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Polysaccharides of Lichens

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It has been noted that lichens generally contain water-soluble polysaccharides in fairly high contents, but until recently, only lichenan, isolichenan and pustulan have been investigated chemically.

Lichenan (= lichenin) $[\alpha]_D + 9 \sim 18°$, $\overline{DP}$ 80 ~ 400, ir_{max}^{KBr} 890 cm^{-1} which was initially isolated from *Cetraria islandica* (Iceland moss) was studied extensively by several workers[2,11,12,15,26] and it has now been recognized as a linear homoglucan with $\beta(1 \rightarrow 3)$ $(1 \rightarrow 4)$ linkages in a ratio of 27 : 73 (3 : 7)[4]. The sequence of those linkages was studied by periodate degradation[6] and acid hydrolysis[21] obtaining glucose, laminaribiose (3-O-β-D-glucosyl glucose), cellobiose, cellotriose, 3 – O – β – cellobiosyl glucose, 4 – O – β – laminaribiosyl glucose, 4 – O – β – laminaribiosyl cellobiose, 3 – O – β – cellobiosyl cellobiose. Enzymatic hydrolysis of lichenan gave precise information about the sequence of linkages by the specificities of enzymes[23].

Cellulase of *Streptomyces* spp. QM B 814 which is known to split the $\beta(1 \rightarrow 4)$ linkage attached to a glucose unit whose 4—position is substituted, yielded 4 β – laminaribiosyl glucose as a main product, accompanied by 4 – O – β – laminaribiosyl cellobiose and 3 – O – β – cellobiosyl cellobiose, while laminarinase of *Rhizopus arrhizus* QM 1032 hydrolysing $\beta(1 \rightarrow 4)$ linkage of a 3 – substituted glucose unit afforded 3 – O – β – cellobiosyl glucose as a main product along with laminaribiose, 4 – O – β – laminaribiosyl cellobiose and 3 – O – β – cellobiosyl cellobiose. Neither laminaritriose nor branched disaccharide were found in the hydrolysate. The above results revealed that lichenan consists of tetrameric Glc $\beta 1 \rightarrow 3$ Glc $\beta 1 \rightarrow 4$ Glc $\beta 1 \rightarrow 4$ Glc as the main unit and pentameric Glc $\beta 1 \rightarrow 3$ Glc $\beta 1 \rightarrow 4$ Glc $\beta 1 \rightarrow 4$Glc $\beta 1 \rightarrow 4$ Glc as the sub-unit.

Isolichenan (= isolichenin) $[\alpha]_D$ +255°, $\overline{DP}$ 34 ~ 43, ir_{max}^{KBr} 925, 845 800 cm^{-1} which occurs in lichens, being accompanied by lichenan, was shown

to be a linear homoglucan with α (1 → 3) (1 → 4) linkages in a ratio of 57 : 43 (3 : 2). Isolichenan is more soluble in cold water than lichenan, and it is characterized by a slight blue colouration with iodine. The main part of isolichenan consists of Glc α 1 → 3 Glc α 1 → 3 Glc α 1 → 4 Glc linkages[7,22].

Earlier investigations showed the occurrence of lichenan and isolichenan in *Cetraria islandica*, *C. nivaris*, *Usnea barbata* and *U. longissima*[17]. Our recent investigations revealed the presence of both glucans in *Cetraria islandica* var. *orientalis*, *C. richardsonii*, *Parmelia tinctorum*, *P. conspersa*, *P. hypotrypella*, *P. nikkoensis*, *Alectoria sulcata*, *A. sarmentosa* and *Usnea bayleyi*.

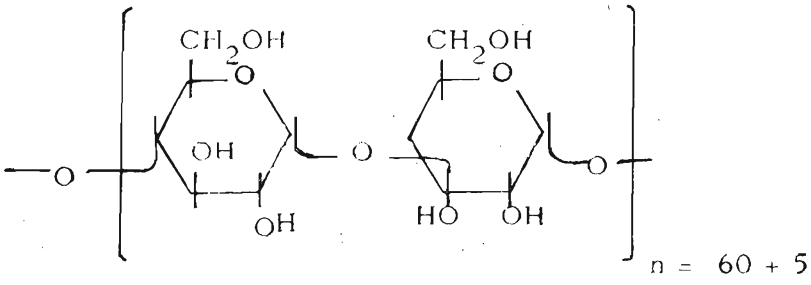
Pustulan was first isolated by Drake[5] in 1943 from *Lasallia pustulata* and *Umbilicaria hirsuta* and studied by Lindberg and McPherson[13] to formulate as linear glucan having β (1 → 6) linkage, since pustulan afforded on hydrolysis glucose, gentiobiose, gentiotriose, and gentiotetraose. This structure was also supported by the degradation of fully methylated pustulan[10]. According to our recent investigation, pustulan has been found characteristically in the lichens of Gyrophoraceae, e.g. *Gyrophora esculenta*, *Umbilicaria angulata*, *U. caroliniana*, *U. polyphylla*[20] and *Lasallia papulosa*.[24]. It has also been shown that pustulan contains 2% of O-acetyl group which is characterized by the ir absorption at 1735 and 1250 cm^{-1} and removed by alkaline hydrolysis with 2% aq. Na_2CO_3 at room temperature. The acetyl content in pustulan was determined by gas chromatography after hydrolysis, to reveal 1 acetyl group for every 10 to 12 glucose units.

By the method of Bouveng,[3] the native polysaccharide swollen in dimethylformamide was treated with phenylisocyanate to form a phenyl carbamate which was methylated by Kuhn's method and treated with $LiAlH_4$ to give a partially methylated glucan. The methyl ether in the 3-position of D-glucose was obtained by the hydrolysis of the partially methylated glucan to indicate, the acetyl group is attached to the 3-hydroxyl[19].

The molecular weight of pustulan has been determined by the equilibrium sedimentation method[33] to be c. 20 000 ($\overline{DP}$ c. 120).

PC-3 type glucan. *Parmelia caperata* contains a cold water insoluble polysaccharide tentatively named PC-3, and a soluble fraction (PC-2) which is identical with isolichenan. The polysaccharide PC-3 was methylated by Hakomori's method and the methyl ether was subjected to methanolysis to afford methyl 2, 3, 6 - tri - O - methyl *and* methyl 2, 4, 6 - tri - O - methyl - D - glucopyranosides, together with a small amount of methyl 2, 3, 4, 6 - tetra - O - methyl - D - gluco - pyranoside. Acetolysis of PC - 3 gave nigerose, maltose, 4 - O - α - nigerosyl D - glucose, and 3 - O - α - maltosyl D - glucose, whereas it gave neither maltotriose nor nigerotriose.

These results revealed that PC-3 differs from isolichenan in the sequence of linkages and is rather similar to nigeran[1] an intracellular polysaccharide of *Aspergillus niger*, but the molecular size, $\overline{DP}$ 300 ~ 350, is bigger than the PC-3 $\overline{DP}$ 100 ~ 130. Therefore, PC-3 is formulated as follows:[31]



PC-3 is contained in *Parmelia caperata*, *P. cetrarioides*, *P. laevior* and *P. saxatilis* along with isolichenan, and in *Cladonia bellidiflora*, *Cl. alpestris*, *Cl. pacifica*, *Cl. squamosa*, *Cl. crispata, and Cl. rangiferina* without co-occurrence of isolichenan.

Everniin. A cold-water insoluble polysaccharide, $[\alpha]_D$ + 138°, of *Evernia prunastri* was named everniin by Stude,[28] and studied by Mićović *and* Stojanović [16] and Stefanovich.[27]

The structure of everniin was formulated as a glucan having $\alpha(1 \rightarrow 3)$ $(1 \rightarrow 4)$ linkages in a ratio of 4 : 1 and molecular weight 1.8 ~ 3.4 × 10^4.

Our recent investigation showed that the polysaccharides of *Evernia prunastri*[30] are separated into three fractions, EP-3 (cold water-insoluble fraction), EP-6 and EP-7 (cold water-soluble fractions). EP-3, $[\alpha]_D$ + 200°, $\overline{DP}$ 70, is similar to everniin except in its specific rotation. Everniin might be a crude mixture of polysaccharides, since the earlier authors, in contrast to our recent results, isolated only everniin as the polysaccharide component of *Evernia prunastri*.

EP-6, $[\alpha]_D$ + 164°, $\overline{DP}$ 160, is an α-glucan having $(1 \rightarrow 3)$ $(1 \rightarrow 4)$, linkages in a ratio of 3 : 2, which is similar to isolichenan, $[\alpha]_D$ + 255° $\overline{DP}$ 34 ~ 43, but it is slightly different in the molecular size.

EP-7, $[\alpha]_D$ + 12°, $\overline{DP}$ 60, is a β-glucan having $(1 \rightarrow 3)(1 \rightarrow 4)$ linkages in the ratio of 3 : 1, which is different from that of lichenan.

Acroscyphan. *Acroscyphus sphaerophoroides* which is a unique lichen growing in some very localized places of high altitudes along the Circum Pacific Region[25] contains, along with a glycopeptide a characteristic glucan for which I would like to propose the name, acroscyphan.

Acroscyphan, $[\alpha]_D$ + 176°, ir 845 cm^{-1}, which is insoluble in cold water gives an intensive blue colouration with iodine. By the methanolysis of the methyl ether, methyl 2, 3, 4, - tri - O - methyl-, methyl 2, 4, 6, - tri - O - methyl—*and* methyl 2, 3, 6 - tri - O - methyl - D - glucopyranosides were afforded along with methyl 2, 3, 4, 6-tetra - O - methyl - D - glucopyranoside.

It has therefore been revealed that acroscyphan is a homoglucan having $\alpha(1 \rightarrow 3)(1 \rightarrow 4)$ $(1 \rightarrow 6)$ linkages[30].

An $\alpha(1 \rightarrow 3)$ $(1 \rightarrow 4)$ glucan (1 : 4) was isolated from *Stereocaulon paschale*[9] Our recent investigation showed that a similar glucan is also contained in *Stereocaulon japonicum* and *St. vesvianum*. A survey work on the polysaccharides of *Stereocaulon* spp. is now under progress.

Glycopeptides are distributed widely in the lichens of *Lobaria* spp. and *Sticta* spp. without co-occurrence of simple glycans[29].

The following lichens were studied in order to find the occurrence of glycopeptides which accompany PC-3 as minor components.

Stictaceae: *Lobaria orientalis, L. isidiosa, L. pseudopulmonaria, L. linita, L. japonica, Sticta gracilis, S. wrightii ;*

Cladoniaceae: *Cladonia bellidiflora, Cl. alpestris, Cl. pacifica, Cl. graciliformis, (Cl. squamosa, Cl. crispata* and *Cl. rangiferina)*

The glycopeptides of *Lobaria orientalis* were separated into LOF-1, $[\alpha]_D$ + 50° ir_{max} 875 cm^{-1}, N, 1.01 %, and LOF-2, $[\alpha]_D$ + 31.5°, ir_{max} 875 cm^{-1}, N, 1.04 %.

The sugar components of LOF-1 determined by a sugar analyzer were glucose and galactose as the main components and mannose, arabinose, xylose and rhamnose as the minor components. LOF-2 contains mainly mannose and galactose.

After treatment of LOF-1 with alkali followed by reduction with $NaBH_4$, the reaction mixture was analysed using an amino acid analyzer. In contrast to the acid hydrolysate of LOF-1, a decrease of serine and threonine which corresponded to the increase of alanine and α-aminobutyric acid was observed by the above treatment.

Thus the carbohydrate portion of LOF-1 is linked with the peptide part by an O—glycosyl linkage with serine and threonine. The methanolysis of permethylates of LOF-1 indicated that $1 \rightarrow 6$ glucan and $1 \rightarrow 3$ mannan are the main components of its carbohydrate moiety.

By the results of our survey a wide distribution of various homo-and heteroglucans in lichens has been demonstrated to suggest their chemotaxonomic characters.

The lichen metabolities of smaller molecular weights are specific species while polysaccharides are generally recognized as being characteristic for a larger group, such as a Family or Genus, as shown in the occurrence of pustulan in Gyrophoraceous lichens. Our experiments of laboratory cultivation of the separated mycobionts of *Ramalina* spp. showed that the lichen polysaccharides might mostly be produced by the mycobionts, and the polysaccharides of phycobionts gave a different character in comparison with those of intact lichens.

One of the most characteristic biological activites of most lichen polysaccharides is their host-mediated antitumour actions[8]. It has almost been established that some polysaccharides of fungi and lichens could inhibit the growth of implanted sarcoma 180 in mice, by the *i.p.* injection once a day during 10 days period to the animal, 24 hr after the implantation of the tumour cells. The results are observed after 5 weeks, measuring the inhibition ratios and the rate of complete regression of tumour in comparision with the control[18].

In general, homoglucans with $\beta(1\rightarrow3)$ $(1\rightarrow4)$ or $\beta(1\rightarrow6)$ linkages are more effective than α-glucans. The molecular size and some fine structures would also be related to the biological activities, since oat-lichenan, dextran of various molecular weights and islandic acid are inactive against the tumour growth.

The mechanism of this host-mediated antitumour activities of fungal and lichen polysaccharides has not yet been established. According to Tokuzen[32], the antitumour active polysaccharides *i.p.* injected to the animal caused extensive outpouring of lymphoid cells, plasma cells and macrophage in the vicinity of the graft about 1 week after implantation of sarcoma 180 in mice, and later invasion of connective tissue cells into the graft about a week after the implantation of tumour cells. The control experiments using the antitumour inactive polysaccharides showed no such characteristic cell action. The polysaccharides having potent suppressing effects against allogenic tumour failed to give suppressive effects against autochotonous tumour. Maeda and Chihara[14] also studied the mechanism of antitumour effects of fungal polysaccharides such as lentinan of *Lentinus edodes*, and concluded that lentinan is an immunopotentiator, and stated that a sensitizing state against serotonine or histamine would have some correlation with the antitumour activity of such polysaccharides.

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