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Note

Assignment of agar or carrageenan structures to red algal polysaccharides

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In screening red algae as sources of new gel-forming polysaccharides[1], it was important to determine whether the polymer belonged to the known carrageenan or agar family. Both families have idealized backbone structures of alternating 3-*O*-linked β-D-galactopyranose and 4-*O*-linked 3,6-anhydro-α-galactopyranose (or α-galactose) residues differing only in the absolute configuration of the latter residue, which is D for carrageenans and L for agars[2]. In reality these structures are "masked" by variable substitution with half-ester sulphate and, to a lesser extent, with methoxyl groups[3,4].

Assignment of configuration to the 4-*O*-linked residue may be determined absolutely by measurements of its specific rotation when a fairly large quantity of pure sample is available, which is seldom the case under screening conditions. This communication illustrates the characterization of small quantities of algal polysaccharides by complementing i.r. spectral data with diagnostic chromatographic mobilities of derivatives of carrabiose (3,6-anhydro-4-*O*-β-D-galactopyranosyl-D-galactose) and agarobiose (3,6-anhydro-4-*O*-β-D-galactopyranosyl-L-galactose), the repeating units from carrageenans and agars respectively[2]. Polysaccharides from 9 species of algae were used to illustrate this procedure.

All i.r. spectra (Fig. 1) exhibited intense bands at 940 cm^{-1} for the 3,6-anhydrogalactose residues[5]. Bands at 805–810 cm^{-1} were representative of axial 2-sulphate groups on 3,6-anhydrogalactose residues, and bands at 820, 830, and 850 cm^{-1} were representative of sulphate groups attached to equatorial O-6, equatorial O-2-, and axial O-4-positions of the 3-linked residues, respectively[6].

These absorption bands typified the known κ- and ι-carrageenans from *Eucheuma cottonii* and *E. spinosum*[3] (Fig. 1a and b) respectively; they suggested an ι-carrageenan structure for the unknown polysaccharide from *Sarcodiotheca furcata* (Fig. 1c); and they illustrated the hybrid nature of the known κ-carrageenan, KCl-insoluble, fraction from *Chondrus crispus*, with the ι-component demonstrated by the low-intensity band at 810 cm^{-1} (Fig. 1i)[7].

Although devoid of bands for sulphate groups, the spectrum of the known

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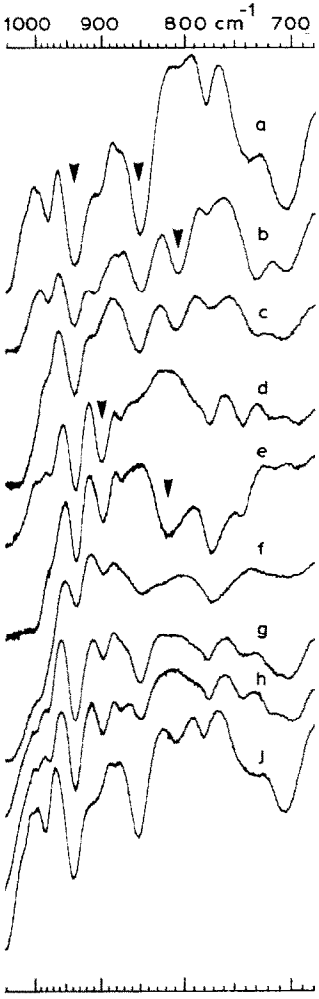

Fig. 1. I.r. spectra (frequency range 700–1000 cm^{-1}) of polysaccharides from, (*a*) *Eucheuma cottonii*, (*b*) *Eucheuma spinosum*, (*c*) *Sarcodiotheca furcata*, (*d*) *Gelidium amansii*, (*e*) *Gloiopeltis furcata*, (*f*) *Odonthalia kamtschatica*, (*g*) *Furcellaria fastigiata*, (*h*) *Endocladia muricata*, and (*i*) the KCl-insoluble fraction from *Chondrus crispus*.

agar from *Gelidium amansii*[8] provided an intense band at 895–900 cm^{-1} (Fig. 1d), which also occurred in the spectrum of the 2- and 6-sulphated agar from *Gloiopeltis furcata*[3] (Fig. 1e). This band, ignored in the literature for algal polysaccharides, occurred in all i.r. spectra of agars we examined, at an intensity above 70% of the 940-cm^{-1} band. Although absent in the spectra of κ-, ι-, and λ-carrageenans, it was not unique to agars as it appeared at decreased intensity, 20% of the 940 cm^{-1} band, in the spectrum of the partially desulphated κ-carrageenan from *Furcellaria fastigiata*[3] (Fig. 1g). The exact nature of the vibration responsible for this band is not known, yet greater absorbance occurred in spectra from agars. Recorded also in i.r. spectra of cellulose, chitin, and xylans, this band has been assigned tenta-

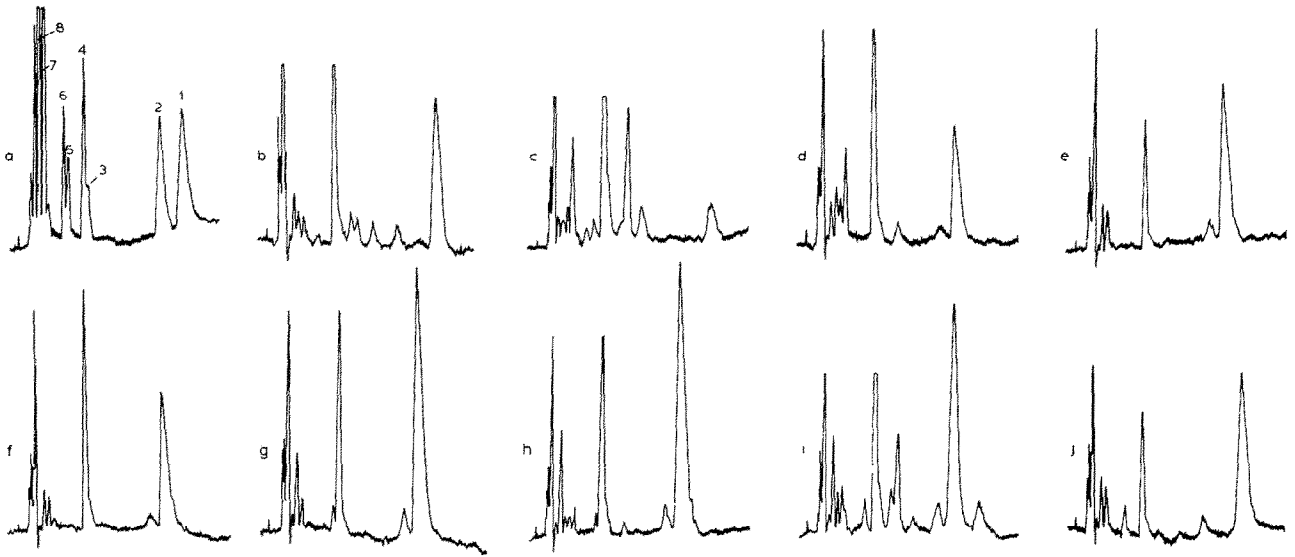

Fig. 2. Liquid chromatograms of (*a*) a mixture of agarobiose dimethyl acetal 1, carrabiose dimethyl acetal 2, methyl β-D-galactopyranoside 3, methyl α-D-galactopyranoside 4, methyl β-D-galactofuranoside 5, methyl α-D-galactofuranoside 6, 3,6-anhydro-D-galactose dimethyl acetal 7, and methyl 3,6-anhydro-α-D-galactopyranoside 8; and l.c. of partial methanolysis products (together with methyl α-D-galactopyranoside as internal standard) from polysaccharides of (*b*) *Gloiopeltis furcata*, (*c*) *Odonthalia kamtschatica*, (*d*) *Sarcodiotheca furcata*, (*e*) the KCl-insoluble fraction from *Chondrus crispus*, (*f*) *Eucheuma spinosum*, (*g*) *Eucheuma cottonii*, (*h*) *Furcellaria fastigiata*, (*i*) and *Endocladia muricata* and (*j*) *Gelidium amansii*.

TABLE I

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF 3,6-ANHYDROGALACTOSE AND GALACTOSE DERIVATIVES[a]

Component	*Relative retention time*	*Relative response factor*
Methyl α-D-galactopyranoside	1.00	1.00
Methyl β-D-galactopyranoside	1.07	1.05
Methyl α-D-galactofuranoside	0.74	1.00
Methyl β-D-galactofuranoside	0.80	0.75
Methyl 3,6-anhydro-α-D-galactopyranoside	0.34	1.95
3,6-Anhydro-D-galactose dimethyl acetal	0.47	1.07
Agarobiose dimethyl acetal	2.36	0.95
Carrabiose dimethyl acetal	2.05	1.04

[a]Concentration of all samples, 5 mg/mL.

tively to an antisymmetric, out-of-phase ring-stretching mode[9-11].

In the spectrum of the unknown polysaccharide from *Odonthalia kamtschatica* (Fig. 1f), the 0.77 ratio of 900/940-cm^{-1} bands suggested an agar structure substituted at axial 4 positions with half-ester sulphate groups as indicated from the band at 850 cm^{-1}. The polymer from *Endocladia muricata* (Fig. 1h), with a 0.30 ratio for these bands, suggested a κ-carrageenan structure partially desulphated at the 4 position of the 3-linked residues, akin to *Furcellaria*. Unequivocal assignment of these polymer types was provided by chromatographic separation of products from partial methanolysis of these polysaccharides.

Preferential cleavage of the 3,6-anhydrogalactosyl bond by mild methanolysis of agar and carrageenan structures led to high yields of the dimethyl acetals of agarobiose[12] and carrabiose[3] respectively. These acetals, as their corresponding hexacetates, had similar retention times in g.l.c. The separation of the underivatized acetals, together with related sugars, proved more effective by high-performance liquid chromatography (l.c., Fig. 2a). Response factors (Table I) based on total peak-areas were similar, with the exception of methyl 3,6-anhydrogalactose, presumably resulting from increased absorbance at 190 nm of this double-ring structure.

L.c. identification of carrabiose dimethyl acetal as a major component of mild methanolysis of polymers from *Chondrus crispus* (Fig. 2e), *Eucheuma spinosum* (Fig. 2f), *E. cottonii* (Fig. 2g), *Furcellaria fastigiata* (Fig. 2h), and the unknown polymer from *Sarcodiotheca furcata* (Fig. 2d) categorized these polysaccharides as carrageenan structures. Similarly, carrabiose dimethyl acetal was the major methanolysis product from *Endocladia muricata* (Fig. 2i), but this more-complex chromatogram indicated the presence of agarobiose dimethyl acetal as 6% of the disaccharide fraction. Complete reproducibility for the methanolysis products from polysaccharides of *E. muricata* from several locations attested to the presence of agarobiose as a minor structural unit in this predominantly carrageenan structure. Transitional structures of this type between representatives of the carrageenan and

agar groups, although rare, have been recorded from algae of the Solieriaceae[13] and Grateloupiaceae[14] families.

Agarobiose dimethyl acetal was identified by l.c. as the major product of mild methanolysis of polymers from *Gelidium amansii* (Fig. 2j), *Gloiopeltis furcata* (Fig. 2b) and the unknown sulphated galactan from *Odonthalia kamtschatica* (Fig. 2c), and characterized them as belonging to the agar group of polymers.

In conclusion, these results demonstrate that algal galactans containing 3,6-anhydrogalactose residues may be categorized conveniently into the carrageenan or agar family of polymers by a combination of i.r.-spectral analysis, using particularly the intensity of the 900 cm^{-1} band, and the l.c. analysis of products from partial methanolysis.

EXPERIMENTAL

Materials. — Polysaccharides studied were extracted from dry algae (10 g) with water (400 mL) for 1.5 h at 98°. Residues isolated by centrifugation were re-extracted twice and the combined centrifugates evaporated to 500 mL, dialyzed overnight against distilled water at 40°, and then concentrated and freeze dried to yield the phycocolloids, which were not purified further. The polymer (5 g) from *Chondrus crispus* was fractionated by addition of potassium chloride (0.3M, 500 mL) and the insoluble κ-fraction recovered by freeze drying after dialysis. A stock solution of methanolic hydrogen chloride (1%) was prepared by adding acetyl chloride (1.95 mL) to dry methanol (98.05 mL) containing 2,2-dimethoxypropane (2 mL).

Carrabiose dimethyl acetal was obtained by boiling the polysaccharide (25 g) from *Furcellaria fastigiata* under reflux in methanolic hydrogen chloride (0.2%, 500 mL) for 45 min. The process was repeated twice on residual polysaccharide. The pooled supernatant solutions were made neutral with silver carbonate, deionized, and evaporated to a syrup. Acetylation of the syrup with acetic anhydride (20 mL) and pyridine (25 mL) for 6 h at 100° yielded the hexaacetate, which was recrystallized from 95% ethanol (m.p. 149–150°). As carrabiose dimethyl acetal is not crystalline, a standard solution was prepared by deacetylation of the hexaacetate (82.5 mg) in dry methanol (19 mL) and 2,2-dimethoxypropane (1 mL) with sodium methoxide (2 mL, containing 35.4 mg sodium in 10 mL of methanol) for 16 h. After neutralization with carbon dioxide, deionization, and evaporation to dryness, the resultant carrabiose dimethyl acetal was dissolved in water (9.8 mL) to provide a 5 mg/mL stock solution.

Agarobiose dimethyl acetal was similarly prepared from agar (25 g, commercial B.B.L.) to yield a crystalline mass that was recrystallized from 95% ethanol as prisms, m.p. 166–168°. Treatment of these crystals (50 mg) with pyridine (5 mL) and acetic anhydride (5 mL) for 5 h at 100° yielded the corresponding hexaacetate, recrystallized from methanol–water, m.p. 87–88°. Methyl glycosides of 3,6-anhydro-α-D-galactose and D-galactose, together with 3,6-anhydro-D-galactose di-

methyl acetal used in this study, were from the laboratory collection of sugars.

Partial methanolysis of polysaccharides. — Agar (50 mg) and κ-carrageenan (50 mg) were heated in sealed tubes for selected periods up to 2 h with methanolic HCl (0.2%, 35 mL) at 65, 70, 80, and 90° to determine yields of agarobiose- and carrabiose-dimethyl acetals respectively and establish the following conditions suitable for both types of polymer.

Dried polysaccharides (50 mg) were methanolyzed (0.2%, 35 mL) for 1 h at 70° in sealed tubes and the resultant mixtures were filtered through microfibre glass, made neutral with silver carbonate, and evaporated to dryness. Water (10 mL) and methyl α-D-galactopyranoside (4.5 mg) were added, and the solutions were deionized with Bio-Rad AG50W-X8 and AG3-X4A resins. After evaporation to dryness, water (1.5 mL) was added and solutions were clarified by using 0.22-μm Millipore MF filters prior to l.c. analyses.

Analyses. — A Spectra Physics SP8100 instrument coupled to a variable-wavelength, nitrogen-purged detector (SP8400) and a computing integrator SP4100 were used for l.c. Separation was with a Brownlee Amino, LiChrosorb (10 μm), column (250 × 4.6 mm i.d., 10-μL injection loop) operated at 39°, eluted with aqueous acetonitrile (93%) at 2 mL/min; detection was at 190 nm.

G.l.c. was performed on a glass column (1.8 m × 0.32 cm) of 3% Silar 10C on Gas Chrom Q (100–120 mesh) operating at 255° with a nitrogen flow-rate of 3 mL/min, using *myo*-inositol hexaacetate as internal standard; *T* 13.06 and 13.31 for the hexaacetates of carrabiose- and agarobiose-dimethyl acetals respectively.

I.r. spectra were recorded on a Perkin–Elmer Infracord instrument, using films prepared by evaporation of aqueous solutions of the polysaccharides (10 mL of 4 mg/mL) in aluminum weighing-dishes contained in a vacuum oven at 40°. Films were mounted on Perkin–Elmer adhesive-film mounts to provide permanent voucher specimens.

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