Pergamon

PII:S0031-9422(97)00060-5

Phytochemistry, Vol. 45, No. 5, pp. 1009–1013, 1997

0031-9422/97 $17.00+0.00

A L-GALACTOSE-CONTAINING CARRAGEENAN FROM CYSTOCARPIC *GIGARTINA SKOTTSBERGII*

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(*Received in revised form* 19 *November* 1996)

Key Word Index—*Gigartina skottsbergii*; Rhodophyta; carrageenans; L-galactose; methylation; ^{13}C NMR.

Abstract—Alkaline treatment of a κ/ι-carrageenan from cystocarpic *Gigartina skottsbergii* and further fractionation with potassium chloride, led to the isolation of a minor product soluble in 2 M KCl with unusual structural features. It contains small amounts of L-galactose and is built up from κ/ι- and λ-blocks.

INTRODUCTION

The structures of the major carrageenans extracted from cystocarpic *Gigartina skottsbergii* have been thoroughly studied [1–3]. In a previous paper, we reported some structural features of an L-galactose-containing product, soluble in 2 M KCl, obtained by alkaline treatment of a partially cyclised μ/ν-carrageenan and further fractionation with potassium chloride [4]. Some unusual galactans with similar characteristics have been isolated from tetrasporophytes [5, 6] and from cystocarpic plants of other algae belonging to the Gigartinaceae [7]. We report herein, the characterization and structural analysis of another 2 M KCl-soluble fraction, isolated when the same procedure described above was carried out on a gelling carrageenan of the same origin.

RESULTS

κ/ι-Carrageenan $1C_1$ [3] was submitted to an alkaline treatment and the modified product ($1C_1T$) was further fractionated with potassium chloride to yield a minor fraction soluble in 2 M KCl (SF). Yield, analysis and optical rotation of SF are shown in Table 1; it contains only galactose and 3,6-anhydrogalactose (molar ratio 1.27:1.00) and the ratio D:L-galactose is 1.00:0.14. Chromatography of SF on DEAE Sephadex A-50 (Cl^-) resulted in irreversible binding of the sample to the resin; SF was not recovered even after treatment of the anion-exchanger with boiling 4 M NaCl or 0.5 M NaOH in 3.5 M NaCl.

SF was converted into the triethylammonium salt [8] and methylated using the Hakomori procedure [8, 9], in the conditions usually employed for carrageenans of the κ family [5]. Table 2 indicates the composition of the permethylated derivative. SF is comprised mainly of 3-linked galactose 4-sulphate and 2-sulphate units in a molar ratio of 1.6:1.0, and 4-linked 3,6-anhydrogalactose and 3,6-anhydrogalactose 2-sulphate residues in a molar ratio of 1.0:1.0; non-substituted β- and α-galactose units also contribute but in minor proportion to the structure of SF. Significant amounts of 2,3,4,6-tetra-*O*-methylgalactose (~4.5%) in permethylated SF would indicate the presence of single stubs of galactose, while 3-*O*-methylgalactose arises from 4-linked galactose 2,6-disulphate units not cyclised during the sequence of alkaline treatment methylation. Minor quantities of other mono-*O*-methylgalactose residues are consistent with the presence of disulphated units in the original product.

The 75 MHz ^{13}C NMR spectrum of SF showed, in the anomeric region, major signals at δ 103.0 (with a shoulder at 102.9), 95.8 and 92.6, and smaller peaks at δ 104.0 and 92.1, which were assigned to κ-, ι- and λ-diads; resonances of the other carbon atoms of these diads are also present in the spectrum (Table 3) [10–12]. The molar ratio of 3,6-anhydrogalactose: 3,6-anhydrogalactose 2-sulphate, calculated from the area of the corresponding anomeric signals, is 1.6:1.0.

DISCUSSION

The matrix galactans of the Rhodophyta consist of linear chains of alternating 3-linked β-galactose (A

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Table 1. Yield, analysis and optical rotation of SF*

Fraction	Yield† (%)	Carbohydrate composition (mol%) D-Gal	L-Gal	D-3,6-AnGal	D-3,6-AnGal 2S	L-3,6-AnGal	Sulphate‡ (as SO_3K) (mol %)	$[\alpha]_D$ (°)
SF	0.9 (3.0)	49.1	6.9	26.8§	17.2§	—	85.0	+24.0
Fs¶	0.2 (1.6)	48.8	24.5	—	8.9	17.8	41.1	−13.0
T_3	8.6 (10.2)	68.6	29.4	←———	2.0 ——→		71.6	+11.2

* Fractions Fs [4] and T_3 [5] were included for comparison.

† Yield from 100 g of whole carrageenan 1C (in parentheses, % of total recovered from 1C) [1].

‡ Moles of sulphate per 100 mol sugar.

§ Determined from areas of corresponding anomeric signals in ^{13}C NMR spectrum.

¶ Fs also contains minor quantities of Rha (1.2%), Xyl (2.4%), Man (4.3%), Glc (5.3%) and 3-*O*-methylgalactose (3.0%).

units) and 4-linked α-galactose (B units). The A units always occur in the D-form, while the B units can occur in either D- (carrageenans) or L- (agars) form. This difference defines two families of polysaccharides which are bridged by a group of polysaccharides containing variable amounts of D- and L-galactose B-units interspersed in the same molecule [13].

Carrageenophytes of the Gigartinaceae and Phyllophoraceae, biosynthesise different structures according to the stage of the life-cycle [13, 14]. For *G. skottsbergii* and *Iridaea undulosa*, it has been proved that carrageenans of the κ-family are produced by cystocarpic plants, whereas tetrasporic plants yield λ-carrageenans [1–3, 5]. This clear-cut scheme has been altered recently by reports of the biosynthesis of minor quantities of L-galactose-containing galactans by cystocarpic [4, 7] and tetrasporic carrageenophytes [5, 6]. In addition, these molecules could be hybrids built up by structures of the κ- and λ-types [4–6].

SF was obtained after an elaborated fractionation procedure which comprised a sharp potassium chloride precipitation (between 0.30–0.31 M KCl) of a κ/ι-fraction ($1C_1$) from the raw carrageenan [1], its alkaline treatment and a further potassium chloride precipitation of the gelling derivatives. SF remained soluble in 2 M KCl and this solubility behaviour seems to be a general characteristic of these galactans [4–6]. Such soluble polysaccharides obtained from cystocarpic plants were always isolated after alkaline treatment of the starting material [4, 7]. This procedure is similar to that of ref. [15], used for the fractionation of a mixture of μ/ν- and λ-carrageenans obtained from an unsorted sample of *Chondrus crispus*. This approach indicates complexation between μ/ν- and λ-structures, which is broken by cyclisation of the 4-linked α-galactose 6-sulphate or 2,6-disulphate units. The interaction between different carrageenan structures has been reported previously [5], showing its influence on the potassium chloride solubilities of the individual products. Moreover, the solubility behaviour of SF would indicate that this product is a block copolymer with κ/ι- and λ-domains as the complexation requires, not only complementary interaction sites but also their arrangement in ordered polyvalent arrays [16]. The unsuccessful anion-exchange chromatography of SF, as well as the electrophoresis of similar galactans obtained from *Chondrus crispus*, *G. canaliculata*, *G. leptorynchos* and *Mastocarpus stellata* [7] are consistent with the presence of a copolymer with κ/ι- and λ-blocks. The existence of these blocks would also explain why a product with mainly a κ/ι-structure (59%) does not have gelling properties.

It may be unusual to attribute a λ-structure to a product obtained after an alkaline treatment but, because the starting material ($1C_1$) was a κ/ι-carrageenan [1-3], the conditions of this treatment were those reported previously for polysaccharides of the κ-family [17]. It is known that the cyclisation reaction is 20–60 times faster for carrageenans of the κ-family than for those of the λ-family [18]. Taking into account the amount of λ-structure in the precursor of SF (given by the percentage of 4,6-di-*O*-methylgalactose in the methylated derivative), it may be estimated from the half-life [18] that ~2.5% of the α-galactose 2,6-disulphate units linked to β-galactose 2-sulphate residues were cyclised during the alkaline treatment and, therefore, SF should still contain ~12% of the former residues. This result is in agreement with the signals found at δ 104.0 and 92.1 in the ^{13}C NMR spectrum of SF and with the detection of ~3% of 3-*O*-methylgalactose in its methylated derivative, because cyclisation proceeds further during methylation. A new alkaline treatment of SF for 24 hr (~eight half-lives for the cyclisation of α-galactose 2,6-disulphate residues in λ-carrageenans) [18] led to an increase of ~3% in the 3,6-anhydrogalactose content, in agreement with the above discussion and with the resistance shown by the α-units to cyclisation.

The small amount of L-galactose is probably interspersed in the carrageenan structure because, if present as traces of a contaminant galactan, it would probably had been lost during the isolation procedure of SF.

In conclusion, cystocarpic *G. skottsbergii* biosynthesises not only the major κ/ι- and μ/ν-carrageenans previously reported [1–3] but also a small amount of a polymer that would be a hybrid containing κ- (41%), ι- (18%) and λ- (29%) structures and single stubs of galactose (~4.5%). Products with similar charac-

Table 2. Composition of sugars produced by permethylation and hydrolysis of SF*

Fraction	Mol % of sugars having methyl groups at the positions indicated															
	Galactose														3,6-AnGal	
	2,3,4,6	2,3,6	2,4,6	3,4,6	2,6	4,6	3,4	2,4	2,3	6	2	3	4	—	2	—
SF†,‡	6.1	3.3	3.5	2.1	←34.0→		tr§	—	1.9	3.7	1.7	←2.7→		tr.	20.3	20.7
SF‖	3.2	2.2	1.8	tr.	23.5	14.6	—	—	←4.8→		3.6	3.5	1.9	tr.	←41.0→	
Fs¶,**	19.5	3.0	5.5	—	18.7	16.2	2.4	—	—	1.1	2.1	—	5.6	—	←25.9→	
T_3¶††	14.0	6.6	9.5	1.6	16.3	10.7	—	25.3	4.3	8.0	1.7	tr	tr	—	←2.0→	

* Composition of permethylated derivatives of Fs and T_3 given for comparison.

† Subjected to reductive hydrolysis and further acetylation.

‡ Minor amounts of 2,3,6-tri-*O*-methylglucose (1.9%) and traces of glucose detected.

§ Percentages lower than 1% are considered as trace (tr).

‖ Hydrolysed with 2 M TFA and derivatised to aldononitrile acetates.

¶ Hydrolysed with 45% HCO_2H and derivatised to aldononitrile acetates.

** Permethylated Fs also contains 2,3,4-tri-*O*-methylxylose (8.0%) and minor quantities of 2,4-di-*O*-methyl- and 2,3-di-*O*-methyl-xylose and 2,3,6-tri-*O*-methylglucose.

†† In T_3 2,3,4-tri-*O*-methylxylose (2.5%) detected.

Table 3. ^{13}C NMR signal assignment of SF

Diad	C-1	C-2	C-3	C-4	C-5	C-6
3-Linked β-D-Gal 4-sulphate	103.0	70.1	79.4	74.8	75.5	61.9
4-Linked α-D-3,6-AnGal	95.8	70.3	79.8	78.9	77.4	70.1
3-Linked β-D-Gal 4-sulphate	102.9*	70.1	77.4	72.8	75.4	61.9
4-Linked α-D-3,6-AnGal 2-sulphate	92.6	75.5	78.5	78.9	77.6	70.3
3-Linked β-D-Gal 2-sulphate	104.0	78.1	76.4	64.8	74.7	61.9
4-Linked α-D-Gal 2,6-disulphate	92.1	75.5	70.1	81.0	69.3	68.8

* Shoulder on signal at δ 103.0.

teristics have been reported in the μ/ν-fraction of the same seaweed [4], in gamethophytes of *C. crispus*, *G. canaliculata*, *G. leptorynchos* and *M. stellata* [7] and in tetrasporophytes of *G. skottsbergii* [6] and *I. undulosa* [5], suggesting that they are usual minor components of the carrageenans produced by seaweeds belonging to the Gigartinaceae.

EXPERIMENTAL

Material. Cystocarpic *G. skottsbergii* was collected in Bahía Camarones (Provincia de Chubut, Argentina) and sorted in the Instituto Nacional Patagónico (CONICET) (Puerto Madryn, Chubut).

General. Sulphate was analysed by the method of ref. [19]. 3,6-Anhydrogalactose was determined, before and after alkaline treatment of the sample, by the resorcinol method [20]. Optical rotations were measured using a 1.1% soln of the sample in H_2O. The monosaccharide composition was determined by reductive hydrolysis of the sample [8] and derivatization for GC. GC of the alditol acetates [21] was performed on a fused-silica capillary column (30 m × 0.25 mm i.d.), WCOT coated with SP-2330 (film thickness 0.20 μm). The oven temp. was 220° and the inj. and FID temps were 235°. N_2 was used as carrier at 1 ml min^{-1} with split ratio of 80:1. Determination of D:L-galactose was carried out by GC of the acetylated 1-deoxy-1-(2′-hydroxypropylamino) alditols [22]. ^{13}C NMR were recorded at 75 MHz with proton decoupling at 80°, with ext. TMS as ref.; a soln of the sample (30 mg) in 1:0.2 H_2O–D_2O (0.4 ml) and a 5 mm-tube were used. Specific parameters include a pulse angle of 90°, an acquisition time of 0.27 sec, no pulse delay and a spectral width of 15 kHz; the number of scans was 720 000.

Alkaline treatment of $1C_1$ and fractionation of the modified product. Alkaline treatment of the sample (4 g) was carried out in 0.5 M NaOH–0.5 M NaCl (3 l) for 90 min at 80° [17]; the soln was neutralised with HOAc with constant agitation. Fractionation of $1C_1T$ was carried out as indicated for whole carrageenan [1]. In order to achieve further cyclisation of α-galactose 2,6-disulphate residues in λ-carrageenan, alkaline treatment was carried out in M NaOH for 24 hr at 80°C [18].

Methylation analysis of SF. The sample (5 mg) was converted into the corresponding triethylammonium salt and methylated by the Hakomori procedure [9] as described in refs [5, 8]. One portion of permethylated SF was subjected to reductive hydrolysis [8] and the partially methylated alditols were acetylated [21]; another portion of the permethylated sample was hydrolysed with 2 M TFA for 2 hr at 121° and the partially methylated galactoses derivatised to the aldononitrile acetates [23]. GC of the alditol acetates and aldononitrile acetates was carried out as described in ref. [4]. GC-EIMS of the alditol acetates [24, 25] and aldononitrile acetates [23] was performed at 70 eV; MS were recorded over the range 40–500 mu. Chromatography was carried out using the SP-2330 column, programmed to run with an initial 1 min hold at 130° and then at 6° min^{-1} to 230°. The He flow rate was 1 ml min^{-1}, the inj. temp. 240° and the split ratio 100:1.

Acknowledgements—We are indebted to Engineer C. Gey from CERMAV-CNRS (Grenoble, France) for running ^{13}C NMR spectrum. This work was supported by grants from CONICET, OAS and the University of Buenos Aires.

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