

Polysaccharides from Lichens: Structural Characteristics and Biological Activity

Elin S. Olafsdottir*, Kristín Ingólfssdottir

Faculty of Pharmacy, University of Iceland, Reykjavik, Iceland

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Abstract: Lichens have been used for medicinal purposes throughout the ages, and beneficial claims have to some extent been correlated with their polysaccharide content. Of 13,500 lichen species growing worldwide, less than 100 species have been investigated for polysaccharide content. Lichen polysaccharides are mainly of three different structural types: β -glucans, α -glucans, and galactomannans. In addition, a few complex heteroglycans have recently been described, such as thamnolan, a water-soluble, immunologically active heteroglycan with a novel rhamnopyranosylgalactofuranan type of structure. A number of investigations have been carried out on biological effects of lichen polysaccharides, most notably antitumour, immunomodulating, antiviral, and memory-enhancing effects. The current review summarizes present knowledge on the structural characteristics and biological activity of lichen polysaccharides.

Key words: Lichens, polysaccharides, structural characteristics, antitumour activity, immunological activity, biological activity.

Introduction

Lichens are slow-growing symbiotic organisms consisting of a fungus and an algae. The symbiosis is beneficial for both partners and allows them to live under harsh conditions such as extremes in moisture and temperature. Lichens count about 13,500 species growing throughout the world. About one third of them have been investigated for low molecular weight compounds and found to produce over 200 different secondary metabolites such as aromatic polyketides, many of which have been shown to be biologically active (1).

Less than 100 species of lichens have been investigated for polysaccharide constituents and found to produce three main structural types: α -glucans, β -glucans, and galactomannans (2–5). Recently complex heteroglycans isolated from lichens have been described (6), (7). Lichen polysaccharides of the β -glucan and galactomannan type have been suggested to be of chemotaxonomic significance (2), (8), (9).

Lichen polysaccharides which can be isolated in considerable yield such as the α -glucans, β -glucans, and galactomannans are generally expected to be of fungal origin (2), (4). This is supported by results of an investigation on the polysaccharide content of several lichen mycobionts and phycobionts grown separately, where it was found that the mycobiont produced polysaccharides similar to those of the parent lichen while the phycobiont produced different polysaccharides (10). However, a polysaccharide similar in monosaccharide composition to the complex lichen heteropolysaccharide, thamnolan, discussed below, has been described as a component of a phycobiont cell wall (11). The localisation of the lichen polysaccharides has not been established, they could either be a part of the fungal cell wall or reserve glucans, and they could be intracellular or a part of the intercellular material which surrounds both algal and fungal cells (12).

Several lichen species, such as *Cetraria islandica* and *Lobaria pulmonaria*, have been used in traditional medicine since ancient times, to treat a variety of illnesses (13). The possible role of polysaccharides in their beneficial action has been suggested (14). All lichen species investigated so far produce polysaccharides in considerable amounts, up to 57% (15), and many of them have been shown to exhibit antitumour, immunostimulating, antiviral as well as other types of biological activity.

Structural Characteristics

Polysaccharides isolated from lichens are primarily linear or scarcely substituted α - or β -glucans. Secondly, several galactomannan-type structures have been reported, and thirdly a few complex heteroglycans, including a totally new rhamnopyranosylgalactofuranan structure, were recently described (6). The lichen species which have been investigated so far for polysaccharide content are grouped according to families and listed in Table 1, with an overall description of their respective structures. The different structures are described in more detail in Tables 2 through 5, together with chemical and physical properties, and discussed in the sections below. Reported NMR shifts are included, especially for the anomeric carbons and protons, as they can be very helpful in analysing structural details of the lichen polysaccharides such as linkage types and linkage type ratios.

Table 1 Lichen species which have been investigated for polysaccharide content – structural types

Family/Species	β -Glucan ^a	Structural types α -Glucan ^a	Galactomannan ratio: Man/Gal/Glc	Others	Ref.
Cladoniaceae					
<i>Cladonia alpestris</i>		nigeran type (1:1)	57:31:12		25
<i>C. amaurocraea</i>	pustulan		66:24:09		25
<i>C. bellidiflora</i>		nigeran type (1:1)			26
<i>C. clathrata</i>		nigeran type (1:1)			27
<i>C. confusa</i>		nigeran type (1:1)	42:51:07		25
<i>C. connexa</i>		nigeran type (1:1)			27
<i>C. crispata</i>		nigeran type (1:1)			28
<i>C. crispatula</i>		nigeran type (1:1)			27
<i>C. furcata</i>		nigeran type (1:1)			27
<i>C. ibitipocae</i>		nigeran type (1:1)	35:60:2		27, 29
<i>C. imperialis</i>		nigeran type (1:1)			27
<i>C. mitis</i>		nigeran type (1:1)			28
<i>C. pacifica</i>		nigeran type (1:1)			26
<i>C. penicillata</i>		nigeran type (1:1)			27
<i>C. rangiferina</i>		nigeran type (1:1)			28
<i>C. signata</i>		nigeran type (1:1)			27
<i>C. squamosa</i>		nigeran type (1:1)			28
<i>C. substellata</i>			27:59:12		29
Parmeliaceae					
<i>Cetraria cucullata</i>	lichenan (1:2)	isolichenan (2:1)	55:35:10		30
<i>C. islandica</i>	lichenan (3:7)	isolichenan (3:2) and Ci-3 (2:1)	47:46:07	Ki-M-7	5, 20, 24, 31
<i>C. nivaris</i>	lichenan	isolichenan			32
<i>C. richardsonii</i>	lichenan (3:7)	isolichenan (3:2)			33
<i>Evernia prunastri</i>	lichenan (3:1)	isolichenan (4:1) and (3:2) nigeran type (1:1) incl. 1,2-linkages isolichenan (6:1) incl. 1,6-linkages	49:42:09		2, 17, 34, 35
<i>Letharia vulpina</i>	lichenan (1:3)	nigeran type (1.2:1)	galman		36, 37
<i>Newropogon aurantiaco-ater</i>	lichenan (1:2)	isolichenan (3:2)	53:44:03		15
<i>Parmelia caperata</i>		isolichenan (3:2), nigeran type (1:1)			28, 38, 39
<i>P. cetrarioides</i>		isolichenan, nigeran type (1:1)			26
<i>P. conspersa</i>	lichenan	isolichenan			26
<i>P. hypotrypella</i>	lichenan	isolichenan			26
<i>P. laevior</i>		isolichenan, nigeran type (1:1)			26
<i>P. nikkoensis</i>	lichenan	isolichenan			26
<i>P. saxatilis</i>		isolichenan (2:1), nigeran type (1.3:1)			40
<i>P. tinctorum</i>	lichenan	isolichenan			26
<i>Parmotrema cetrarum</i>	lichenan (1:1.9)		46:45:09		2, 34
<i>P. araucaria</i>			50:44:6, 49:44:7		34
<i>P. sulcata</i>			42:44:12		37
<i>Usnea barbata</i>	lichenan	isolichenan			32
<i>U. baylei</i>	lichenan	isolichenan			26, 41
<i>U. facitata</i>		isolichenan			42
<i>U. longissima</i>	lichenan	isolichenan			43
<i>U. meridionalis</i>			35:42:23; 52:35:13		34
<i>U. rubescens</i>	lichenan (3:7)				28
<i>Usnea</i> sp.	lichenan (1:3)		33:47:21, 35:61:4		36, 37
Peltigeraceae					
<i>Peltigera aphthosa</i>			38:44:11		37
Umbellariaceae					
<i>Actinogyra muehlenbergii</i>	pustulan		58:37:05		36, 37
<i>Gyrophora esculenta</i>	pustulan				44
<i>Lasallia papulosa</i>	pustulan				44
<i>L. pennsylvanica</i>	pustulan				28
<i>Umbilicaria angulata</i>	pustulan				45
<i>U. caroliniana</i>	pustulan				45
<i>U. hirsuta</i>	pustulan				44, 46
<i>U. polyphylla</i>	pustulan				45
<i>U. pustulata</i>	pustulan		40:20:30		46, 47
<i>U. spodochoera</i>			32:19:32		47

Table 1 cont.

Family/Species	β -Glucan ^a	Structural types α -Glucan ^a	Galactomannan ratio: Man/Gal/Glc	Others	Ref.
Ramalinaceae					
<i>Ramalina celastri</i>	laminaran type	isolichenan (3:1), nigeran type (1:1)			48
<i>R. ecklonii</i>		isolichenan (3:1)	36:50:14		34, 49
<i>R. scopulorum</i>					41
<i>R. usnea</i>	laminaran type	isolichenan (3.8:1) incl. 1,2-linkages	38:44:18		31
Lichen imperfecti					
<i>Thamnolia subuliformis</i>				thamnolan	6
Caliaciaceae					
<i>Acrosyphus sphaerophoides</i>		(2:3) incl. 6% 1,6-linkages acrosyphan			33
Alectoriaceae					
<i>Alectoria sulcata</i>	lichenan	isolichenan			17
<i>A. sarmentosa</i>	lichenan	isolichenan			17
Sphaerophoraceae					
<i>Sphaerophorus globosus</i>		(2:3) incl. 6% 1,6-linkages acrosyphan type			33
Stereocaulaceae					
<i>Stereocaulon excutum</i>		isolichenan (3:1)			50
<i>St. japonicum</i>		isolichenin (2:1)			33, 51
<i>St. paschale</i>		acrosyphan type (2:5)	52:36:12		37, 52
<i>St. ramulosum</i>	laminaran type	nigeran type (1:1)	57:43:00		53, 54
<i>St. soreidiferum</i>		isolichenan (3:1)			
Rocellaceae					
<i>Rocella montagnei</i>	lichenan	isolichenan			43
Lobariaceae					
<i>Pilophoron ocellularis</i>		isolichenan (2:1)			33
<i>Pseudocyphellaria aurata</i>			61:30:19		34
<i>Sticta</i> sp.	no glucan	no glucan	63:21:16		55
Dictyonemataceae					
<i>Cora pavonia</i> (now: <i>Dictyonema glabratum</i>)	(1→3),(1→6)-glucan			CP-heteroglycan	7
Pysciaceae					
<i>Tornabenia intricata</i>			93:00:09		34
Collemaataceae					
<i>Collema leptosporum</i>	(1→3),(1→6)-glucan		35:00:65, 82:18:00	CL-heteroglycan	23

^a Ratio of (1→3) and (1→4) linkages in parenthesis.

β -Glucans

The first polysaccharide fraction isolated from a lichen species was a mixture of lichenan and isolichenan isolated from *Cetraria islandica* in 1813 by Berzelius (16). Lichenan is a cold-water insoluble, gel-forming, linear (1→3)-(1→4)- β -D-glucan with a linkage ratio of 3:7 (Table 2). A number of β -glucans with lichenan-type of structures differing in the ratio of (1→3) and (1→4) linkages, are listed in Table 2. The cold water-soluble lichenan-type of β -glucan from *Evernia prunastri* has a linkage ratio of 3:1 with the (1→3)-linkage dominating, which is reversed compared to lichenan (17). Pustulan, which is found in almost all species investigated belonging to the family of Umbilicariaceae (Table 1) is a linear (1→6)- β -D-glu-

can which may be O-3-acetylated (Table 2). The acetyl groups give characteristic bands in the IR spectrum and a high field signal at $\delta = 22.1$ ppm in the ¹³C-NMR spectrum and at $\delta = 2.1$ ppm in the ¹H-NMR spectrum.

Other β -glucans found in lichens are linear (1→3)- β -D-glucans (laminaran-type) currently found in three lichen species (Tables 1 and 2), and an O-6-substituted (1→3)-(1→6)- β -D-glucan from *Cora pavonia* and *Collema leptosporum* (Table 2). The mean molecular weights of the β -glucans are reported to be between 20 and 62 kD. Optical rotation measurements of β -glucans give positive, low values, except for pustulan which has a negative optical rotation of -37° (Table 2). The three-dimensional structure of lichenan has been analysed by X-ray

Table 2 β -Glucans from lichens – structural characteristics and analytical data (see Table 1 for further references)

Structural type	Linkages in main chain	Linkage ratio	Side chains or substitution	M_r in kD	$[\alpha]_D$ deg.	Sol. in cold H_2O	IRmax cm^{-1}	NMR-shifts (anomeric and others) 1H -NMR ^{13}C -NMR	NMR sol/temp	NMR ref.
lichenan	(1→3), (1→4)	(3:7)	linear	20–35	+8	insol.	890	4.44 103.4, 102.4, 102.5, 87.0, 80.2, 79.9, 60.4	DMSO- d_6 / 60 °C	30, 56
lichenan	(1→3), (1→4)	(1:3)			–8	sol.				
lichenan	(1→3), (1→4)	(1:2)			+14	insol.				
lichenan (branched)	(1→3), (1→4)	(1:1.9)	branched (not pure)		+49	sol.				
lichenan	(1→3), (1→4)	(3:1)			+12	sol.	890			
pustulan (acetylated or not)	(1→6)		O-3-acetylated (ca. 10%)	20	–37	insol.	1735, 1250 ^a	2.1 ^a 104.6, 77.4, 76.6, 74.8, 71.7, 70.6, 22.1 ^a	D ₂ O/70 °C	4
laminaran type	(1→3)		linear	62	+10	insol.		103.2, 86.4, 76.6, 73.2, 68.7, 61.1	NaOD-D ₂ O/ 30 °C	48
<i>Cora pavonia</i> glucan	(1→3), (1→6)		branched at O6 (ca. 20%)		+13	sol.		104.5, 86.5, 68.7, 62.6	D ₂ O/70 °C	7
<i>Collema leptosporum</i> glucan	(1→3), (1→6)		branched at O6 (ca. 23%)	57	0	sol.		104.6, 104.4, 86.4, 70.6, 62.5	D ₂ O/30 °C	23

^a From the acetyl group.

crystallography and the conformation shown to be a triple helix. Triple helical structures have also been demonstrated for (1→3)- β -glucans from several fungi. The introduction of short side chains or (1→4) or (1→6) linked residues into the (1→3)- β -glucan backbone does not seem to interrupt this triple helix conformation (5), (12).

α -Glucans

Isolichenan was the first α -glucan described from lichens. Isolichenan is a cold-water soluble (1→3)-(1→4)- α -D-glucan originally isolated from *Cetraria islandica* and reported to have M_r of about 6–8 kD and early reports (18), (19) disagree on the linkage ratio (from 3:2 to almost 1:1). A recent investigation could not confirm the existence of such a small α -glucan in *C. islandica*; instead, a much larger isolichenan with a linkage ratio of 2:1 according to NMR data was found (20). The definitions “isolichenan-type” polysaccharide or sometimes only “isolichenans” have been used for α -D-glucans having (1→3)-(1→4)-linkages in their main chain. By studying Table 3, it seems that lichens produce isolichenan-type polysaccharides with considerable variation in linkage ratios as well as M_r , even within the same species. Occasionally these α -glucans can be branched at O2, O3 or O6. Nigeran-type polysaccharides can be defined as a subgroup of the isolichenan-type α -glucans having a linkage ratio of 1:1. Accordingly, acrosyphan-type α -glucans can be considered as a subgroup of the isolichenan-type having a high proportion of (1→4)-linkages in the main chain. The nigeran-type α -glucans are insoluble in cold water. The optical rotation measurements of the lichen α -glucans always give positive high values (Table 3).

Galactomannans

A polysaccharide fraction from *Cetraria islandica* containing galactose and mannose was recognized as early as 1906 (21) but more detailed structural investigations on this group of lichen polysaccharides were mainly carried out over the past 15 years. Currently, galactoglucomannans have been isolated

from at least 24 different species of lichens (Table 1). The main chain always consists of (1→6)-linked α -D-mannopyranosyl units. The ratio of Man/Gal/Glc is variable. However, in most cases mannose or almost equivalent proportions of mannose and galactose predominate. The amount of glucose reported in these heteroglycans should be considered with caution as it is possible that the samples might be contaminated with small amounts of accompanying glucans from the lichen. The mannopyranosyl units in the main chain are branched mainly at O2 or O4 by units of α - or β -Galp, α -Manp or Glcp and more rarely by β -Galf or α -Manf (Table 4). Galactomannans with no glucose units attached have been isolated from two lichen species, *Collema leptosporum* and *Stereocaulon ramulosum* with monosaccharide Man/Gal ratios of 82:18 and 57:43, respectively. More details on their structure can be seen in Table 4. Glucomannans have been reported from *Tornabenia intricata* and *C. leptosporum*. The monosaccharide Man/Glc ratios are 93:7 and 35:65, respectively (Table 4).

Complex heteroglycans

A few complex heteroglycans have been isolated from lichens. Polysaccharides containing monosaccharides other than, or in addition to galactose, glucose, mannose are grouped here. The first was isolated in 0.06% yield from the Basidiomycetous lichen *Cora pavonia* in 1987 by Iacomini et al. (7) and the structure was shown to be predominated by Manp and Xylp with a main chain of (1→3)-linked α -D-mannopyranosyl units. More details on the structure can be found in Table 5.

Another totally different heteropolysaccharide, thamnolan, was recently isolated in 0.05% yield from *Thamnia subuliformis* (now: *Thamnia vermicularis* var. *subuliformis*) by Olafsdottir et al. (6). Thamnolan is a rhamnopyranosylgalactofuranan with a structure predominated by (1→3)-linked β -D-galactofuranosyl units with complex rhamnopyranosyl side chains and terminal xylose units as described in Table 5. The optical rotation of thamnolan which is not previously published, was found to be $[\alpha]_D^{23} = -63^\circ$ (c. 1.0, in water) and is in accordance with the negative literature values reported for

Table 3 α -Glucans from lichens – structural characteristics and analytical data (see Table 1 for further references)

Structural type	Linkages in main chain	Linkage ratio	Side chains and comments	M_r in kD	$[\alpha]_D$ deg.	Sol. in cold H ₂ O	IRmax cm ⁻¹	NMR-shifts (anomeric and others) ¹ H-NMR ¹³ C-NMR	NMR sol/temp	NMR ref.
isolichenan	(1→3), (1→4)	(3:2)	linear	6–8	+255	sol.		101.6, 100.9, 100.6, 82.1, 81.9, 79.3	D ₂ O/70 °C	31
isolichenan (Ci-3)	(1→3), (1→4)	(2:1)	linear	2000	+264	sol.		102.2, 101.6, 101.3, 82.2, 81.8, 79.2	D ₂ O/25 °C	20
isolichenan	(1→3), (1→4)	(3:1)	linear, irregular distribution of linkages	294	+213	sol.		101.3, 100.7, 100.6, 100.3, 81.4, 81.3, 80.9, 78.4	D ₂ O/70 °C	48
isolichenan	(1→3), (1→4)	(3.8:1)	5% branched at O2		+243	sol.		101.6, 101.0, 100.6, 82.1, 81.9, 79.3	D ₂ O/70 °C	31
isolichenan (everniin)	(1→3), (1→4)	(4:1)	linear	26	+138	insol.	925, 845, 780			
isolichenan nigeran type (PC-3)	(1→3), (1→4)	(6:1)	incl. (1→6)-linkages	21	+201 ^a	insol.				
“	“	“	linear	69	+155 ^b	insol.		100.2, 99.3, 82.5, 78.8, 60.4, 59.9	DMSO- <i>d</i> ₆ /70 °C	48
nigeran/isolichenan nigeran type	(1→3), (1→4)	(1.2:1)	linear			insol.				
acrosyphyan	(1→3), (1→4)	(2:3)	incl. 6% (1→6)-linkages		+176	insol.	845			
acrosyphyan-type	(1→3), (1→4)	(2:5)	ca. 3% branched at O3	24	+233	sol.				

^a 2N NaOH ^b 1% NaOH.**Table 4** Galactomannans from lichens – structural characteristics and analytical data (see Table 1 for further references)

Structural type	Linkages in main chain	Ratio: Man/Gal/Glc	Side chains or substitution	M_r in kD	$[\alpha]_D$ deg.	Sol. in cold H ₂ O	Anomeric ¹³ C NMR shifts	NMR sol/temp	Ref.
Galactoglucmannans in general (have been isolated from at least 24 species of lichens)	(1→6)-linked α -D-Manp	variable, Man or Gal are always dominating	different pattern of substitution mainly at O2 or O4, by units of α - or β -Galp or α -Manp or Glcp, more rarely by β -Galp or α -Manf	ca. 2000 was recently published for two glycans from <i>Cladonia</i> sp.	+20 to +115	sol.	109.5 (β -Galp-(1→4)) 104.7 (β -Galp-(1→4) and β -Glcp-(1→4)) 103.7 (α -Manp-(1→2)- α -D-Manp) 102.8 (α -Galp-(1→2)) 102.2 (α -Manp-(1→2)- α -Manp-(1→2)) 101.0 (unsub. (1→6)-linked α -Manp units) 99.9 (sub. at O2, (1→6)-linked α -Manp units)	D ₂ O/70 °C	2, 4
CL-galactomannan	(1→6)-linked α -D-Manp	82:18:00	partly sub. at O4 and/or O2 by end units of β -Galp (19%), α -Manp (39%) and β -Galp (0.3%)	140	+40	sol.	109.2, 107.1, 104.7, 104.0, 103.5, 102.0, 100.4, 99.5	D ₂ O/30 °C	23
StR-galactomannan	(1→6)-linked α -D-Manp	57:43:00	partly sub. at O4 and/or O2 by end units of β -Galp and α -Manp		+73				53
TI-glucomannan	(1→6)-linked α -D-Manp	93:00:07	partly sub. at O2 with α -D-Manp and a small amount of α -D-Glcp	ca. 100	+74	sol.			
CL-glucomannan	not determined	35:00:65	not determined			sol.			23

β -D-galactofuranosyl units (22). A third heteroglycan was isolated from *Collema leptosporum* (23) but the structure was not characterized except for the monosaccharide composition which revealed that this glycan, in contrast to the other complex heteroglycans, contains no rhamnosyl units.

Ki-M-7 is a galactomannan isolated from the alkali extract of *Cetraria islandica* by Ingólfssdóttir et al. (24). This galactomannan is grouped with the complex heteroglycans as it contains a small proportion of rhamnosyl units (Table 5).

Biological Activity

Polysaccharides from plants and other natural sources have long been known to exert antitumour, immunomodulatory, anticoagulant, and other types of biological activity (57–59). Research over the last 30 years, much of which has been performed in Japan, has shown lichen polysaccharides to possess similar types of biological activity, whilst having a low toxicity level.

Table 5 Complex heteroglycans from lichens – structural characteristics and analytical data

Structural type	Monosaccharide comp. ratios in parantheses	Main structural characteristics	M _r kD	[α] _D deg.	Sol. in cold H ₂ O	Anomeric and other characteristic ¹³ C NMR shifts ^a	NMR sol/temp	Ref.
Thamnolan	Gal:Rha:Glc:Xyl:Man (40:31:13:10:6)	Dominated by a (1→3)-linked β-D-Galp with sidechains in position 6 for about 7% of the units, and an (1→2)-linked α-L-Rhap with branches on either C3 or C4. Xyl is only present as a terminal unit and after partial hydrolysis, the trisacch. XylGlcGlc was detected.	1450	-63 ^b	sol.	109.3 (C1 in Galf) 19.0 (C6-Me in Rhap) peaks around 102 (C1 in Rhap)	D ₂ O/25 °C	+6
CP-heteroglycan	Gal:Rha:Glc:Xyl:Man:Fuc (17:4:8:32:29:10)	A main chain dominated by (1→3)-linked α-D-Manp, monosub. at O4 (10%) or disub. at O2 and O4 (10%) with β-D-Xylp		+25	sol.	105.2 (C1 in β-D-Xylp) 103.0, 100.9, 99.7, 80.1, 17.3	D ₂ O/70 °C	7
CL-heteroglycan	Gal:Glc:Xyl:Man (24:27:18:31)	Not described further			sol.			23
Ki-M-7	Gal:Man:Rha (57:39:4)	Composed of two blocks Firstly: (1→6)-linked α-D-Manp units partly sub. at O2 or O4. Secondly: (1→6)-linked α-D-Galp units, partly 2,4-di-O-sub. Terminal units of α-D-Galp or β-D-Galf.	18	+112	sol.	107.9 (C1 in β-D-Gal-f) 103.1 (C1 in α-D-Galp) 101.4 (C1 in 1,6-α-D-manp) 99.9 (C1 in 1,4,6-α-D-Manp) 99.3 (C1 in 1,6-α-D-Galp) 99.0 (C1 in 1,2,4,6-α-D-Galp)	D ₂ O/25 °C	24

^a ¹H-NMR shifts for thamnolan: 5.22 (*J* < 2.0 Hz, H1 in Galf), 1.32 (s, C6-Me in Rhap).

^b Not previously published. Measured in D₂O at 23 °C (c 1.0 mg).

Antitumour and immunological activity

Japanese scientists have studied lichen polysaccharides extensively for non-cytotoxic, host-mediated antitumour activity. In the first of these investigations (60), crude polysaccharide fractions from 9 lichen species, obtained as ethanol precipitates from aqueous extracts, were investigated for activity against subcutaneously implanted sarcoma-180 ascites tumour in mice. The polysaccharides were administered (200 mg/kg) intraperitoneally daily for 10 days starting 24 hours after tumour implantation. After 5 weeks the average tumour weights in the treated and control groups were compared. Many of the polysaccharide fractions suppressed tumour growth, often with a very high rate of complete regression.

Further fractionation of active polysaccharide fractions showed both isolichenan-rich and lichenan-rich fractions (Tables 2 and 3) from *Cetraria islandica* var. *orientalis* to be highly active. No direct cytotoxic activity was detected and the effects were proposed to be host-mediated. Partially acylated β-(1→6) pustulan-type glucans (Table 2) were obtained through further fractionation of active fractions from *Gyrophora esculenta* (GE-3) and *Lasallia papulosa* (44).

The acyl group was later identified as *O*-acetyl attached in the 3-position of approximately 10% of the glucose units (41), this being the first example of acetylated polysaccharides isolated from lichens. Structure-activity investigations revealed that deacetylation reduced the antitumour activity whereas replacement of *O*-acetyl groups by *O*-methyl groups, or complete acetylation of the glucan, caused total loss of activity (41).

Further investigations by the Japanese team headed by Nishikawa involved the isolation of highly active, partially acylated pustulan-type glucans from *Umbilicaria* species (45), shown to have a close resemblance to GE-3. Furthermore, a highly ac-

tive β-glucan (UR-1-1) identified as lichenan was isolated from *Usnea rubescens*, an active GE-3-type glucan (L-Pe-1-1) was isolated from *Lasallia pensylvanica* and moderately active heteropolysaccharides consisting chiefly of mannose, galactose, and glucose units were found in *Cladonia* species (28).

In order to establish whether the antitumour activity of GE-3 and UR-1-1 was achieved through stimulation of immune responses, the polysaccharides were subjected to testing in the *in vivo* carbon clearance test. Intraperitoneal administration of GE-3 to mice at a dose of 100 mg/kg resulted in a significant increase in the rate of colloidal carbon elimination, suggesting significant activation of the reticuloendothelial system (61). No activity was observed for the lichenan UR-1-1. Lauroyl derivatives of GE-3 were less effective, and a cold-water soluble carboxymethyl derivative was inactive.

Two isolichenan-type polysaccharides, EP-3 and EP-6, differing in the ratio of (1→3)-, (1→4)-linkages, in addition to a lichenan (EP-7) were isolated from a crude extract of *Evernia prunastri* (Table 1) which had shown activity against sarcoma-180 (17). Testing of the purified polysaccharides revealed that the isolichenans were inactive while EP-7 showed significant growth-inhibitory effects. Inactive α-glucans were isolated from active crude polysaccharide fractions from *Acroscyphus sphaerophoroides*, *Alectoria sulcata*, and *Alectoria sarmentosa*, while active lichenan was isolated from both *Alectoria* species.

Of the water-soluble polysaccharides from *Lobaria* species (Stictaceae) showing antitumour activity against transplanted sarcoma 180, two glycopeptides (LOF-1, LOF-2) were identified from *L. orientalis* (62). The main carbohydrate components of LOF-1 were shown to be a (1→6)-glucan and a (1→3)-mannan, linkage to the peptide moiety occurring through *O*-glycosyl linkages with serine and threonine. An inhibition of 81.6% was exhibited by LOF-1 at a dose of 10 mg/kg.

Lichenan and pustulan were chosen as representatives of polysaccharides active against allogenic tumours (e.g., sarcoma 180) to see whether they would also be active against syngeneic tumours in mice. Results showed that pustulan was active against three types of syngeneic tumours at daily doses of 25–75 mg/kg, suggesting immuno-mediated activity, whereas lichenan was inactive (63).

An increase in α_1 -acid glycoprotein (α_1 -AG) levels in the serum of ascites tumour-bearing mice treated with lichen polysaccharides was later observed (64). It was suggested that this might be linked to the antitumour activity of the polysaccharides as purified α_1 -AG inhibited the growth of tumour cells *in vitro*. Lichenan, GE-3, and urea-treated GE-3 (UGE-3) all caused pathological changes to the liver and spleen, liver necrosis becoming evident soon after *i.p.* administration. Leukopenia was further induced, followed by leukocytosis. Earlier (65) it had been observed that the liver of mice receiving injections of β -glucans from lichens became enlarged and multifocal mesenchymal cell accumulation occurred, the severity and duration being dose-dependent. No liver changes were observed on administration of α -glucans such as isolichenan. When pustulan and lichenan were tested for activity against syngeneic tumours as referred to above (63), the liver and spleen weights of mice treated with pustulan increased considerably more than in those treated with lichenan, the effects of pustulan being correlated with an increase in number and activity of macrophages.

The first report (24) of a chromatographically purified polysaccharide from lichens being tested for immunostimulating activity was that of the galactomannan Ki-M-7 (Table 5). The polysaccharide (100 μ g/ml), which was shown to be free of LPS contamination, showed enhancement of granulocytic phagocytosis amounting to 68% in an *in vitro* phagocytosis assay performed with human granulocytes. When tested for reticuloendothelial phagocytic activity in the *in vivo* carbon clearance test, a significant increase occurred in the rate of colloidal carbon elimination (24).

As the immunologically active Ki-M-7 was isolated from an alkali extract, an investigation was undertaken to determine whether polysaccharides extractable with hot water, i.e., constituents of traditional preparations made by boiling the lichen in water, also had immunomodulating properties (66) and might substantiate to some extent pharmacological claims for the plant. Polysaccharides from a hot aqueous extract of Iceland moss were fractionated by ethanol precipitation and ion-exchange chromatography. Several of the major fractions exerted significant *in vitro* anti-complementary activity and pronounced enhancement of granulocytic phagocytosis. All fractions were subjected to affinity chromatography prior to testing to remove any trace of potential endotoxin impurities.

Further purification of the active polysaccharide fractions led to the isolation of a homogenic cold-water soluble isolichenan-type α -glucan, Ci-3 (Table 3). Results of *in vitro* testing of Ci-3 for phagocytic and anti-complementary activity showed that, at concentrations of 100 μ g/ml, Ci-3 stimulated granulocytic phagocytosis about 50% compared with stimulation by fMLP set as 100% and reduced complementary-induced hemolysis by about 80% (20).

At concentrations of 100 μ g/ml and 1000 μ g/ml, the complex and novel polysaccharide, thamnolan (Table 5), stimulated *in vitro* granulocytic phagocytosis about 36% and 91%, respectively, compared with stimulation by fMLP set as 100% (6). The polysaccharide was free from LPS contamination. This activity is comparable to that of Ci-3 (20), but a little less than that reported for Ki-M-7 (24). Results of *in vitro* anti-complementary testing show that at concentrations of 100 μ g/ml, thamnolan reduces complementary-induced hemolysis about 90% (6).

Cytotoxicity

In addition to non-cytotoxic antitumour activity as referred to above, lichen α -glucans have been shown to exhibit cytotoxicity. An α -(1 \rightarrow 3)-(1 \rightarrow 4)-glucan obtained from *Ramalina celastri* as well as its sulfated derivative showed cytotoxic activity against HeLa cells (67). Signs of cell death induced by apoptosis were seen.

Antiviral activity

Lichenan from Iceland moss exhibited antiviral activity against the following viruses which had been mechanically transmitted into *Nicotiana* species: tobacco mosaic- and etchviruses (TMV, TEV), potato viruses X and Y (PVX, PVY), as well as cucumber mosaic virus (CMV) (68). Partly hydrolysed lichenan with different degrees of polymerisation (6–30, 30–60, 60–90, >190) was equally effective in reducing the number of local (TMV) and systemic (PV4) infections compared to the native glucan. Other mixed-linkage β -glucans, including schizophyllan and pachyman from fungi, and laminaran from algae, were less active than lichenan. In contrast, pustulan as well as α -glucans were ineffective. The mechanism of action of lichenan is not clear, but results indicated that early events of viral replication were affected (69).

Hippocampal potentiation

In studies on the effects of glucans on hippocampal synaptic plasticity in rodents, isolichenan from *Parmelia caperata*, administered orally and intravenously, enhanced the electrophysiological model of spatial memory in rats, i.e., the formation of hippocampal long-term potentiation (39). Isolichenan from *Cetrariella islandica*, on *i.v.* administration, significantly enhanced short-term potentiation evoked by a subthreshold tetanus, without any effect on basal evoked synaptic potential (70). The β -glucans, lichenan and GE-3, were inactive. Orally administered isolichenan was further shown to improve memory acquisition in mice, the learning ability of which had been impaired by ethanol, as well as in rats in which memory impairment had been induced by beta-amyloid peptide (70), (71).

Biological activity of polysaccharide derivatives

Derivatives of the linear β -D-glucans which had proven particularly effective against sarcoma-180, i.e., GE-3 and UR-1-1, were prepared and evaluated for antitumour activity (72). Results showed that treatment with urea caused no reduction in activity whereas on carboxymethylation, the antitumour activity was greatly reduced. Lauroyl derivatives with different degrees of substitution were also prepared from GE-3. Derivatives with lauroyl contents less than 3.3% exhibited strong an-

titumour activity, while the more highly esterified products were inactive.

A sulfate derivative, GE-3-S, prepared by chlorosulfonic acid treatment of GE-3 has been shown to inhibit the replication of human immunodeficiency virus (HIV) *in vitro* (73). GE-3-S inhibited the cytopathic effect of HIV and suppressed HIV-antigen expression in Molt-4 cells without inhibiting HIV-reverse transcriptase. Sulfate derivatives of lichenan from *Cetraria islandica* and PC-3 from *Parmelia caperata* were inactive against HIV as were the unsulfated counterparts.

As referred to above, cytotoxic activity has been demonstrated by a sulfated derivative of an α -glucan from *Ramalina celastri*.

Discussion and Conclusion

By studying Table 1, a pattern of distribution of several lichen polysaccharide structural types can be observed for the three most frequently studied lichen families. The glucan pustulan is characteristic for Umbilicariaceae, lichenan-type glucans are the only β -glucans found in Parmeliaceae, and the only α -glucan isolated from Cladoniaceae is of the nigeran type. Thus, polysaccharides from lichens such as pustulan and lichenan, have been suggested to be of taxonomic importance at genus and family level (2), (8). Chemotypes of lichen galactomannans have also been considered as an aid for classification and identification of lichens by analysis of the anomeric region of their ^{13}C -NMR spectra (2), (9).

It is exciting to know that, even though only a fraction of the world's lichen species has been investigated for polysaccharide constituents, unusual structures have already emerged. The recently investigated *Thamnia subuliformis* belonging to Lichen imperfecti has been shown to contain a water-soluble heteroglycan, thamnolan, with a novel kind of structure not described earlier, either from lichens, fungi, bacteria or plants (6). Thamnolan has been shown to be immunologically active but remains to be studied for further biological activity.

Polysaccharides from lichens have shown various types of biological activity such as potent antitumour activity, general stimulating activity on the unspecific immune system, antiviral activity and recently memory enhancing effects. In some cases activity has been improved by chemical modification.

The most active antitumour lichen polysaccharides on *i.p.* administration to mice appear to be (1 \rightarrow 3)- β -glucans. This is perhaps not surprising, as (1 \rightarrow 3)- β -D-glucans from other sources have been found effective against allogeneic, syngeneic and autochthonous tumours (74). Antitumour activity of lichen polysaccharides was initially thought to be host-mediated, but this has not been confirmed, *cf.* stimulation of the reticuloendothelial system in mice is only exhibited by pustulan-type polysaccharides but not by lichenans. This might indicate that the mechanism of antitumour activity differs between β -glucans. The possible contributing role of cytotoxicity to the antitumour activity of lichen polysaccharides should not be dismissed, although direct proof of such activity is scarce and limited to *in vitro* testing.

The importance of the three-dimensional structure of β -glucans for antitumour activity is well established and has been

demonstrated for lentinan, a (1 \rightarrow 3)- β -glucan from the fungus *Lentinus edodes*, which has been used clinically for adjuvant cancer therapy in Japan (75). It can be expected that the same applies for other (1 \rightarrow 3)- β -glucans including the lichen polysaccharides.

Immunomodulating effects, i.e., increased phagocytic activity as well as anti-complementary activity have been confirmed for chromatographically purified lichen polysaccharides, namely an α -glucan, galactomannan and rhamnogalactan. Taking into account the use of lentinan and other immunologically active polysaccharides in adjuvant cancer therapy (75), it is worthwhile to study in more detail the *in vivo* immunological activity of these and other lichen polysaccharides.

Although studies of antiviral activity of lichen polysaccharides are limited, it is noteworthy to observe the difference in antiviral effects expressed by different types of glucans. Noteworthy also is the fact that similar antiviral effects are expressed by lichenans of differing degrees of polymerisation.

The effects of isolichenan on hippocampal function certainly deserve further attention, not least in light of the proposed connection of increased density beta amyloid protein in deterioration associated with Alzheimer's disease.

In view of the diverse biological activity expressed by lichen polysaccharides in limited studies, it seems likely that therapeutic effects claimed from the use of certain lichens, such as *Cetraria islandica*, *Lobaria pulmonaria*, and *Umbilicaria* species, can be in part attributed to the polysaccharides. Lichen polysaccharides definitely deserve further study with regard to biological activity, including studies into mechanism of action and structure-activity relationships. Other lichen species should be investigated, both as a potential source of new chemical structures and biological activity.

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Dr. E. S. Olafsdottir

Faculty of Pharmacy
University of Iceland
Hagi, Hofsvallagata 53
107 Reykjavik
Iceland
E-mail: elinsol@hi.is
Fax: +354 525 4071