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Note

Structural studies on the soluble polysaccharide of *Chondrus canaliculatus*. ^{1}H-n.m.r. study of hexa-*O*-acetylcarrabiose dimethyl acetal*

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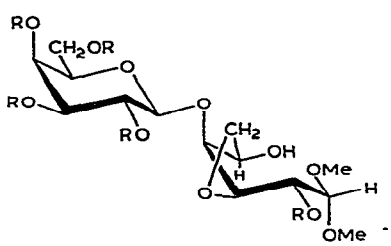
Chondrus canaliculatus is a red seaweed, of the family Gigartinaceae, that grows in the northern and central parts of the Chilean coast. A preliminary study on this seaweed was performed by Marini-Bettólo and Ibáñez[2], who found that it contains a soluble polysaccharide that behaves like the carrageenan from *Chondrus crispus*.

From the hydrolyzate of the soluble polysaccharide, D-galactose was isolated, and from the methanolyzate, methyl α-D-galactopyranoside (**1**), 3,6-anhydro-D-galactose dimethyl acetal (**2**), and O-β-D-galactopyranosyl-(1→4)-3,6-anhydro-*aldehydo*-D-galactose (carrabiose) dimethyl acetal (**3a**) were obtained. Carrabiose dimethyl acetal was identified by its physical constants, and by a ^{1}H-n.m.r. study (270 MHz) of its per-*O*-acetyl derivative (**3b**).

The ^{1}H-n.m.r. spectrum of **3b** showed that it has six acetyl and two methoxyl groups; the other signals integrated well for 14 protons, in agreement with the structure shown.

A spectrum (500-Hz sweep-width) of the region outside that for the acetyl and methoxyl groups permitted making the assignments shown in Table I, wherein



3a R = H

3b R = $COCH_3$

*Polysaccharides from Chilean Seaweeds, Part VI. For part V, see ref. 1.

TABLE I

^{1}H-N.M.R. DATA FOR HEXA-*O*-ACETYLCARRABIOSE DIMETHYL ACETAL (**3b**), METHYL 2,3,4,6-TETRA-*O*-ACETYL-*β*-D-GALACTOPYRANOSIDE (**4**), AND 2,4,5-TRI-*O*-ACETYL-3,6-ANHYDRO-D-GALACTOSE DIMETHYL ACETAL (**5**) IN $CDCl_3$

Compound	*Chemical shifts (δ)*									*Coupling constants (Hz)*						
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	*OAc*	*OMe*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
3b[a]	4.61	5.20	5.03	5.40	—	—	—	[b]		8.0	10.0	3.5	—	—	—	—
3b[c]	4.50	5.09	—	—	5.30	—	—	[b]	3.38 3.44	7.0	3.0	—	—	—	—	—
4	4.41	5.20	5.03	5.40	3.93	4.14	4.21	1.98 2.06 2.06 2.15	3.52	7.5	10.5	3.3	0.8	6.5	6.5	6.5
5	4.52	5.31	4.06	5.10	5.13	3.94	4.02	2.08 2.10 2.16	3.36 3.43	7.3	3.1	~4.7	2.8	<1.5	4.6	10.0

[a]Data for the 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl group. [b]Chemical shifts for the acetyl groups of **3b**. [c]Data for the 2,5-di-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal residue.

TABLE II

MOLAR RELATIONS OF MONOSACCHARIDES AND SULFATE GROUPS, AND I.R. DATA, FOR FRACTIONS AND WHOLE POLYSACCHARIDE OF *Chondrus canaliculatus*

Material	*Galactose*	*3,6-Anhydro-galactose*	*Sulfate*	ν_{max} *(cm^{-1})*
Whole polysaccharide	1.0	0.6	1.1	930, 840
Fraction 1	1.0	0.7	1.2	930, 840
Fraction 2	1.0	1.2	1.5	930, 840
Fraction 3	1.0	0.3	1.0	930, 840–810

the proton chemical-shifts of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**4**) and of 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal (**5**) are included[3,4]. These two compounds (**4** and **5**) may be considered to be models for the analysis of the ^{1}H-n.m.r. spectrum of **3b**.

For the galactopyranosyl ring, the positions of the signals of H-2, H-3, and H-4 do not show any change in relation to those of the same protons in the spectrum of **3b**; only H-1 shows a moderate, downfield shift, which is consistent with the fact that a methoxyl group has a higher shielding effect than a glycosyl group[5–9]. The change of the H-1 signal to lower field could be taken as an indication that, in fact, the glycosidic bond involves C-1 of the tetra-*O*-acetyl-β-D-galactopyranosyl group.

For the 3,6-anhydro ring of the dimethyl acetal, it may be seen that the signals of H-1, H-2, and H-5 are only slightly shifted from the resonance positions of the same protons in compound **5**, while the signal of H-4 is shifted to a much higher field (δ 4.04 or less) in relation to the same proton in compound **6** (δ 5.08). This upfield shift, of at least 1.04 p.p.m., can only be attributed to the effect of the replacement of the acetyl group at O-4 by the tetra-*O*-acetyl-D-galactopyranosyl group. The aforementioned, upfield shift is in good agreement with that shown[10] by H-4 (δ 4.01) of the 2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl group in the disaccharide methyl hepta-*O*-acetyl-α-maltoside in relation to the signal-position of H-4 in the spectrum of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside. The moderate shift-differences shown by H-2 and H-5 of the anhydro ring of **3b** could be explained by assuming that the glycosidic linkage forces those protons into magnetic environments influenced by the spatial proximity of the other ring residue[11].

The composition (molar proportions) of the whole polysaccharide, as well as those of the fractions (see Table II) separated by the addition of solutions of potassium chloride[12] of increasing concentration, is also in agreement with a general structure of the carrageenan type for the soluble polysaccharide of *Chondrus canaliculatus*.

EXPERIMENTAL

General. — Melting points were measured with an Electrothermal melting-point apparatus, and are uncorrected. Optical rotations were measured with a Hilger–

Watts polarimeter. Evaporations were performed *in vacuo*, the bath temperature being kept below 45°. Infrared spectra (films) were recorded with a Perkin–Elmer Model 621 spectrometer. Proton-n.m.r. spectra were recorded, and integrated at 100 MHz and 270 MHz, respectively, with a Varian HA-100 spectrometer and with a 270-MHz spectrometer built at the University of British Columbia with Nicolet parts. Sample concentrations were 6–8% in deuteriochloroform, and tetramethylsilane was used as the internal, reference standard. Analytical and preparative paper chromatography were performed on Whatman No. 1 and No. 3 papers, respectively. Preparative t.l.c. was performed on glass plates coated with silica gel (type 60, Merck). The solvent systems (v/v) used were *A* (4:1:2 1-butanol–ethanol–water), *B* (4:1:5 1-butanol–acetic acid–water), *C* (8:2:1 ethyl acetate–pyridine–water), and *D* (4:1 benzene–ethyl acetate). Centrifugations were conducted at 18,000 r.p.m. in an MSE Superspeed Centrifuge at room temperature. Gels obtained by precipitation with potassium chloride were dissolved in sodium acetate solution, and dialyzed first against the same solution, and then against distilled water. The percentage of 3,6-anhydrogalactose was determined by the procedure reported by Yaphe[13]. Sulfate was analyzed according to the procedure reported by Schöniger[14].

Extraction and hydrolysis of the polysaccharide. — The dried, ground seaweed (220 g) was extracted with water (2 L); the resulting mixture was filtered through muslin, and the extraction process was repeated twice. The mixture was centrifuged and, after dialysis against distilled water, the supernatant liquor was evaporated to a syrup which was poured into acetone, giving 27.2 g (12.3%) of a fibrous polysaccharide. This product was dissolved in water, and reprecipitated by pouring the solution into ethanol.

A portion of the polysaccharide was hydrolyzed with 0.5M sulfuric acid, giving a syrup that showed galactose as the sole monosaccharide in paper chromatography with solvents *B* and *C*. After preparative paper-chromatography, D-galactose, m.p. 164–165°, $[\alpha]_D^{19}$ +78.3° (water), was isolated; a sample of this product was reduced, and the alditol acetylated to give hexa-*O*-acetylgalactitol, m.p. and mixed m.p. 166–167°.

Methanolysis. — This was performed on 3.0 g of polysaccharide according to the procedure reported by Araki and Hirase[15]. The resulting syrup was chromatographed on a column (70 × 5 cm) of cellulose with solvent *A*; 10-mL fractions were collected.

On evaporation, fractions 36–40 gave **2** as a syrup (0.16 g) which could not be crystallized from any solvent; $[\alpha]_D^{22}$ +33.3° (*c* 1.2, water); R_F 0.66 (solvent *A*); lit.[15,16] for 3,6-anhydro-D-galactose dimethyl acetal: $[\alpha]_D$ +36.5° (water); R_F 0.67 (solvent *A*). A sample of this syrup was acetylated, giving **5**; its ^{1}H-n.m.r. spectrum was in full agreement with published data[4] for 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal.

On evaporation, fractions 46–50 gave a syrup (0.47 g) which was dissolved in hot ethanol; on cooling, **1** was obtained as crystals, whose m.p., specific rotation, and ^{1}H-n.m.r. spectrum were in agreement with published data for methyl α-D-

galactopyranoside, as were the physical constants of its 2,3,4,6-tetra-*O*-acetyl derivative.

Fractions 51–65 were evaporated, and the resulting syrup was purified by preparative paper-chromatography (solvent *A*), affording 0.77 g of a chromatographically pure syrup. A sample of this was acetylated, and the resulting acetate was purified by preparative t.l.c. with solvent *D*. The resulting product was crystallized from 85% ethanol, affording **3b** as crystals, m.p. 154–155°, $[\alpha]_D^{22}$ −20.5° (*c* 0.4, benzene); lit.[17] for hexa-*O*-acetylcarrabiose dimethyl acetal: m.p. 153–154°, $[\alpha]_D$ −16.5° (benzene); ^{1}H-n.m.r.* ($CDCl_3$, 270 MHz): δ 1.98 (s, 3 H, acetyl), 2.04 (s, 3 H, acetyl), 2.08 (s, 3 H, acetyl), 2.10 (s, 3 H, acetyl), 2.15 (s, 3 H, acetyl), 2.16 (s, 3 H, acetyl), 3.38 (s, 3 H, methoxyl), 3.44 (s, 3 H, methoxyl), 3.90–4.06 (5 H), 4.14 (m, 2 H), 4.50 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1′), 4.61 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.03 (dd, 1 H, $J_{3,2}$ 10.0, $J_{3,4}$ 3.3 Hz, H-3), 5.09 (dd, 1 H, $J_{2,3}$ 3.0, $J_{2,1}$ 7.0 Hz, H-2′), 5.20 (dd, 1 H, $J_{2,3}$ 10.0, $J_{2,1}$ 8.0 Hz, H-2), 5.30 (m, 1 H, H-5′), and 5.40 (dd, 1 H, $J_{4,3}$ 3.4, $J_{4,5}$ 1.0 Hz, H-4).

Fractionation of the polysaccharide. — A solution of the whole polysaccharide in water was fractionated by addition of a solution of increasing concentration of potassium chloride, according to the procedure reported by Pernas *et al.*[12]; three fractions were obtained: Fraction 1 (precipitated by 62mM potassium chloride), Fraction 2 (precipitated by 0.25M potassium chloride), and Fraction 3 (soluble in 0.25M potassium chloride).

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*Protons of the 3,6-anhydro dimethyl acetal ring are denoted by primed numbers.

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