[Chem. Pharm. Bull. 26(9)2668—2670(1978)]

UDC 547.458.02 : 581.192

A Polysaccharide of the Lichen, *Stereocaulon japonicum*[1]

Itsuro Yokota[2a] and Shoji Shibata[2b]

Faculty of Pharmaceutical Sciences, University of Tokyo[2]

(Received January 25, 1978)

The polysaccharides of *Stereocaulon japonicum* Th.Fr. were studied to find that a cold water-soluble fraction, SJ-2-I, is an α (1→3) (1→4) glucan (2.7: 1) ($\overline{DP}$: 64) partially branched at 3,4- or 2,3-positions.

Keywords——lichen polysaccharide; *Stereocaulon japonicum* Th.Fr.; Smith's degradation; glucan; methylation analysis

A water-soluble polysaccharide of *Stereocaulon paschale* (L.) Hoffm. reported by Hauan and Kjølberg[3] as being α (1→3) (1→4) glucan (4: 1) (mol. wt.: 22000) is the only polysaccharide of *Stereocaulon* spp. so far studied.

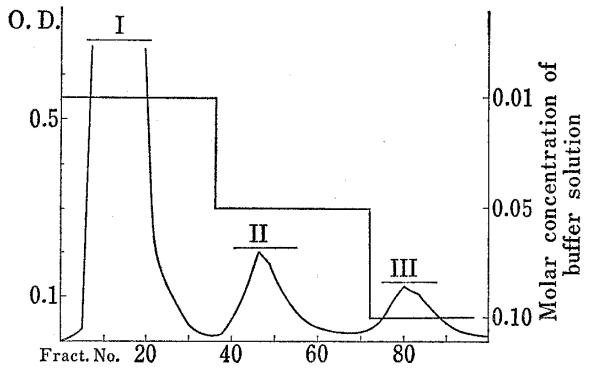

Fig. 1. The Chromatogram of Fraction SJ-2 on DEAE-Cellulose

Column size: 2.5×23.5 cm; Total amount fed for separation: 200 mg; Fraction: 15 ml each; Reagent: Phenol–H_2SO_4; O.D. measured at 490 nm.

The present paper concerns mainly the water-soluble polysaccharides of *Stereocaulon japonicum* Th. Fr. From aqueous extracts of this lichen a crude polysaccharide fraction (SJ-1) was obtained by the addition of ethanol. SJ-1 was subjected to the freezing and thawing procedure[4] to separate into a cold water-soluble (SJ-2) and an insoluble (SJ-3) fraction. SJ-2 fraction was chromatographed on a DEAE-cellulose column eluted with a phosphate buffer solution (pH 6.6) to obtain SJ-2-I, SJ-2-II and SJ-2-III fractions. By the acid hydrolysis followed by the determination of the hydrolyzed prod-

Table I. Properties of the Polysaccharides of *Stereocaulon japonicum*

Fract. No.	Yield (%)[a]	N (%)	IR (cm^{-1})	$[\alpha]_D$	Sugar components
SJ-1	15.0	0.5	840	+180°	Man Gal Glc (2:1:13)
SJ-2	9.0	0.4	840	+174°	Man Gal Glc (2:1:13)
SJ-3	0.06	1.0	843	+76°	Man Gal Glc (1:2:4)
SJ-2-I	5.5	0.3	850	+201°	— — Glc
SJ-2-II	0.5	Nil	810, 870	+70°	Man Gal Glc (3:1:1)
SJ-2-III	0.1	—	860	+46°	Man — Glc (3:1)

a) Calcd. from the dry weight of lichen thalli.

1) Part VII in the series of Polysaccharides of Lichens and Fungi. Part VI: K. Takahashi, T. Takeda, S. Shibata, M. Inomata, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), **22**, 404 (1974).
2) Location: *7–3–1 Hongo, Bunkyo-ku, Tokyo, 113, Japan*; *a*) Present address: *Nippon Chemiphar. Co., Ltd., 1-22 Hikokawato, Misato-shi, Saitama-ken, 341 Japan*; *b*) Present address: *Meiji College of Pharmacy, 1–35–23 Nozawa, Setagaya-ku, Tokyo, 154, Japan.*
3) E. Hauan and O. Kjølberg, *Acta Chem. Scand.*, **25**, 2622 (1971).
4) N.B. Chada, E.L. Hirst, and D.J. Manners, *J. Chem. Soc.*, **1957**, 1951.

ucts with a sugar analyzer, SJ-2-I was proved to be an α-homoglucan, and its molecular weight was determined by the Park-Johnson method[5] (mol. wt.: 10000; $\overline{DP}$=64).

Fully methylated SJ-2-I prepared by a combination of the Hakomori and Kuhn Methods[6] was methanolyzed, and the products were analyzed by a gas–liquid chromatography (GLC) to prove the formation of methyl 2,4,6-tri-O-methylglucopyranoside, methyl 2,3,6-tri-O-methylglucopyranoside (2.7:1), methyl 2,3,4,6-tetra-O-methylglucopyranoside together with a small amount of methyl di-O-methylglucopyranoside. The acetolysis of SJ-2-I afforded a trisaccharide peracetate which was separated by a column chromatography over silica gel. The trisaccharide peracetate was converted into a permethylate by the action of dimethylsulphate and alkali. The GLC of the methanolysis products derived from the trisaccharide permethylate gave a proportionally larger peak of methyl di-O-methylglucopyranoside than that observed in the methanolysis products of the permethylate of SJ-2-I to reveal that the branching point was not cleaved by the acetolysis of SJ-2-I to afford a peracetate of a branched trisaccharide.

The peak of methyl di-O-methylglucopyranoside in the GLC disappeared by further treatment of the methanolysis product with periodate to indicate that it must be methyl 2,6-di-O-methyl- or 4,6-di-O-methylglucopyranoside. Thus the major part of SJ-2-I has been

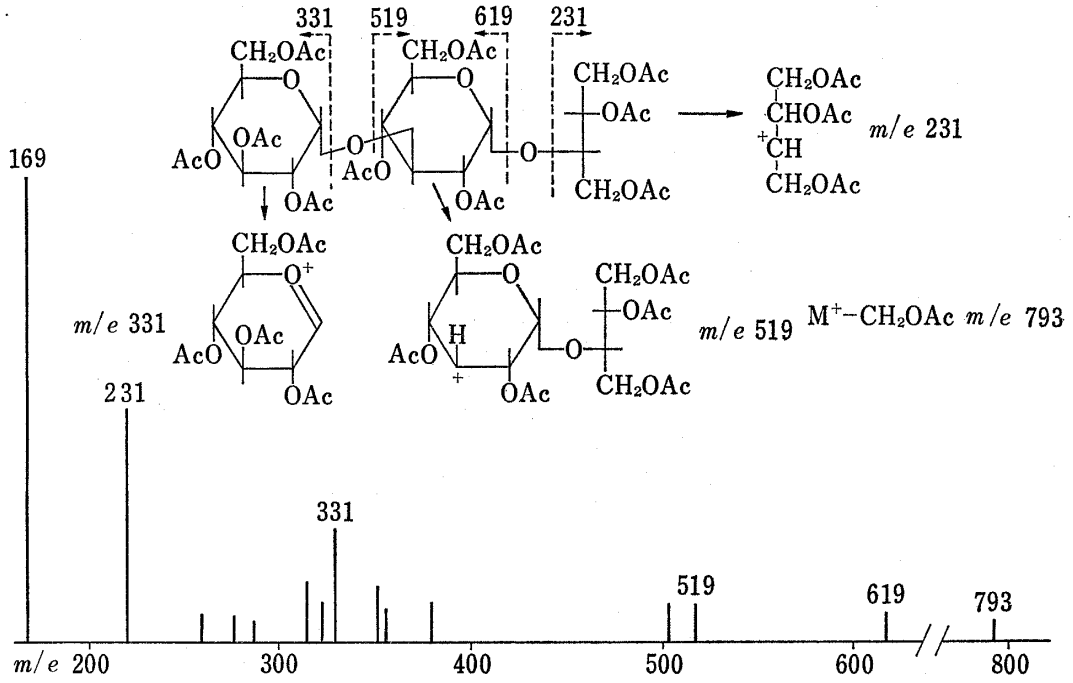

Fig. 2. The Mass Spectrum of Acetate of the Smith Degradation Product of SJ-2-I

linear portion:
major
→3)αD-Glc-(1→4)αD-Glc-(1→3)αD-Glc-(1→3)αD-Glc-(1→3)αD-Glc-(1→4)αD-Glc-(1→
minor
→3)αD-Glc-(1→4)αD-Glc-(1→3)αD-Glc-(1→3)αD-Glc(1→4)αD-Glc-(1→
branched portion:
(A) αD-Glc-(1→4) / αD-Glc-(1→3) } αD-Glc(1→ or (B) αD-Glc-(1→3) / αD-Glc-(1→2) } αD-Glc-(1→

Chart 1. Partial Structure of SJ-2-I

5) J.T. Park and M.J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).
6) S. Hakomori, *J. Biochem*, **55**, 205 (1964); R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955), K. Wallenfelds, G. Bechtler, R. Kuhn, H. Trischmann, and H. Eggi, *Angew. Chem., Int. Ed.* **2**, 515 (1963).

shown to be an α (1→3) (1→4) glucan (3: 1) partially branching at either 3,4 or 2,3-positions, (A) or (B). SJ-2-I was submitted to the controlled Smith degradation followed by acetylation to give a fraction which was separated by a preparative thin-layer chromatography (TLC). This fragment was proved by mass spectrometry (MS) to be a peracetate of nigerosyl erythritol. Therefore, α (1→3) (1→4) glucan (2: 1) would be present as a minor portion of SJ-2-I.

Experimental

Apparatus——Sugar analysis was performed using a JEOL liquid chromatographic autoanalyzer Model JLC-6AH. The infrared (IR) spectra were measured with a JASCO Model DS-402G spectrophotometer. The specific rotations were taken with a Yanagimoto polarimeter Model OR-50.

The GLC analysis was carried out with a Shimadzu Gas Chromatograph Model GC-5A with a hydrogen flame ionization detector. Mass spectra were measured with a Hitachi RMU-6L MS spectrometer.

Isolation and Purification of Polysaccharide Fractions of *Stereocaulon japonicum*——The lichen thalli of *Stereocaulon japonicum* collected in Fuji lake district (400 g) were extracted with acetone (3 l, 6 hr × 2) and 80% EtOH (3 l, 6 hr × 2) sequentially in order to remove soluble components, and the residual thalli were extracted further with dist. H_2O (3 l, 8 hr × 3) on a boiling water-bath. The hot extract was treated with EtOH to form precipitates which were collected by centrifugation, and dried to a pale brownish water-soluble substance (polysaccharide fraction SJ-I) (66.1 g).

SJ-I (10.0 g) was separated into a cold water-soluble SJ-2 (5.9 g) and an insoluble SJ-3 fraction (0.27 g), by the freezing and thawing procedure.[4] SJ-2-fraction (1.0 g) was chromatographed on a column of DEAE cellulose (6 cm × 30 cm) (phosphate form) to give an elution diagram indicating 3 peaks (SJ-2-I (0.62 g), SJ-2-II (0.06 g) and SJ-2-III (0.01 g)), estimated by phenol-H_2SO_4 method (OD measured at 490 nm).

Methylation of SJ-2-I——SJ-2-I (220 mg) was methylated by Hakomori-Kuhn combination method[6] treating with NaH (1.2 g), DMSO (50 ml) and MeI (5 ml × 2) for 72 hr, and then with MeI (10 ml + 5 ml) and Ag_2O (2 g × 2) in $CHCl_3$ (10 ml) for 24 hr. A fully methylated fraction which gave no OH absorption in IR spectrum was separated.

Methanolysis of fully Methylated SJ-2-I——SJ-2-I permethylate (100 mg) was treated with 5% MeOH-HCl (5 ml) at 80° for 6 hr. The methanolysis products were examined on GLC using a 2% XE-60 column at 150°.

The major and minor products were identified, respectively, as being methyl 2,4,6-tri-O-methylglucopyranoside (6.5 and 9.1 min), methyl 2,3,6-tri-O-methylglucopyranoside (7.4 and 8.2 min) and methyl 2,3,4,6-tetra-O-methylglucopyranoside (3.7 and 5.1 min) in comparison with authentic samples. Methyl di-O-methylglucopyranoside was assigned by the retention time (25 min).

Acetolysis of SJ-2-I——SJ-2-I (1.0 g) was acetolysed with AcOH (8 ml), Ac_2O (2 ml) and conc. H_2SO_4 (0.5 ml) at 0° for 1 hr. A trisaccharide peracetate fraction (50 mg) (MS *m/e*: 907 (M^+), 894, 865, 847, 805, 619, 605, 557, 331. NMR ($CDCl_3$) δ ppm: 2.0 (33H, $COC\underline{H}_3$), 3.4—5.6 (21H, $>C\underline{H}-$) obtained by a column chromatography over Si gel using C_6H_6 and MeOH (10: 0 to 10: 1) as the solvents was converted into a permethylate by the action of Me_2SO_4 (4 ml) and 30% NaOH (8 ml) in acetone (5 ml) for 4.5 hr. The solvent was distilled off below 20° and the residue was extracted with $CHCl_3$ to obtain the product.

The permethylated product was subjected to methanolysis under the same condition as described above for fully methylated SJ-2-I. The products were determined by GLC to give the same retention times for methyl 2,4,6-tri-O-methyl, methyl 2,3,6-tri-O-methyl, methyl 2,3,4,6-tetra-O-methyl, and methyl di-O-methylglucopyranoside, respectively, and the proportion of methyl di-O-methylglucopyranoside to others was larger than that given in the case of direct methanolysis of fully methylated SJ-2-I.

Controlled Smith Degradation of SJ-2-I Followed by Acetylation——SJ-2-I (500 mg) was treated with $NaIO_4$ (800 mg in 50 ml H_2O) under stirring in the dark for 125 hr, and then with ethylene glycol (3 ml) for 0.5 hr. The reaction mixture was dialysed and then reduced with $NaBH_4$ (0.18 g) for 20 hr. After neutralization with AcOH followed by dialysis, the reaction mixture was hydrolyzed with 0.1 N H_2SO_4 at room temperature for 6 hr. After neutralization with ion exchange resin (CG-4B (OH^-)), the hydrolyzate was acetylated with Ac_2O and pyridine. The product of acetolysis separated by a column chromatography over si gel (C_6H_6: MeOH 19: 1 to 9: 1) and by a preparative TLC (si gel, C_6H_6: MeOH 10: 1) to obtain a major fraction (SA, Rf=0.6).

Mass Fragmentation of Fraction SA——The fraction SA, a major fraction of the acetolysis products derived from SJ-2-I by the controlled Smith degradation, was analyzed mass spectrometrically to give ion peaks, 793 ($M^+ - CH_2OAc$), 519 (hepta-acetate of glucosyl erythritol—OAc), 331 (pentaacetylglucose—OAc), 231 (tetraacetyl-erythritol—OAc), and 169 (base peak). SA has, therefore, been proved to be nigerosyl erythritol peracetate.

Acknowledgement The authors wish to thank Dr. S. Kurokawa, National Science Museum, Tokyo, and Dr. M. Nuno, Yakurikenkyukai, for their kind help in identification of lichens. Thanks are also due to Ministry of Health and Welfare for grant.