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EVIDENCE FOR KAPPA CARRAGEENAN IN *HALARACHNION LIGULATUM*

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Key Word Index—*Halarachnion ligulatum*; Furcellariaceae; Rhodophyceae; κ-carrageenan; neocarrabiose sulphate unit.

Abstract—The red alga, *Halarachnion ligulatum* contains a κ-like carrageenan. The IR and ^{13}C NMR spectra indicate that the polysaccharide contains neocarrabiose sulphate units.

INTRODUCTION

Carrageenans are cell wall polysaccharides which can be extracted with water from members of the Rhodophyta (red alga). They are composed of D-galactose residues linked alternately by α-1,3 and β-1,4 bonds and are classified according to the substitutions that occur on the free hydroxyl groups [1]. These substitutions are generally the addition of ester sulphate, or the formation of a 3–6 anhydride on the 4-linked residue.

Of the few members of the Furcellariaceae examined for their chemical composition, those of the genus *Furcellaria* contain a 'furcellaran' which is a Kappa desulphated carrageenan and constitutes a source of commercially valuable phycocolloid. We report now on the chemical composition of *Halarachnion ligulatum*.

RESULTS AND DISCUSSION

H. ligulatum was collected in the gametangial phase when its thalli are composed of erect fronds. The yield of polysaccharide was 13% of the dry weight. Acid hydrolysis of the polysaccharide gave galactose (96.8%) and xylose (3, 2). An $[\alpha]_D$ of $+30°$ for the polysaccharide and its IR spectrum suggested the presence of 4-O linked, 3,6-anhydro-α-D-galactopyranose units (A-unit) alternating with 3-*O*-linked β-D-galactopyranose (G-unit) units as found in κ-carrageenan (Fig. 1). The IR spectrum was very similar to that of a commercial sample of κ-carrageenan (ex. *Eucheuma cottonii*) with absorptions at 1240 (S-O vibration of sulphates), 930 (3,6-anhydrogalactose units) and 845 cm^{-1} (secondary axial 4-sulphate on galactose units). Quantitative analysis of the galactan of *H. ligulatum* after alkali modification showed that the relative proportions of galactose, its 3,6-anhydride, and ester sulphate, were 100:91:82. The treatment with alkali [2] eliminates the primary sulphate ester at C-6 of the 4-linked galactopyranose unit (μ precursor) giving rise to 3,6-anhydrogalactose. ^{13}C NMR spectroscopy can be applied to the analysis of red algal polysaccharides with the range δ95–105 being assigned to the anomeric carbon atoms of sugar residues [3]. The signal at δ 102.4 was assigned to G-1 and that at δ95.3 to A-1 of the neocarrabiose sulphate repeating structure (Fig. 2).

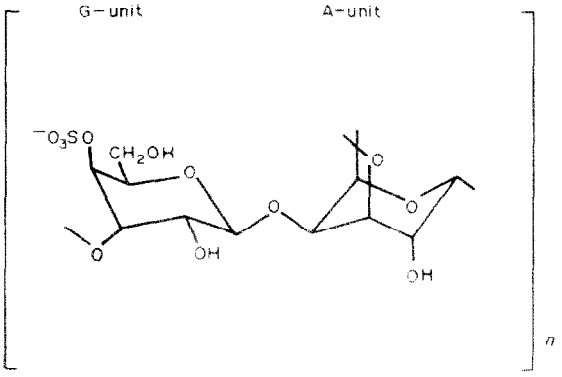

Fig. 1. Disaccharide repeating units in κ-carrageenan.

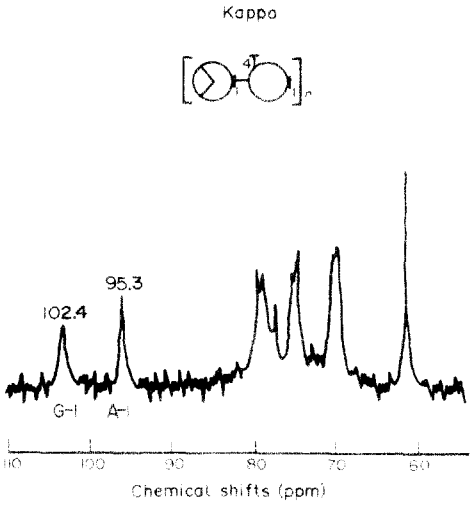

Fig. 2. ^{13}C NMR spectra of carrageenan from *H. ligulatum*.

EXPERIMENTAL

Algal material. Samples of *Halarachnion ligulatum* were harvested in June at Logonna Daoulas, near Brest, Brittany.

Extraction. The alga (20 g) was extracted with 600 ml 16% NaOH at 90° for 5 hr. Diatomaceous earth was added, the mixture filtered, and the polysaccharide recovered by addition of 2.5 vol isopropanol at 80°.

Hydrolysis of the polysaccharide and sugar analysis. The polysaccharide (1 g) in 0.5 M H_2SO_4 (5 ml) was hydrolysed for 12 hr at 90°. The hydrolysate was neutralized, the neutral sugars reduced with $NaBH_4$ and the resultant alditols acetylated with Ac_2O–C_5H_5N (1:1). The alditol acetates were analysed by GC: glass column (1.8 mϕ1/8) containing 3% SP 2340 on supercoport (100/200 mesh); 210°; N_2 30 ml per min. Inositol acetate was used as the internal standard.

Sulphates was determinated by a turbidometric method [4]. The 3,6-anhydrogalactose residues were analysed by the resorcinol method using fructose for the standardization [5].

The total sugar content was measured by the phenolsulphuric method [6]. IR spectroscopy was performed on films prepared by evaporating (60°) 0.25% solns of the carrageenans on polyvinyl chlorate plates (Afco dur) ^{13}C NMR: 22.6 MHz, 30 mg/ml in D_2O at 95°, relative to int. DSS and converted to external TMS.

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STEREOCONFIGURATION OF SEQUOYITOL BY HIGH RESOLUTION ^{1}H NMR

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Key Word Index—*Podocarpus sellowii*, Podocarpaceae; sequoyitol; 5-methoxy-*myo*-inositol; *myo*-inositol.

Abstract—The stereoconfiguration of sequoyitol, isolated as the pentaacetate from the leaves of *Podocarpus sellowii*, has been established by comparing its ^{1}HNMR spectrum with that of *myo*-inositol hexaacetate.

INTRODUCTION

Because of very poor solubility in common organic solvents and of extreme polarity, degradative methods have always been used for establishing the stereochemistry of the inositols. However, it has been shown recently [1] that high resolution ^{1}H NMR spectroscopy can be successfully exploited for elaborating the stereoconfigurations of the inositols by using the respective acetates as starting materials.

RESULTS AND DISCUSSION

In continuation of our work in this field [1], sequoyitol was isolated by dehydrating the water extract of the hydrophilic portion of the total ethanol extract of the leaves of *Podocarpus sellowii* after separating the diterpenes [2]. The gummy material upon acetylation followed by usual work-up furnished the pentaacetate (**1a**), $C_{17}H_{24}O_{11}$ mp 200°, m/z 404 $[M]^+$, which showed in the IR spectrum peaks for acetoxy and methoxy functions at 1760, 1430, 1370 and 1235 cm^{-1} besides the characteristic absorptions in the finger print region at 1168, 1140, 1094, 1065, 951 and 912 cm^{-1}, typical for sequoyitol pentaacetate as was reported in ref. [3]. The integration of all the resonance intensities in the 360 MHz ^{1}H NMR spectrum, as well as the resonance pattern, suggests that the three intense singlets at δ 2.19 (3H), 2.08 (6H) and 1.99(6H) are assigned for the five acetoxy groups, the former is axial and the latter two for four acetoxy functions, all equatorial. The other intense singlet at δ 3.45 represents the methoxyl protons. The two pairs of doublets at 4.99 and 5.02 ($J = 2.80$ Hz) are for the two axial