroxide solution in a boiling water bath for 1 hr. The seaweed was then blended to a paste and heated for a further 3 hr. A filter aid[1] (50 g) was added, and the mixture was stirred continuously for 30 min and then filtered with pressure. The filtrate, about 700 ml–1 liter, was about 1% carrageenan.

A sodium chloride solution (10%, 50 ml) was added, and the mixture was poured into twice its volume of 85% isopropanol with rapid stirring. The precipitate was strained through nylon cloth, washed twice with 600 ml of the 85% isopropanol, and dried at 60°. The dried polysaccharide was ground to a powder, yielding 11.92 g (59.6%); SO_3Na, 31.86%; 3,6-anhydrogalactose ($C_6H_{10}O_4$).

The modified carrageenan was prepared by treating the dry seaweed (20 g) in water (700 ml) with lime (4 g) instead of a 2% sodium hydroxide solution and heating and filtering as already described. The filtrate was adjusted to pH 8.5–9.0 with dilute hydrochloric acid. Addition of 10% sodium chloride was not necessary before precipitation of the polysaccharide with 85% isopropanol. The yield was 11.86 g (59.3%); SO_3Na, 26.29%; 3,6-anhydrogalactose.

Fractionation of Carrageenan—The fractionation method used was that of Stancioff and Stanley (1). The powdered carrageenan (8.00 g) was dispersed by continuous stirring for 1 hr at room temperature in 2.5% potassium chloride solution, and the mixture was allowed to sit overnight. A filter aid (40 g) was stirred into the mixture for 1 hr, the mixture was filtered with suction, the filter cake was washed with potassium chloride solution (2.5%, 100 ml), and the filtrate and washing were combined and evaporated to 600 ml and precipitated with 2.5 volumes of 85% isopropanol. The precipitate was washed twice with 300-ml portions of the solvent, dried at 60° for 5 hr, and ground into a powder, yielding 1.13 g (15.76%); SO_3Na, 31.12%; 3,6-anhydrogalactose, 11.00%.

The filter cake was then suspended in hot water (800 ml), the mixture was stirred for 1 hr at 85–100° and filtered with pressure, and the cake was washed with hot water (100 ml). The filtrate and washing were combined and heated to 70°, and sodium chloride solution (10%, 50 ml) was added with stirring. The hot mixture was poured into twice its volume of 85% isopropanol with vigorous stirring. The coagulum was squeezed, washed twice with 500-ml por-

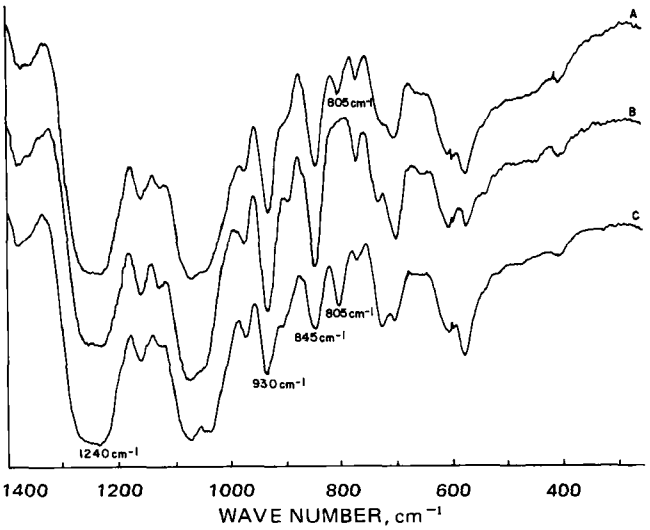
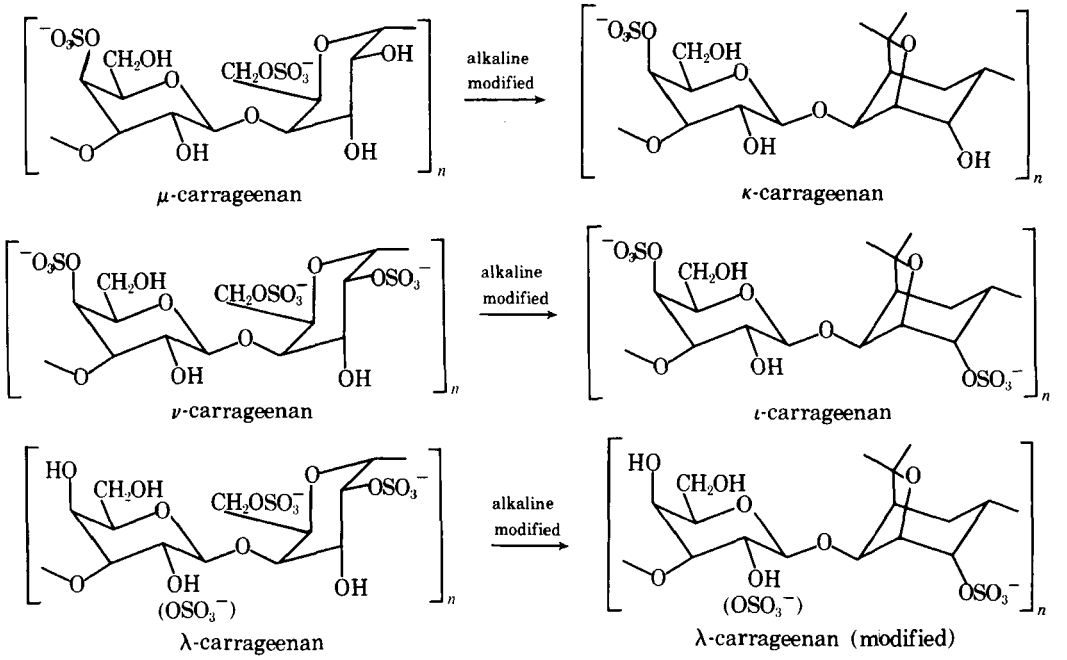


Figure 1—*IR spectra of: A,* A. concinna *carrageenan; B, κ-carrageenan from* Eucheuma cottonii; *and C, ι-carrageenan from* Eucheuma spinosum.

[1] Diatomite.



Scheme I

tions of isopropanol, and dried in a 60° oven for 5 hr, yielding 6.04 g (84.24%); SO_3Na, 33.13%; 3,6-anhydrogalactose, 27.03%.

Sodium Borohydride Treatment of Potassium Chloride-Soluble Fraction—The Rees method (6) was used in which the polysaccharide (1.6 g) was dissolved in water (600 ml), sodium borohydride was added (0.2 g), and the solution was allowed to sit overnight at room temperature. More sodium borohydride (7.2 g) and sodium hydroxide (24 g) were added to the solution with cooling. After the rapid evolution of gas had subsided, the solution was heated on a water bath at 80° for 2 hr. The solution was neutralized with acetic acid and then poured, with stirring, into 2.5 times its volume of 85% isopropanol. The coagulum was squeezed, washed twice with isopropanol, and dried at 60° for 5 hr, yielding 1.46 g (91.25%); SO_3Na, 24.37%; 3,6-anhydrogalactose, 23.63%.

IR Determination—The polysaccharide (30 mg) was dissolved in distilled water (15 ml), and 3 ml of the solution was poured onto mercury (4 ml) contained in a 15-ml porcelain crucible. The solution was dried to a thin film in a heated vacuum desiccator (70°). The mercury was poured off, the film was mounted between two 15 × 5-cm pieces of cardboard provided with 2.5 × 1.3-cm windows, and the spectra were determined[2].

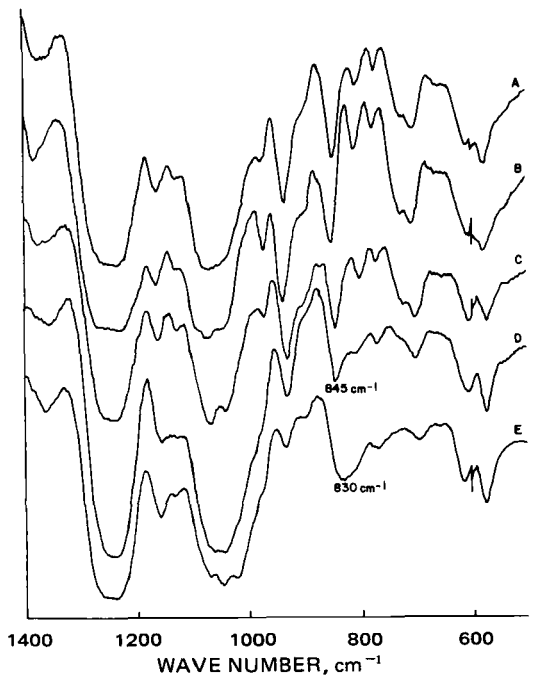


Figure 2—*IR spectra of: A,* A. concinna *carrageenan; B, the modified* A. concinna *carrageenan; C, the potassium chloride-insoluble fraction; D, the potassium chloride-soluble fraction; and E, λ-carrageenan.*

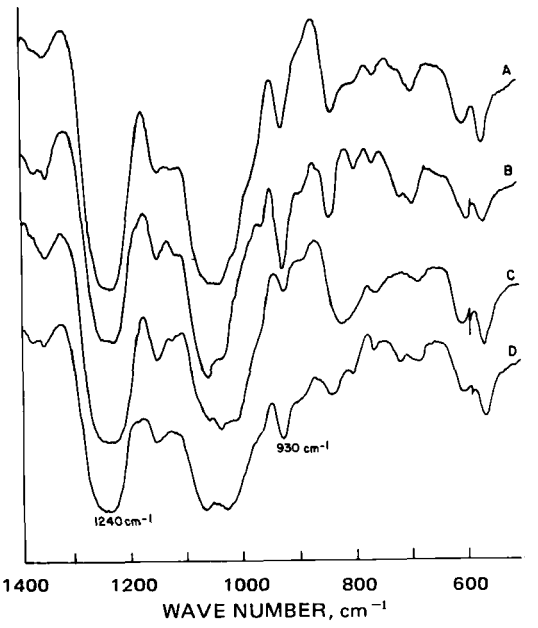


Figure 3—*IR spectra of: A, potassium chloride-soluble fraction of* A. concinna *carrageenan; B, modified potassium chloride-soluble fraction; C, λ-carrageenan; and D, modified λ-carrageenan.*

[2] Beckman IR 467 spectrophotometer.

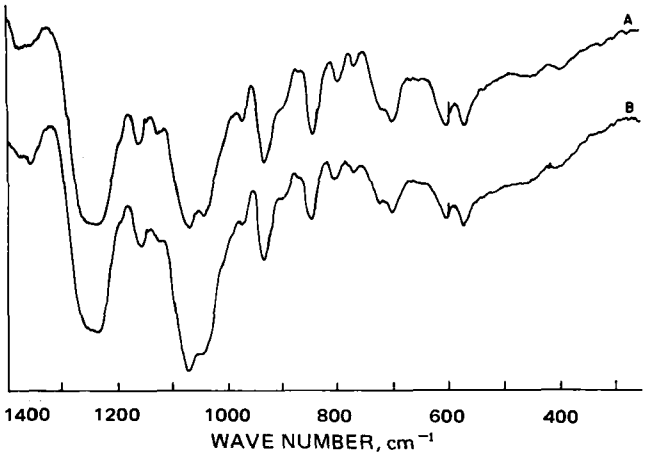


Figure 4—*IR spectra of: A, potassium chloride-insoluble fraction of* A. concinna *carrageenan; and B, modified potassium chloride-soluble fraction.*

RESULTS AND DISCUSSION

The IR spectra of the polysaccharide from *A. concinna* showed absorption bands characteristic of a carrageenan with: 1240 cm^{-1}, S—O stretching vibration; 930 cm^{-1}, typical of the presence of 3,6-anhydrogalactose; and 845–850 cm^{-1}, due to the secondary axial 4-sulfate absorption. But it is neither a κ- nor an ι-carrageenan as shown in Fig. 1. As shown in Fig. 2, the broadening at the base of the 850- and 805-cm^{-1} absorptions was possibly due to the presence of the primary equatorial 6-sulfate absorption at 820 cm^{-1}, which disappeared upon alkaline modification with the elimination of the primary 6-sulfate and the formation of the 3,6-anhydrogalactose (1, 2, 7–16).

Fractionation of the polysaccharide with 2.5% aqueous potassium chloride yielded the potassium chloride-insoluble and potassium chloride-soluble fractions. The IR spectrum (Fig. 2) of the potassium chloride-insoluble fraction is very similar to that of modified carrageenan. The potassium chloride-soluble fraction could not be λ-carrageenan, because the broad absorption at 850–800 cm^{-1} has a maximum at 850 cm^{-1} while that of the λ-carrageenan is at 830 cm^{-1}. The broad absorption band at 850–800 cm^{-1} of the potassium chloride-soluble fraction was resolved into two sharp peaks (Fig. 3) at 850 and 805 cm^{-1} upon modification with sodium borohydride, and the IR spectrum of the product (Fig. 4) was very similar to that of the potassium chloride-insoluble fraction. However, the potassium chloride-soluble fraction of the *A. concinna* carrageenan cannot possibly be the precursor of the potassium chloride-insoluble fraction because of the low sulfate and 3,6-anhydrogalactose content of the modified carrageenan.

There is a possibility that the structure of the carrageenan of *A. concinna* could be identical or closely related to that of *A. durvilliae*, which Lawson *et al.* (3) investigated and classified as a deviant ι-carrageenan.

According to the physical properties and IR studies, the polysaccharide of *A. concinna* is definitely a carrageenan; however, further studies are still needed to determine the different monosaccharide units in the molecule and to clarify the chemical structure.

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[x] To whom inquiries should be directed.