Carbohydrate Research, 36 (1974) 201–204

Note

The carrageenan system of *Gigartina skottsbergii* S. et G.

Part IV. Methylation analysis of a partially desulphated derivative

ALBERTO S. CEREZO

Departamento de Química Orgánicà, Facultad de Ciencias Exactas y Naturales, Pab. 2, Ciudad Universitaria, Buenos Aires (Argentina)

(Received September 24th, 1973; accepted for publication in revised form, February 15th, 1974)

In earlier work[1], a fraction of carrageenan (previously referred to as fraction A) was isolated from *Gigartina skottsbergii* by precipitation with 0.3–0.4M potassium chloride[1]. Studies carried out on this polysaccharide and its partially desulphated derivative suggested the presence of galactose 2-sulphate residues[1]. However, methylation analysis[2] of fraction A showed that all the sulphated D-galactose was 3-linked and sulphated at C-4. The latter conclusion has now been verified by methylation analysis of a partially desulphated fraction A.

Repetition of the desulphation[1] of fraction A (sulphate as SO_3Na, 29.4%), but using Dowex-50(H^+) resin[3] and hydrolysis for 12 h only, increased the yield of the desulphated derivative to 60.5–75.0% (extreme values for different batches). The product (SO_3Na, 16.4–18.7%) was used for further work.

The partially desulphated carrageenan was reduced and methylated as previously described[2]. After acid hydrolysis, 2,6-di-*O*-methyl- and 2,4,6-tri-*O*-methyl-D-galactose were separated and characterized[2]. A small amount of tetra-*O*-methyl-D-galactose was also separated; this was undoubtedly due to the shortened chain-length caused by methanolysis during desulphation. Trace of another tri-*O*-methylated-D-galactose, which showed the chromatographic behaviour of 2,3,6-tri-*O*-methyl-D-galactose, were detected. All these products were also isolated[2] after hydrolysis of methylated fraction A.

The isolation of 2,6-di-*O*-methyl-D-galactose and 2,4,6-tri-*O*-methyl-D-galactose confirms the presence[2] of 3-linked D-galactose 4-sulphate residues and 3-linked, non-sulphated D-galactose residues in fraction A. The fact that the yield of the trimethylated sugar was higher, and that of the dimethylated derivative was lower, than those obtained in the hydrolysis of methylated fraction A shows that sulphate-ester groups linked to C-4 of D-galactose residues were split off during the heterogeneous, acid hydrolysis.

The oxidative, acid hydrolysis of the methylated, partially desulphated fraction A was carried out by a modification of the procedure described previously[2,4].

3,6-Anhydro-2-*O*-methyl-D-galactonic acid and 3,6-anhydro-D-galactonic acid were subsequently isolated and characterized[2]. Paper chromatography indicated the presence of small quantities of 3,6-anhydro-2,4-di-*O*-methyl-D-galactonic acid.

The isolation of 3,6-anhydro-2-*O*-methyl-D-galactonic acid and 3,6-anhydro-D-galactonic acid confirmed previous results[1,2], showing the existence of sulphated and non-sulphated 3,6-anhydro residues in the original polysaccharide and (accepting that position 4 is engaged in a galactosidic linkage) that the sulphate ester is located at position 2. Although many of the fractions separated were mixtures and a complete assessment of the yields of the products is impossible, the quantity of 3,6-anhydro-2-*O*-methyl-D-galactonic acid in the present experiments appeared to be greater, and the amount of 3,6-anhydro-D-galactonic acid less, than in the previous experiments[2]. This suggests that some of the sulphate groups of the 3,6-anhydrogalactose residues were split off during the heterogeneous, acid hydrolysis.

EXPERIMENTAL

For general methods, physical constants, and details of the characterization of the relevant sugar derivatives, see ref. 2.

Partial desulphation of fraction A. — Fraction A (5 g), which was obtained[1] from *Gigartina skottsbergii* by precipitation with 0.3–0.4M potassium chloride, was suspended in absolute methanol (500 ml) together with dried Dowex-50(H^+) resin (20 g) and dry sodium chloride (3 g). The mixture was shaken at room temperature for 12 h, after which time a considerable amount of material remained insoluble. The resin and the insoluble material were separated and shaken with water (2 × 50 ml) until all the degraded polysaccharide was dissolved. The aqueous and the methanolic solutions were combined, dialysed against running tap-water until free from salts, concentrated, and freeze-dried, and the residue was dried at room temperature, *in vacuo*, over phosphorus pentaoxide. Yields from different batches ranged from 60.5–75.0% (after discounting the loss of sulphate), with sulphate contents (as SO_3Na) of 16.4–18.7%; $[\alpha]_D$ +53.5 ±3° (*c* 0.6, water). The i.r. spectrum showed an absorption centered at 845–850 cm^{-1} which did not extend over 830 cm^{-1}.

Methylation of partially desulphated fraction A. — The partially desulphated fraction A was methylated in essentially the same way[2] as fraction A. After six methylations, the starting material (5 g) yielded 4.5 g of a methylated derivative (methoxyl, 21.0%). When this material was subjected to another five methylations, the final product (3.8 g) had 20.95% of methoxyl and 17.86% of sulphate (as SO_3Na).

Hydrolysis of methylated, partially desulphated fraction A and identification of the acid-stable products. — Methylated, partially desulphated fraction A (2 g) was treated with 45% formic acid, the hydrolysate was worked up, and the residue was fractionated by cellulose-column chromatography, as detailed previously[2], to give the following fractions.

Fraction 1 (0.4 g), R_F 0.77–0.80 (solvent B), was a mixture of degradation products, possibly formed from the 3,6-anhydro-D-galactose residues.

Fraction 2 was a syrup (0.02 g), $[\alpha]_D^{22}$ +104° (*c* 0.8, water). Paper chromatography (p.c.) (solvent B) indicated the presence of only one compound (R_F 0.68) with a mobility identical to that of 2,3,4,6-tetra-*O*-methyl-D-galactose. Reaction of the syrup with aniline yielded 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactopyranosylamine, m.p. 188–190°, $[\alpha]_D^{20}$ +36.5° (acetone); lit.[5] m.p. 192°, $[\alpha]_D$ +37.7° (acetone).

Fraction 3 (0.02 g), on p.c. (solvent B), was found to contain two substances, R_F 0.39 and 0.48, which were identified (p.c.) as 2,4,6-tri-*O*-methylgalactose and 2,3,6-tri-*O*-methylgalactose, respectively.

Fraction 4 (0.28 g) was a syrup containing only one substance (2,4,6-tri-*O* methyl-D-galactose), R_F 0.40. It was extracted with hot, dry, light petroleum to give material having m.p. 103–105° (from light petroleum), $[\alpha]_D$ +88° (*c* 0.5, water).

Fraction 5 (0.34 g), on p.c. (solvent B), was shown to contain only one substance (R_F 0.18), which was chromatographically identical to 2,6-di-*O*-methyl-D-galactose. On treatment with methanol, it crystallized, and recrystallization from ethyl acetate gave material having m.p. 103–105°, $[\alpha]_D$ +82° (*c* 0.5, water).

Oxidative hydrolysis of the methylated, partially desulphated fraction A. — Methylated, partially desulphated fraction A (2 g) was dissolved in 0.125M sulphuric acid (75 ml) containing 0.25 ml of bromine. The hydrolysis was carried out following the same procedure[2] used for fraction A, although the hydrolysis of the 3,6-anhydrogalactosidic linkages was continued for 41 h. The reaction was then stopped, although the reaction for combined 3,6-anhydrogalactose was still positive. The mixture of aldonic acids was separated from the neutral sugars by using diethylaminoethyl-Sephadex, and fractionated by cellulose-column chromatography (solvent C). Fractions (5 ml) were collected, and those of similar composition (p.c., solvent C) were combined and concentrated to dryness to give the following fractions.

Fraction 1 (94 mg), on p.c. (solvent C, reagent b), was shown to contain a major spot (R_F 0.71). With reagent c, two spots of R_F 0.39 and 0.72 appeared, which were identical chromatographically to 2,6-di-*O*-methyl-D-galactonic acid (R_F 0.38) and 3,6-anhydro-2,4-di-*O*-methyl-D-galactonic acid (R_F 0.72) (see ref. 2).

A solution of the syrup in 0.1M sodium hydroxide was kept for 6 h at room temperature. After neutralization with Dowex-50(H^+) resin, p.c. showed a major spot of 3,6-anhydro-2-*O*-methylgalactonic acid (R_F 0.46), together with lesser spots of 2,6-di-*O*-methylgalactonic acid (R_F 0.38) and 3,6-anhydro-2,4-di-*O*-methylgalactonic acid (R_F 0.72).

Fraction 2 (62 mg) was a syrup which contained the component of R_F 0.72 present in fraction 1, and another of R_F 0.81 (reagent b). After treatment as described above, 3,6-anhydro-2-*O*-methyl-D-galactonic acid together with 2,6-di-*O*-methyl-D-galactonic acid and minor amounts of 3,6-anhydro-D-galactonic acid were revealed by p.c. (reagent c).

Fraction 3 (0.24 g) was a syrup, $[\alpha]_D$ +78° (*c* 0.6, methanol), which crystallized on storage. Recrystallisation from ethyl acetate gave 3,6-anhydro-2-*O*-methyl-D-galactonic acid, m.p. 138–140°.

Fraction 4 (36 mg) was a mixture of 2,6-di-*O*-methyl-D-galactonic acid (major) and 3,6-anhydro-2-*O*-methyl-D-galactonic acid (minor).

Fraction 5 (84 mg) was 3,6-anhydro-D-galactonic acid.

ACKNOWLEDGMENTS

The author is indebted to Dr. Oscar Kühnemann (Centro de Investigaciones de Biología Marina, INTI) for the gift of the seaweed. This work was supported by a grant from the Consejo Nacional de Investigaciones Cientificas y Técnicas.

REFERENCES

1 A. S. CEREZO, *J. Chem. Soc., C*, (1967) 2491.
2 A. S. CEREZO, *Carbohyd. Res.*, 26 (1973) 335.
3 S. HIRANO, P. HOFFMAN, AND K. MEYER, *J. Org. Chem.*, 26 (1961) 5064.
4 N. S. ANDERSON, T. C. S. DOLAN, AND D. A. REES, *J. Chem. Soc., C*, (1968) 596.
5 W. N. HAWORTH, J. V. LOACH, AND C. W. LONG, *J. Chem. Soc.*, (1927) 3146.