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Carrageenan from the tetrasporic stage of *Gigartina decipiens* (Gigartinaceae, Rhodophyta)

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ABSTRACT

The structure of the polysaccharide isolated from tetrasporophytic plants of the New Zealand red alga *Gigartina decipiens* has been determined by chemical and spectroscopic techniques. It is a linear polymer composed primarily of alternating 3-linked β-D-galactopyranosyl 2-sulphate and 4-linked α-D-galactopyranosyl 2,6-disulphate residues. About 15% of the 3-linked residues have an additional sulphate ester group at the 6-position. Aside from this small extra sulphate substitution, the structure is that of the idealised λ-carrageenan. Good quality solution-state ^{13}C NMR spectra were recorded and interpreted for this carrageenan and for the carrageenans produced from it by solvolytic desulphation and alkali modification.

INTRODUCTION

Red seaweeds in the family Gigartinaceae are a rich source of the sulphated galactans known as carrageenans that have applications as gelling, thickening, and suspending agents in food processing. A number of *Gigartina* species are endemic to New Zealand, and *G. decipiens* is relatively abundant. In order to describe carrageenan structures, a system of nomenclature has been developed in which idealised disaccharide repeating units are referred to by Greek letters. Native carrageenans are often hybrids of more than one of these repeating units. Variations in carrageenan structure occur not only between different Gigartinacean algae, but also between the life stages of the same algae. Gametophytes contain κ-type carrageenans, while tetrasporophytes contain λ-type carrageenans[1].

The idealised λ-carrageenan structure is that shown in Fig. 1A, in which a 3-linked β-D-galactopyranosyl 2-sulphate residue alternates with a 4-linked α-D-galactopyranosyl 2,6-disulphate residue. The λ-type carrageenan from *Chondrus crispus* tetrasporophytes, for example, differs from this idealised structure in that

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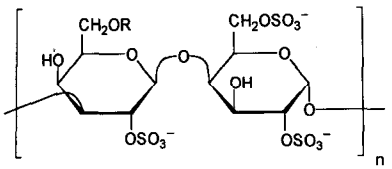

Fig. 1. The structure of λ-carrageenans. A: idealised, (R = H); B: from tetrasporophytic *Gigartina decipiens*, (R = 85% H, 15% SO_3^-).

about 30% of its 3-linked β-D-galactopyranosyl residues do not have the sulphate at the 2-position[2]. In contrast, the tetrasporophytes of certain previously examined *Gigartina* species, such as *G. atropurpurea*, *G. canaliculata*, and *G. chamissoi*, also contain ξ-carrageenan in which the 6-sulphate ester of the idealised λ-carrageenan is absent[3].

The structural analysis of red algal galactans has been greatly simplified by the advent of ^{13}C NMR spectroscopy applied to hot aqueous solutions of these materials[4]. This technique, however, has so far not been of significant help in identifying λ-carrageenans. Native λ-carrageenans generally yield very viscous solutions even when hot, due probably to their high molecular weights and possibly to interactions with minor components, such as proteins, in the samples[5]. The resulting ^{13}C NMR spectra are generally composed of broad envelopes rather than clearly defined signals.

Recent advances in chemical methodology for the analysis of sulphated galactans have shown promise in the analysis of a commercial "λ"-carrageenan[6]. Accordingly, we have employed these methods to characterise the polysaccharide from *Gigartina decipiens* tetrasporophytes. We also report on the utilisation of ^{13}C NMR spectroscopy in the analysis of this polymer.

EXPERIMENTAL

Materials.—Specimens of the material studied have been deposited in the Herbarium of the Museum of New Zealand. Tetrasporophytic specimens of *Gigartina decipiens* were collected at Eastbourne, Wellington in March, 1991 (WELT A19497) and in September, 1991 (WELT A19835) and were air-dried.

Isolation of polysaccharide.—Samples of air-dried weed (1–10 g) were heated in $NaHCO_3$ solution (0.05 M, 60 mL/g of weed) at 90°C for 4 h. Amyloglucosidase (from *Rhizopus* mould, Sigma Chemical Co.) was added to the cooled mixture to digest any floridean starch present. After 16 h at room temperature, the extract was then boiled again briefly to deactivate the enzyme, filtered hot through a Whatman GF/D membrane, dialysed against several changes of distilled water to remove salts and glucose, then lyophilised.

Infrared spectroscopy.—A Perkin–Elmer 580 spectrophotometer was used. Samples were analysed as films, prepared by drying 0.4% w/v solutions on silanised glass dishes.

^{13}C NMR spectroscopy.—Spectra were recorded on 3% w/v solutions in 50:50 D_2O–H_2O at 90°C on a Bruker AC 300 spectrometer (75 MHz, 0.885-s acquisition time, 0.5-s delay time, and 80° pulse width). Chemical shifts are quoted relative to internal Me_2SO as standard at 39.4 ppm.

Sulphate analysis.—Samples were dried in vacuo over P_2O_5 for 16 h prior to analysis. Polysaccharide samples (~ 3 mg) were weighed accurately into tubes with PTFE-lined screw-caps. Nitric acid (70%, 0.25 mL) containing anhyd Na_2CO_3 (10 mg/mL) was added, the tubes were sealed, and the contents were refluxed for 15 min in a sand bath at 200°C. After this time, the caps were removed and the tubes were returned to the sand bath. The liquid was evaporated off over a period of 1 h with occasional assistance from a stream of dry air. The residues were cooled and dissolved in deionised water (50 mL). Sulphate content was determined by ion chromatography using a Dionex QIC fitted with Dionex AG4A and AS4A columns, and AMMS-1 membrane suppressor eluted with $NaHCO_3$ (0.86 mM) and Na_2CO_3 (2.2 mM).

Constituent sugar analysis.—Polysaccharide samples (~ 1 mg) were analysed for constituent sugars using the reductive hydrolysis method of Stevenson and Furneaux[6]. This technique combines hydrolysis (with CF_3CO_2H) and reduction (with 4-methylmorpholine-borane), followed by acetylation, to produce alditol acetates. Derivatised samples were analysed by GLC using a Hewlett–Packard 5890 Series II chromatograph with a Supelco 2330 column (15 m × 0.25 mm) at 220°C and a flame-ionisation detector (FID). Identification of components was by comparison of retention times with authentic standards.

Methylation analysis.—Samples in the triethylammonium salt form (~ 1 mg) were methylated, and alditol acetate derivatives were prepared from the methylated polysaccharides by reductive hydrolysis[6]. In certain cases, particularly when estimating the relative amounts of 2,3,6-Gal and 2,4,6-Gal, it was necessary to prepare C-1 deuterated alditol acetates. This necessitated the use of $NaBD_4$ and a different method of alditol acetate preparation[6]. Derivatised samples were analysed by GLC as above and also by GLC–MS on a Hewlett–Packard MSD 5970 with a Hewlett–Packard Ultra-2 column (20 m × 0.20 mm) at 50°C (2 min), 50°C/min to 180°C (1 min), 50°C/min to 220°C (15 min), and electron impact (EI) ionisation at 70 eV. Partially methylated, alditol acetates were identified by comparison of their retention times with those of an authentic standard mixture, prepared by the method of Doares et al.[7], and mass spectral data[8].

Quantitative sugar analysis.—The operation of the gas chromatograph on split injection mode affected the relative FID response factors of the various derivatives of interest so that published effective carbon response (ECR) factors were not applicable. Response factors were determined experimentally from authentic standards whenever quantitative data was required using *myo*-inositol hexaacetate as

an internal standard with a given response factor of 1. It was assumed that, for example, all three tri-*O*-acetyl-tri-*O*-methyl-galactitols gave the same response. The following standards were available from earlier studies[6]: 1,2,4,5-tetra-*O*-acetyl-3,6-anhydro-D,L-galactitol, 1,4,5-tri-*O*-acetyl-3,6-anhydro-2-*O*-methyl-L-galactitol and 1,5-di-*O*-acetyl-3,6-anhydro-1-deuterio-2,4-di-*O*-methyl-D-galactitol. Each standard was checked for purity by GLC and had the expected EI-mass spectrum[6].

*1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol.*—Methyl α-D-galactopyranoside was methylated, hydrolysed, reduced, and acetylated[7]. The crude product was purified by flash chromatography on silica gel, eluting with 1:3 hexane–EtOAc. Fractions were analysed by GLC and those which contained pure compound were combined and dried to yield a colourless syrup which had the expected EI-mass spectrum[8].

*1,3,4,5-Tetra-*O*-acetyl-2,6-di-*O*-methyl-D-galactitol.*—Methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside[9] was methylated, hydrolysed, reduced, and acetylated[7]. The crude product was purified by flash chromatography on silica gel, eluting with 1:1 hexane–EtOAc. Fractions were analysed by GLC, and those that contained pure compound were combined and dried to yield a white solid that had the expected EI-mass spectrum[8].

*1,5,6-Tri-*O*-acetyl-1-deuterio-2,3,4-tri-*O*-methyl-D-galactitol.*—1,6-Anhydro-β-D-galactopyranose was *O*-permethylated, hydrolysed, reduced (with $NaBD_4$) and acetylated[7]. The crude product was purified by flash chromatography on silica gel, eluting with 1:1 hexane–EtOAc. Fractions were analysed by GLC as above. A white solid was obtained that had the expected EI-mass spectrum[10].

*1,2,3,4,5-Penta-*O*-acetyl-6-*O*-methyl-D-galactitol.*—1,2 : 3,4-Di-O-isopropylidene-α-D-galactopyranoside[11] was methylated, hydrolysed, reduced, and acetylated[7]. The product was crystallised from EtOH and had the expected EI-mass spectrum[8].

*1,2,3,4,5,6-Hexa-*O*-acetyl-galactitol.*—Galactitol was acetylated[6], and the product was purified by flash chromatography on silica gel. The resulting white crystals had the expected EI-mass spectrum[8].

Alkali modification.—Two different sets of reaction conditions were used: (*1*) polysaccharide (100 mg) was dissolved in distilled water (20 mL), and $NaBH_4$ (20 mg) added. The mixture was stirred overnight. $NaBH_4$ (60 mg) and aq NaOH (3 M, 10 mL) were added, and the mixture was heated at 80°C for 7 h[12]. The mixture was cooled, neutralised with glacial acetic acid, dialysed against distilled water (×3), then lyophilised to give GdT-AM1; (*2*) polysaccharide (25 mg) was dissolved in distilled water (2.5 mL), then a solution of NaOH (200 mg) and $NaBH_4$ (20 mg) in distilled water (2.5 mL) was added. The mixture was heated at 100°C for 3.5 h, then cooled, neutralised with glacial acetic acid, dialysed against distilled water (×3), and lyophilised to give GdT-AM2.

Solvolytic desulphation.—The pyridinium salt form of the native polysaccharide from *G. decipiens* was prepared by dialysis against aqueous pyridinium hydrochloride (~ 0.1 M, adjusted to pH 6.5) several times, then against distilled water, and

finally lyophilised. A sample of the resulting material was dissolved in 89:10:1 Me_2SO–MeOH–pyridine, 0.4 mL/mg. The mixture was heated for 4 h at 100°C[13]. The sample was cooled, dialysed against tap water, then distilled water (×3), and isolated by lyophilisation to give GdT-DS.

RESULTS AND DISCUSSION

Analysis of native polysaccharide.—The amyloglucosidase-treated, freeze-dried extract of the March, 1991 sample of *Gigartina decipiens* tetrasporophyte (GdT) was a white fluffy solid obtained in 55% yield from air-dried seaweed and had a sulphate content of 41%. The infrared spectrum showed an intense band at 1250 cm^{-1} characteristic of sulphate esters generally and a broad band at 820–840 cm^{-1} due to equatorial 2- and 6-sulphate ester groups which are characteristic of λ-type carrageenans[12] (Fig. 2).

The high viscosity of aqueous λ-carrageenan solutions, even when hot, makes it difficult to obtain well resolved ^{13}C NMR spectra. Five spectra have been reported previously[14–18]. Rochas in 1982 partially hydrolysed a commercial "λ"-carrageenan (Sigma) to improve the NMR resolution[14]. Despite this, the spectrum still appeared complex due to the hybrid nature of the sample (Stevenson and Furneaux later showed that Sigma "λ"-carrageenan also contained sugar residues characteristic of ξ- and π-carrageenans[6]). De Lestang Bremond et al. published a spectrum of a λ-carrageenan fraction from *Chondrus crispus*[15]. This spectrum contained only a single intense peak in the anomeric region at 100 ppm, rather than two peaks expected for a polymer with a disaccharide repeating unit. McCandless and Gretz obtained a "good spectrum containing only twelve signals" from λ-carrageenase-treated λ-carrageenan from *Iridaea cordata* although the spectrum, itself, was not published[16]. Rochas and Lahaye in 1989 used ultrasonication to reduce the viscosity of the λ-carrageenan from *Gigartina canaliculata* prior to NMR spectroscopy[17]. The resulting spectrum was well resolved but complex, indicative of a hybrid structure. Furneaux and Miller[18] also obtained a poorly resolved spectrum of Sigma "λ"-carrageenan, but noted a characteristic signal at 66.4 ppm. Due to the external tetramethylsilane referencing system used in this work[18], this chemical shift value is ca. 1.6 ppm larger than it would be when referencing to internal Me_2SO at 39.4 ppm as for spectra reported herein. An exceptionally clear ^{13}C NMR spectrum of native GdT has been obtained (Fig. 3). We attribute this to a fortuitous mild depolymerisation of the sample during spectral accumulation at 90°C. The same result was obtained on spectroscopic examination of a second sample of the same polysaccharide, but in a third experiment a much noisier spectrum with broader resonances was recorded. λ-Carrageenans are rather susceptible to depolymerisation, as can be observed during their extraction from seaweed. In the absence of an alkaline buffer (e.g., $NaHCO_3$), their hot aqueous solutions can become acidic and lose viscosity. Anecdotal evidence (Dr. Dimitri J. Stancioff, personal communication) points to an oxidative free-radical reaction

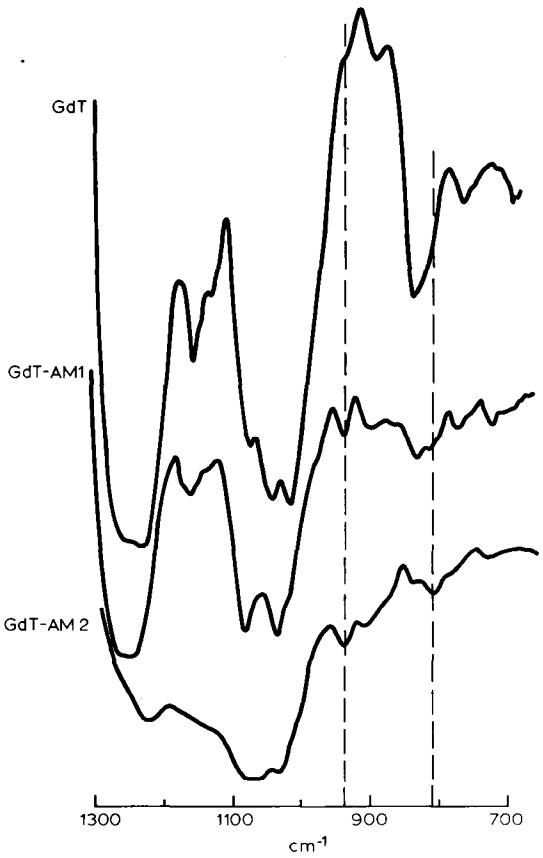

Fig. 2. Infrared spectra of polysaccharide samples from tetrasporophytic *Gigartina decipiens*. GdT (native); GdT-AM1 (alkali modified, Method 1); GdT-AM2 (alkali modified, Method 2). Dotted lines indicate 805 and 935 cm^{-1}.

being involved in the onset of depolymerisation, since it is accelerated by iron ions or tannins and by increased mixing with air (oxygen). Depolymerisation of the polymer during NMR analysis may be associated with degradation so the accumulation of spectral data was monitored visually. After approximately three hours, changes (e.g., in the position of the C-1 resonances) were observed in the spectrum, which suggested that the polysaccharide was being degraded. The spectrum reported here is that seen before any changes had occurred.

Such depolymerisation in situ of the GdT sample is indeed fortuitous, because the simple twelve line ^{13}C NMR spectrum that can thus be obtained confirms that the polysaccharide is largely composed of a single repeating disaccharide unit. Two resonances are observed in the anomeric region, as would be expected from such a disaccharide repeating unit. They occur at 103.4 and 91.6 ppm for the 3-linked and 4-linked residues, respectively (Table I). These positions are quite different from those for κ-, ι-, μ-, and ν-carrageenans[19,20]. The spectrum also contains a peak at

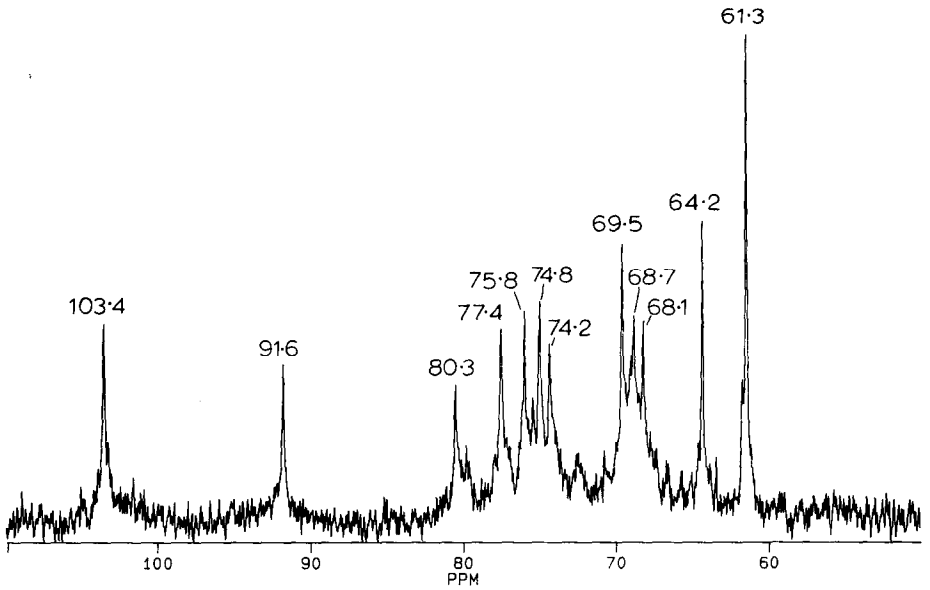

Fig. 3. ^{13}C NMR spectrum of λ-carrageenan (GdT) from tetrasporophytic *Gigartina decipiens* (5448 scans).

64.2 ppm, which is in the same characteristic region mentioned above. The ^{13}C NMR spectrum of GdT has been tentatively assigned (Table I).

Constituent sugar analysis showed that the native polysaccharide consisted almost solely of galactose (Table II). Methylation analysis of the polysaccharide in the triethylammonium salt form was complete in one step, and the results are shown in Table III. This analysis revealed largely 2,3-Gal (i.e., 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl-galactitol) corresponding to 2-sulphated, 3-linked galactopyranosyl residues and 2,4,6-Gal (i.e., 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-galactitol) corresponding to 2,6-disulphated 4-linked galactopyranosyl residues, with a lesser but significant amount of 2,3,6-Gal (corresponding to 2,6-disulphated 3-linked galactopyranosyl residues). While the latter has been previously observed[6,21], its occurrence has not been widely associated with λ-carrageenans. In the classical

TABLE I

Assignment of chemical shifts in the ^{13}C NMR spectra of native (GdT), alkali modified (GdT-AM2), and desulphated (GdT-DS) polysaccharide samples from tetrasporophytic *Gigartina decipiens*

Sample	3-Linked residue						4-Linked residue					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
GdT	103.4	77.4 [a]	75.8 [a]	64.2	74.2	61.3	91.6	74.8	69.5	80.3	68.7 [b]	68.1 [b]
GdT-AM2	100.3	77.6 [c]	77.2 [c]	67.8	74.7	61.1	95.6	74.7	77.4 [c]	79.5	77.0 [c]	69.8
GdT-DS	104.7	70.5	78.8	65.7	75.2	61.2	96.2	69.2	70.9	78.6	70.4	61.4

[a,b,c] Assignments may be interchanged.

TABLE II

Constituent sugar analysis (normalized mol%) of the polysaccharide from tetrasporophytic *Gigartina decipiens* and various derivatives

Constituent sugar [a]	GdT	GdT-AM1	GdT-AM2 [b]	GdT-DS
AnGal	1	35	40 (46)	1
Gal	99	65	60 (54)	99

[a] AnGal determined as 1,2,4,5-tetra-*O*-acetyl-3,6-anhydro-D-galactitol, Gal as D-galactitol hexaacetate. [b] Numbers in parentheses refer to the results obtained with extra reducing reagent.

structure for λ-carrageenan, derived from the polysaccharide from tetrasporophytic *Chondrus crispus*, 30% of the 3-linked galactosyl units are not 2-sulphated[2]. Very little 3-Gal was observed in the methylation analysis of GdT, however, indicating that the polysaccharide is almost fully 2-sulphated on its 3-linked galactosyl residues. No differences were observed in the analysis of the September, 1991 sample.

Alkali-modified polysaccharide.—Treatment of polysaccharides containing 4-linked galactosyl 6-sulphate residues with hot alkali results in the formation of 3,6-anhydrogalactosyl residues through intramolecular displacement of the 6-sulphate group[22]. Alkali modification of idealised λ-carrageenan would yield a polysaccharide composed of alternating 3-linked β-D-galactosyl 2-sulphate and 4-linked 3,6-anhydro-α-D-galactosyl 2-sulphate residues. The native polysaccharide

TABLE III

Glycosyl-linkage analysis (normalized mol%) of the polysaccharide from tetrasporophytic *Gigartina decipiens* and some derivatives thereof

Constituent sugar and deduced substitution [a]	GdT	GdT-AM1	GdT-AM2 [d]	GdT-DS
3-Gal	1	2		47
2,3-Gal [b]	39	39	41 (39)	
3,6-Gal				1
2,3,6-Gal [c]	8	7	7 (6)	
4-Gal	1			44
2,4-Gal	3	2	3 (3)	2
2,4-AnGal	1	35	38 (41)	
2,4,6-Gal [c]	42	8	1 (1)	
T-AnGal				1
T-Gal				3
3,4-Gal [b]	Tr	Tr	Tr	2
2,3,4-Gal	3	4	4 (4)	
2,3,4,6-Gal	2	3	6 (6)	

[a] 2,4-Gal means a 2,4-disubstituted and/or linked galactopyranosyl residue, analysed as 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-galactitol; T-Gal means a terminal sugar unit, analysed as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol etc. [b] 2,3- and 3,4-Gal coelute from a Supelco 2330 column[7]. A trace amount of 3,4-Gal was detected in each sample containing 2,3-Gal by GLC–MS analysis. [c] Enantiomeric, partially methylated alditol acetates differentiated and determined by deuterium labelling[6]. [d] Numbers in parentheses refer to the results obtained with extra reducing reagent.

from tetrasporophytic *G. decipiens* was treated with alkali using the method of Craigie and Leigh[12] (Method 1) to give GdT-AM1, which had a sulphate content of 34.8%. Infrared analysis of GdT-AM1 showed a new peak at 935 cm^{-1} characteristic of 3,6-anhydrogalactosyl residues, although a peak remained at 820–830 cm^{-1} (Fig. 2). Previously published spectra of alkali-modified λ-carrageenans[12,23] show peaks of only weak intensity in this region. This peak would thus appear not to be associated with an equatorial 2-sulphate (from 3-linked residues). If it was attributed to 6-sulphate substituents, this would suggest that anhydrogalactose formation was incomplete. A further small peak at 805 cm^{-1} was attributed to 3,6-anhydrogalactosyl 2-sulphate residues[12].

Constituent sugar analysis of GdT-AM1 now revealed both galactosyl (Gal) and 3,6-anhydrogalactosyl (AnGal) residues (Table II), but the latter were present in a smaller proportion than would have been expected if all of the 4-linked 2,6-disulphated residues had been converted. Methylation analysis showed that 2,4-AnGal had indeed been formed, but also that 2,4,6-Gal residues remained (Table III). Evidently the alkali modification had been incomplete under these "standard" conditions. An alternative explanation for the residual 2,4,6-Gal residues would be the existence of branches attached at the 6-position of 2-sulphated, 4-linked galactosyl residues[21]. However, a consequence of branches would be the presence of terminal sugar residues that were not observed in the methylation analysis. Although the alkali modification reaction appeared to be incomplete, the ^{13}C NMR spectrum of the alkali-modified polysaccharide was well resolved with twelve major peaks visible. The positions of the two major resonances in the anomeric region had changed to 100.3 and 95.6 ppm for the 3- and 4-linked residues, respectively, although small peaks corresponding to λ-carrageenan remained. The positions of certain other peaks had also altered due to the structural changes caused by alkali modification.

Alkali modification of the polysaccharide under more forcing conditions (Method 2) produced GdT-AM2 in 79% yield. Constituent sugar analysis showed that the 3,6-anhydrogalactosyl (AnGal) content had risen to 40% (Table II). The Gal:AnGal ratio is now close to, but not exactly, 1:1. However, the known protective effect of a 2-sulphate ester group on the adjacent 3,6-anhydrogalactosidic bond (cf., ι-carrageenan)[6] makes it difficult to release all the AnGal residues under the conditions of reductive hydrolysis before the reducing agent has been consumed. Addition of extra reducing agent (4-methylmorpholine–borane; 0.05 mL) to the sample before and after the 80°C prehydrolysis step, led to a higher recovery of AnGal (46%, Table II). Methylation analysis of GdT-AM2 showed that very little 2,4,6-Gal remained (Table III), and thus alkali modification was almost complete under these conditions. Again the amount of 2,4-AnGal was increased by the addition of extra reducing agent, but to a lesser extent. The amount of unmethylated galactose residues present in this singly methylated sample of GdT-AM2 was relatively high, but after a second methylation the amount of 2,4-AnGal had declined and T-Gal had appeared, indicating polymer degradation.

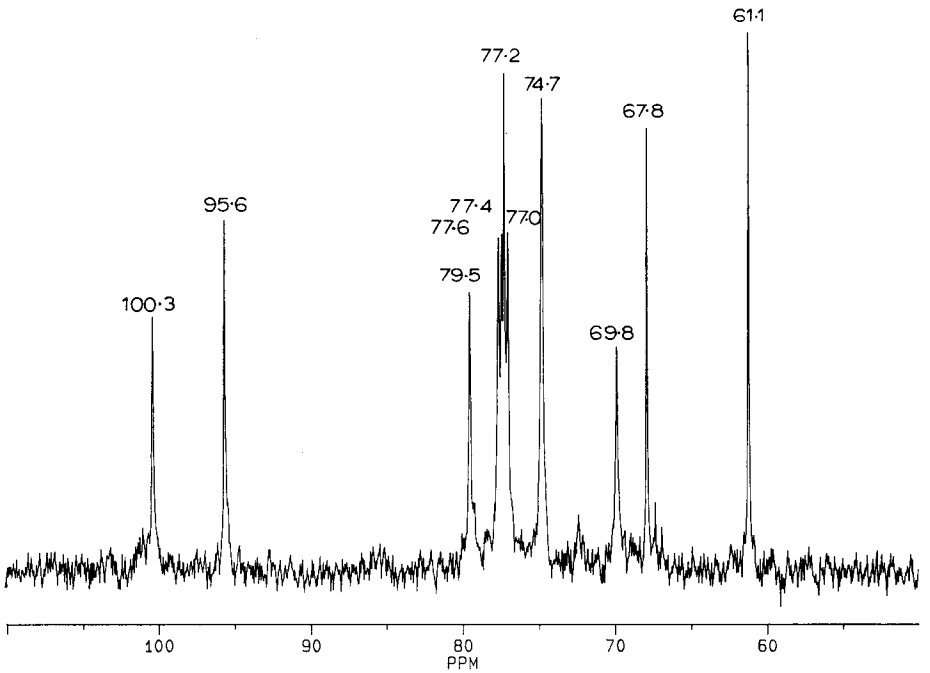

Fig. 4. ^{13}C NMR spectrum of alkali modified λ-carrageenan (GdT-AM2) from tetrasporophytic *Gigartina decipiens* (5114 scans).

Infrared analysis of this material showed bands at 805 and 935 cm^{-1} (Fig. 2), which were assigned to 3,6-anhydrogalactosyl-2-sulphate and to 3,6-anhydrogalactosyl residues, respectively[12], as above. The weak band observed at 830 cm^{-1} must correspond to the equatorial 2-sulphate and the small amount of 6-sulphate on the 3-linked residues. The percentage of 2,4,6-Gal in GdT-AM2 was much lower than in GdT-AM1. This suggested that the formation of 3,6-anhydrogalactosyl residues under alkaline conditions was merely more difficult than expected, rather than impossible due to the existence of sugar branches on O-6 of 4-linked residues. Independent confirmation that the rate of alkali modification for λ- compared with κ-family polysaccharides is 20–60 fold slower, was presented in a recently[24]. This rate difference was unexpected. The only difference between λ-carrageenan and ν-carrageenan (the ι-precursor) is in the position (O-2 vs. O-4) of a sulphate ester on the 3-linked residues adjacent to the 4-linked residue undergoing reaction.

The ^{13}C NMR spectrum of GdT-AM2 is shown in Fig. 4. The same twelve major signals are observed as in GdT-AM1. Our assignments for the ^{13}C NMR spectrum of GdT-AM2 are given in Table I. Recently, Stortz and Cerezo developed a computer program which calculated the expected ^{13}C NMR chemical shifts for a range of structural units including those of native and alkali-modified λ-carrageenans[25]. Whilst such an exercise offers a useful guide to potential chemical shifts, it is dependent on the extrapolation of existing values from known

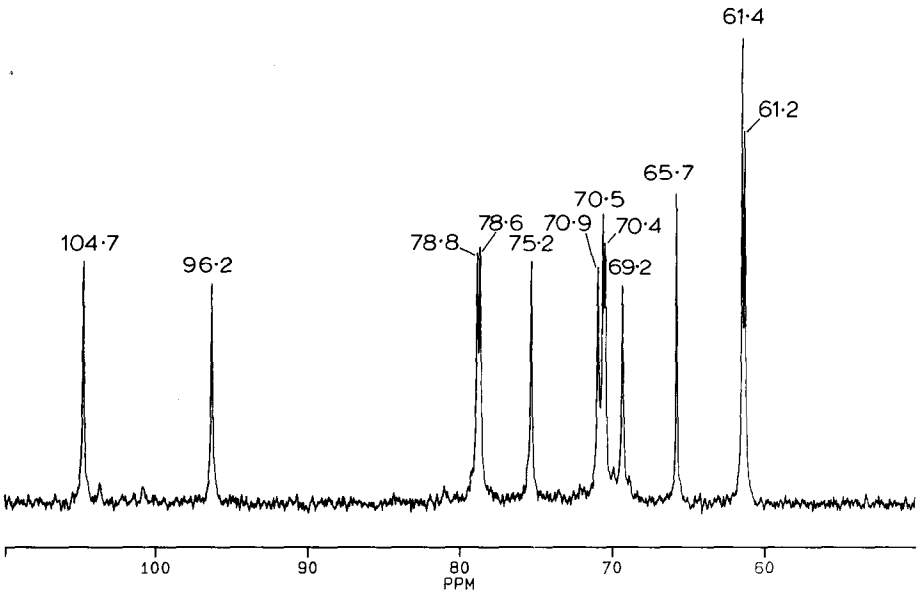

Fig. 5. ^{13}C NMR spectrum of desulphated λ-carrageenan (GdT-DS) from tetrasporophytic *Gigartina decipiens* (5758 scans).

structures. It will, therefore, require revision in the light of the experimental data reported here for λ-carrageenan and its alkali-modified derivative, both of which differ significantly from the calculated values.

Desulphated polysaccharide.—The sulphate groups were removed solvolytically from the native polysaccharide by heating its pyridinium salt form in a Me_2SO–MeOH–pyridine mixture to give GdT-DS, which had a sulphate content of 1.9%. Desulphation of idealised λ-carrageenan would yield a polysaccharide composed of 3-linked β-D-galactopyranosyl and 4-linked α-D-galactopyranosyl residues. The ^{13}C NMR spectrum of the desulphated polysaccharide was composed of twelve, well-resolved peaks (Fig. 5). The chemical shifts of these peaks (Table I) corresponded to those assigned by Usov et al.[4] in 1980 for a methanolic HCl-desulphated commercial λ-carrageenan, which was a linear polymer composed of alternating 3-linked β-D- and 4-linked α-D-galactopyranosyl residues. Constituent sugar analysis showed that no 3,6-anhydrogalactose was formed during the desulphation process (Table II). Methylation analysis confirmed the presence of equimolar amounts of 1,3-linked and 1,4-linked galactosyl residues (Table III), which is consistent with a structure composed of repeating disaccharide units. No 4,6-Gal was observed, which confirmed the absence of sugar branches on O-6 of the 4-linked galactose residues. Very little 3,6-Gal and no 2,3-Gal were observed, which suggested that the unusual 2,3,6-Gal residues observed in the methylation analysis of the native polymer were due to 2,6-disulphated 3-linked galactose residues.

CONCLUSIONS

The use of modern analytical techniques has facilitated the identification of the polysaccharide from tetrasporophytic *Gigartina decipiens*. The results show that the polysaccharide is predominantly a λ-type carrageenan. It has a trisulphated repeating disaccharide unit in which the 2- and 6-positions of the 4-linked residues and the 2-position of the 3-linked residues are fully sulphated. In addition, a small proportion of 3-linked residues also have a sulphate ester group at the 6-position (Fig. 1B).

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