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Note

Chemotaxonomic studies on the polysaccharides of lichens. Polysaccharides of stereocaulaceous lichens†

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Since the pioneering work of Berzelius[2], several polysaccharides of lichens have been studied The water-soluble polysaccharides, generally contained to the extent of $>10\%$ in lichen thalli are classified chemically into 3 groups (*1*) homoglucans[3–7], (*2*) heteroglycans[10] and (*3*) glycopeptides[18,19] The lichen homoglucans have been studied more extensively than other groups, as tabulated in Table I. nevertheless heteroglycans and glycopeptides are also known to be distributed in lichens

Some lichen polysaccharides show host-mediated, antitumor activity against implanted Sarcoma 180 in mice, and the structure–activity relationships have been discussed[10,18]

On the other hand the polysaccharides of lichens can be used to some extent as reference compounds for the chemotaxonomic classification of lichens, the most remarkable example of such use has been provided by the characteristic occurrence of pustulan, a partially acetylated (1→6)-β-glucan, in umbilicaliaceous lichens[10] The polysaccharides of stereocaulaceous lichens have been investigated in this respect, and the structure of a water-soluble polysaccharide, SJ-2-1, of *Stereocaulon japonicum* Th Fr has been reported[14,15] as being that of a (1→3), (1→4)-α-glucan (3 1) partially branched at either positions 3 4 or 2 3 as the main component, accompanying one having (1→3), (1→4)-α-linkages (2 1) as a minor structure A similar glucan having (1→3), (1→4)-α-linkages (5 2) was reported earlier[20] to have been isolated from *St pascale* (L) Hoffm

The present paper concerns further chemical screening of the cold-water-soluble polysaccharides of some other members of stereocaulaceous lichens, and certain chemotaxonomic considerations

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TABLE I

THE STRUCTURES AND DISTRIBUTIONS OF HOMOGLUCANS FROM LICHENS

Name	*Linkages*	*Ratio of linkages*	$\overline{dp}$	$[\alpha]_D$ *(degrees)*	*Lichens*	*References*
Lichenan	β-(1→3) (1→4)	3 7	80–400	+9 — +18	*Cetraria* spp *Parmelia* spp (Parmeliaceae)	3–5
Isolichenan	α-(1→3) (1→4)	3 2	34–43	+255	*Usnea* spp , *Alectoria* spp (Usneaceae)	3,6,7
Pustulan (GF 3)	β-(1→6) (partially acetylated)		120	−38	*Umbilicaria* spp , *Lasallia* spp (Umbilicariaceae)	8–10
Evernan (EP 7)	α-(1→3) (1→4)	3 2	70	+200	*Evernia* spp (Everniaceae)	11–13
PC-3	α-(1→3) (1→4)	1 1	100–130	+201	*Parmelia* spp (Parmeliaceae), *Cladonia* spp (Cladoniaceae)	16,19
Acroscyphan	α (1→3) (1→4) (1→6)		—	+176	*Acroscyphus sphaerophoroides* (Sphaerophoraceae)	17
SI 2-1	α (1→3) (1→4)	3 1	64	+201	*Stereocaulon* spp (Stereocaulaceae)	14,15
—	α (1→3) (1→4)	5 2	140	+233	*Stereocaulon* spp (Stereocaulaceae)	20

EXPERIMENTAL

General — I r spectra were recorded with a JASCO Model DS-420-G, specific rotations determined with a JASCO Model ORD/CD spectrometer, and the elemental analyses (%N) made with a Perkin–Elmer M 240 Analyzer Sugar analyses were performed with a JEOL chromatographic, fully automatic, analyzer Model JLC-6AH The ^{13}C-n m r spectra were recorded at 15 MHz at room temperature with a JEOL FX-60 NMR spectrometer using 10% solutions in D_2O in 10-mm (o d) tubes The n m r shifts were related to internal Me_4Si Mass-spectral analyses were performed with a JEOL Gas Chromatograph–Mass Spectrometer system (Model D-300) coupled with a JEOL data-analysis system (Model JMA-2000 disc system) In g l c –e i m s , the acceleration voltage was 3 00 kV the ionization energy, 70 eV, the ion-source temperature 190°, and the vacuum 100 ntorr, and in g l c –c i m s , the acceleration voltage was 200 eV , the ionization gas NH_3 the ionization energy, 3 00 kV, the ion-source temperature, 190°, and the vacuum. 10 μtorr

Preparation of DEAE-cellulose column — DEAE-cellulose (Whatman DE-23, 200 g) was suspended in water (15 vol), and degassed for 2 h in an ultrasonic-wave bath The fully stirred slurry was poured into a glass column (60 mm, o d × 1 m),

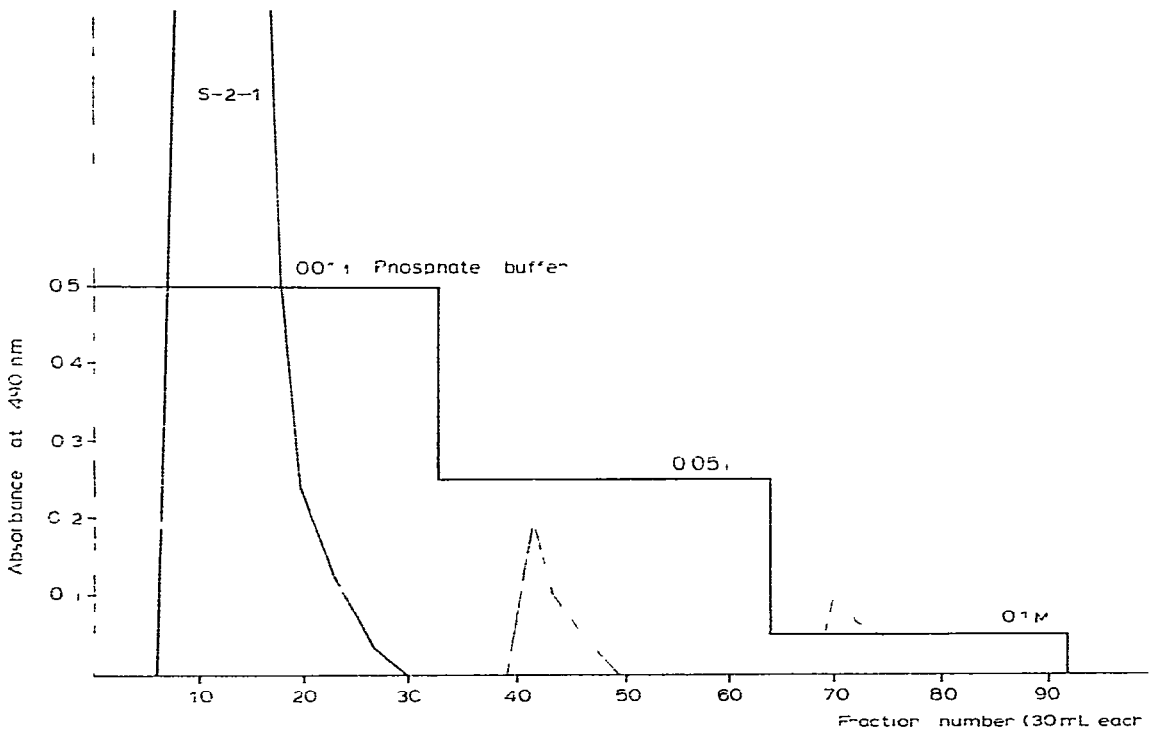


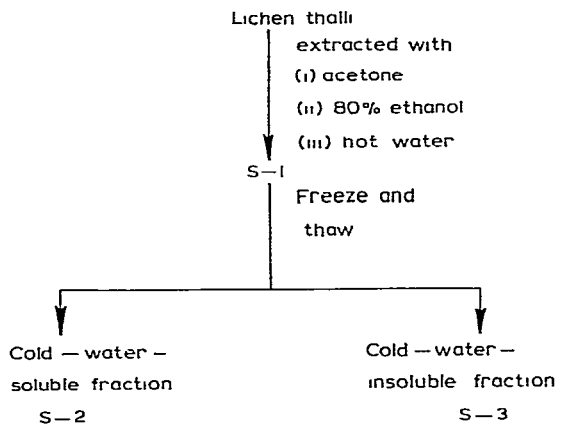
Fig 1 Elution diagram of S-2 on DEAE-cellulose, determined by phenol–sulfuric acid (as the reagent) and the absorbance at 490 nm

and successively eluted with 0 5M HCl (1 5 L) and distilled water to give an effluent of pH 4 0, and then with 0 5M NaOH (3 0 L) and distilled water to pH 7 0 The column was equilibrated with 0 1M phosphate buffer (3 0 L), and finally treated with the first buffer solution (0 01M, pH 6 8)

Materials — The following lichens were used *Stereocaulon sorediferum* Hue (collected at Ichijoji, Kyoto), *St exutum* Nyl (collected at Nikko), *St tomentosum* Fr (collected at Squamish, B C , Canada), *St intermedium* (Sav) Magn (collected at Mt Bandai), and *St vesuvianum* Pers (collected at Saiko, Mt Fuji) The results of the previous study[1] on *St japonicum* Fr (collected at Saiko Mt Fuji) were referred to, for comparison

Isolation and purification — Powdered lichen thalli (400 g) were twice extracted with acetone (3 L) during 6 h, and twice with 80% ethanol (3 L) during 6 h, in order to remove soluble components, and the residue was twice extracted with distilled water (3 L) during 8 h on a boiling-water bath The hot extracts were treated with ethanol, giving precipitates that were collected, and dried, to afford water-soluble polysaccharides (Fraction S-1) The crude polysaccharide was separated by the freeze–thaw method into a cold-water-soluble fraction (Fraction S-2) and an insoluble fraction (Fraction S-3)

A solution of fraction 2 (500 mg) in water (30 mL) was chromatographed (see Fig 1) on a column of DEAE-cellulose, using stepwise elution with a phosphate buffer (pH 6 8) of gradient concentrations (*i*) 0 01M (1 L), (*ii*) 0 05M (1 L), and (*iii*) 0 1M (1 L), each effluent collected was dialyzed, and the solution lyophilized (see Scheme 1)



Scheme 1 General fractionation process for lichen polysaccharides

Sugar analysis — Neutral-sugar components of each polysaccharide fraction were determined with a sugar analyzer as follows The polysaccharide was hydrolyzed with M H_2SO_4 for 12 h at 100°, and the hydrolyzate made neutral with Amberlite IRA-47 (OH^-), and evaporated to dryness The residue was dissolved in 0 13M borate buffer (2 mL), and examined with a sugar analyzer Determination of the

sugar components was made by the orcinol–sulfuric acid method, the absorbances at 510 and 425 nm being automatically recorded

Methylation analysis — The first fraction (50 mg) collected by chromatography on a column of DEAE-cellulose was methylated twice by the Hakomori method[21], to yield fully methylated polysaccharide that gave no OH absorption in the ir spectrum. The methylated polysaccharide was hydrolyzed with M sulfuric acid (5 mL) for 12 h at 100° the acid neutralized with Amberlite IRA-47 (OH^-), the filtrate evaporated to dryness and the residue reduced for 3 h with a solution of $NaBH_4$ (200 mg) in water (10 mL), with stirring After treatment with Amberlite IR-120 (H^+) until evolution of gas ceased. boric acid was removed by codistillation with methanol The product was dried *in vacuo*, and acetylated with acetic anhydride (5 mL) and pyridine (5 mL) for 12 h at room temperature The acetylation product was submitted to glc–ms in a column (2 mm × 2 m) of 2% of OV-225 under a pressure of carrier gas (N_2) of 2 kg cm^{-2}

TABLE II

CHARACTERIZING PROPERTIES OF LICHEN POLYSACCHARIDES ISOLATED FROM *Stereocaulon* SPP

Lichens	*Polysaccharide fraction No*	N(%)	$[\alpha]_D$ *(degrees)*	ν *(cm⁻¹)*	*Components*			*Yield* (%)
					Man	*Gal*	*Glc*	
St japonicum	SJ-1	0 5	+180	840	2	1	13	15 0
	SJ-2	0 4	−174	840	2	1	13	8 9
	SJ-2-1[a]	nil	+201	850	—	—	1	6 1
	SJ-3	1 0	−76	843	1	2	4	0 04
St soredulerum	SS-1	nil	−170	840	2	1	14	5 3
	SS-2	nil	−178	845	2	1	10	3 6
	SS-2-1[a]	nil	+230	845	—	—	1	1 8
	SS-3	2 2	−169	848	—	1	12	0 1
St evolutum	SE-1	—	+172	850	1	—	3	17 3
	SE-2	1 0	+183	850	1	—	3	11 3
	SE-2-1[a]	nil	−160	845	—	—	1	5 4
	SE-3	—	+54	843	2	1	7	0 6
St vesuvianum	SV-1	0 4	−43	845,891	3	7	9	5 7
	SV-2	0 9	+55	845,895	4	4	3	2 6
	SV-2-1[a]	0 7	−23	800,870	1	1	1	1 3
	SV-3	0 8	+74	843,895	4	4	3	0 2
St tomentosum	ST-1	0 9	−57	850,890	1	2	1	3 9
	ST-2	0 7	+88	840,880	4	3	2	1 9
	ST-2-1[a]	2 8	−80	800,890	5	4	3	0 9
	ST-3	0 8	+74	806,890	2	3	3	0 6
St intermedium	SI-1	—	+73	845,896	3	2	2	9 2
	SI-2	0 3	+88	840,880	—	—	—	6 5
	SI-2-1[a]	0 9	+70	800,890	7	5	8	0 9
	SI-3	—	+81	893 810	—	—	—	0 01

[a]The main fraction of the cold-water-soluble portion, separated by DEAE-cellulose column chromatography, this fraction was used for identification

RESULTS AND DISCUSSION

Some physicochemical properties of the crude polysaccharide and the fractions separated by freeze–thawing, followed by chromatography on a column of DEAE-cellulose, are given in Table II The series of purified water-soluble polysaccharides of *Stereocaulon* spp so far examined was grouped into α-glucans and β-dominant heteroglycans, by analysis of the sugar components nitrogen content (%) values of optical rotation, and i r -spectral absorptions

The structures of the α-glucans (SS-2-1 and SE-2-1) were investigated by ^{13}C-n m r spectroscopy, which revealed that they are very similar to[14,15] SJ-2-1 their structures having α-(1→3)-(1→4)-linkages (3 1) as the main sequence (see Table III)

In addition, methylation analysis was also performed to verify the nature of the linkages The permethylated α-glucan prepared by the Hakomori method was hydrolyzed, and the products were transformed into alditol acetate methyl ethers in the usual way For example, the retention times in g l c of the *O*-methylalditol acetates derived from SS-2-1 are shown in Table IV Each g l c fraction of *O*-methyl-alditol acetates was directly measured by e i m s The major peaks of these mass fragments agreed well with the results given earlier by Bjorndal *et al*[22], but the molecular-ion peak of each *O*-methylalditol acetate did not appear However, using c i m s , with ammonia as the ionization reagent-gas, QM^+ ions of *O*-methylalditol acetates were given The results of methylation analysis, based on the g l c–m s analysis, agreed with the structure of SS-2-I revealed by the ^{13}C-n m r spectra

In conclusion, the lichens of Stereocaulaceae so far tested may be classified into 2 groups according to their water-soluble, polysaccharide constituents One group, involving *Stereocaulon japonicum* *St sorediferum*, and *St exutum*, contains α-(1→3)-(1→4)-glucan (3 1), and a group comprising *St vesuvianum*, *St tomentosum*,

TABLE III

THE ASSIGNMENTS OF ^{13}C-N M R SIGNALS OF LICHEN HOMOGLUCANS ISOLATED FROM *Stereocaulon* SPP

Homoglucan fractions			*Assignment*
SS-2-1	*SJ-2-1*	*SE-2-1*	
101 0	101 2	101 0	C-1 (1→4)
100 4	100 3	100 3	C-1 (1→3)
81 0	80 9	80 9	C-3 (1→3)
78 2	78 2	78 2	C-4 (1→4)
74 4	74 4	74 5	C-3 (1→4)
73 6	73 8	73 6	C-5 (1→3)
72 7	72 7	72 7	C-2 (1→4)
71 6	71 6	71 6	C-2 (1→3)
			C-4 (1→3)
61 6	61 6	61 6	C-6 (1→3)
			C-6 (1→4)

TABLE IV

G L C–M S. DATA FOR *O*-METHYLALDITOL ACETATES DERIVED FROM SS-2-1 IN METHYLATION ANALYSIS

	O-Methylalditol acetates					
	1 5-Di-O-Ac-2,3,4 6-tetra-O-Me-D-glucitol		*1,3,5-Tri-O-Ac-2 4 6-tri-O-Me-D-glucitol*		*1,4 5-Tri-O-Ac-2 3,6-tri-O-Me-D-glucitol*	
	Retention time (min)					
	16 5		26 5		31 0	
	Mode					
m/z	*e i*	*c i*	*e i*	*c i*	*e i*	*c i*
43	1000[a]		1000[a]		1000[a]	
45	29		209		175	
71	119		318			
80		162				
87	154		214		179	
99					91	
101	543					
113					305	
117	320		512		669	
129	273		540			
145	242					
161	313		268			
205	104					
228				180		
233					243	
242						310
263		675				
291				300		210
340		1000[b]				
368				1000[b]		1000[b]

[a]Relative intensity to max 1000 [b]QM$^+$ ion $[M + NH_4]^+$

and *St intermedium* is characterized by the presence of a β-dominant heteroglycan containing D-mannose, D-galactose, and D-glucose The present results provide a guide for further chemotaxonomic investigations of Stereocaulaceae lichens

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