Polysaccharides from Lichens: Structural Characteristics and Biological Activity

Elin S. Olafsdottir*, Kristín Ingólfsdottir

Faculty of Pharmacy, University of Iceland, Reykjavik, Iceland

Received: June 19, 2000; Accepted: October 8, 2000

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 polysaccahrides.**

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).

Planta Med 67 (2001) 199–208

ISSN: 0032-0943

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

Table 1 cont.

Family/Species	Structural types β-Glucan[a]	 α-Glucan[a]	Galactomannan ratio: Man/Gal/Glc	Others	Ref.
Ramalinaceae					
Ramalina celastri	laminaran type	isolichenan (3:1), nigeran type (1:1)			48
R. ecklonii		isolichenan (3:1)	36:50:14		34, 49
R. scopulorum					41
R. usnea	laminaran type	isolichenan (3.8:1) incl. 1,2-linkages	38:44:18		31
Lichen imperfecti					
Thamnolia subuliformis				thamnolan	6
Caliaciaceae					
Acroscyphus sphaerophoides		(2:3) incl. 6% 1,6-linkages acroscyphan			33
Alectoriaceae					
Alectoria sulcata	lichenan	isolichenan			17
A. sarmentosa	lichenan	isolichenan			17
Sphaerophoraceae					
Sphaerophorus globosus		(2:3) incl. 6% 1,6-linkages acroscyphan type			33
Stereocaulaceae					
Stereocaulon excutum		isolichenan (3:1)			50
St. japonicum		isolichenin (2:1)			33, 51
St. paschale		acroscyphan type (2:5)	52:36:12		37, 52
St. ramulosum	laminaran type	nigeran type (1:1)	57:43:00		53, 54
St. sorediiferum		isolichenan (3:1)			
Rocellaceae					
Roccella montagnei	lichenan	isolichenan			43
Lobariaceae					
Pilophoron ocicularis		isolichenan (2:1)			33
Pseudocyphellaria aurata			61:30:19		34
Sticta sp.	no glucan	no glucan	63:21:16		55
Dictyonemataceae					
Cora pavonia (now: *Dictyonema glabratum*)	(1→3),(1→6)-glucan			CP-heteroglycan	7
Pysciaceae					
Tornabenia intricata			93:00:09		34
Collemataceae					
Collema leptosporum	(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-glucan which may be O-3-acetylated (Table **2**). The acetyl groups give characteristic bands in the IR spectrum and a high field signal at δ = 22.1 ppm in the ^{13}C-NMR spectrum and at δ = 2.1 ppm in the ^{1}H-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 ^{1}H-NMR	^{13}C-NMR (anomeric and others)	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_2O/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_2O/ 30 °C	48
Cora pavonia glucan	(1→3), (1→6)		branched at O6 (ca. 20%)		+13	sol.			104.5, 86.5, 68.7, 62.6	D_2O/70 °C	7
Collema leptosporum glucan	(1→3), 1→6)		branched at O6 (ca. 23%)	57	0	sol.			104.6, 104.4, 86.4, 70.6, 62.5	D_2O/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, acroscyphan-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-Gal*p*, α-Man*p* or Glc*p* and more rarely by β-Gal*f* or α-Man*f* (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 Man*p* and Xyl*p* 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 *Thamnolia subuliformis* (now: *Thamnolia 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 $[\alpha]_D^{23} = -63°$ (*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_2O	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_2O/70 °C	31
isolichenan (Ci-3)	(1→3), (1→4)	(2:1)	linear	2000	+264	sol.		5.39, 5.44	102.2, 101.6, 101.3, 82.2, 81.8, 79.2	D_2O/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_2O/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_2O/70 °C	31
isolichenan (everniin)	(1→3), (1→4)	(4:1)	linear	26	+138	insol.	925, 845, 780				
isolichenan	(1→3), (1→4)	(6:1)	incl. (1→6)-linkages		+152	insol.					
nigeran type (PC-3)	(1→3), (1→4)	(1:1)	linear	21	+201[a]	insol.	925, 845, 780				
"	"	"	linear	69	+155[b]	insol.			100.2, 99.3, 82.5, 78.8, 60.4, 59.9	DMSO-d_6/ 70 °C	48
nigeran/isolichenan	(1→3), (1→4)	(1.2:1)	linear			insol.					
nigeran type	(1→3), (1→4)	(1:1)	incl. (1→2)-linkages		+217	insol.					
acroscyphan	(1→3), (1→4)	(2:3)	incl. 6% (1→6)-linkages		+176	insol.	845				
acroscyphan-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_2O	Anomeric ^{13}C NMR shifts	NMR sol/temp	Ref.
Galactoglucomannans in general (have been isolated from at least 24 species of lichens)	(1→6)-linked α-D-Man*p*	variable, Man or Gal are always dominating	different pattern of substitution mainly at O2 or O4, by units of α- or β-Gal*p* or α-Man*p* or Glc*p*, more rarely by β-Gal*f* or α-Man*f*	ca. 2000 was recently published for two glycans from *Cladonia* sp.	+20 to +115	sol.	109.5 (β-Gal*f*-(1→4)) 104.7 (β-Gal*p*-(1→4) and β-Glc*p*-(1→4)) 103.7 (α-Man*p*-(1→2)-α-D-Man*p*) 102.8 (α-Gal*p*-(1→2)) 102.2 (α-Man*p*-(1→2)-α-Man*p*-(1→2)) 101.0 (unsub. (1→6)-linked α-Man*p* units) 99.9 (sub. at O2, (1→6)-linked α-Man*p* units)	D_2O/70 °C	2, 4
CL-galactomannan	(1→6)-linked α-D-Man*p*	82:18:00	partly sub. at O4 and/or O2 by end units of β-Gal*p* (19%), α-Man*p* (39%) and β-Gal*f* (0.3%)	140	+40	sol.	109.2, 107.1, 104.7, 104.0, 103.5, 102.0, 100.4, 99.5	D_2O/30 °C	23
StR-galactomannan	(1→6)-linked α-D-Man*p*	57:43:00	partly sub. at O4 and/or O2 by end units of β-Gal*p* and α-Man*p*		+73				53
TI-glucomannan	(1→6)-linked α-D-Man*p*	93:00:07	partly sub. at O2 with α-D-Man*p* and a small amount of α-D-Glc*p*	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 Ingólfsdottir 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, anticoagulent, 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	$[\alpha]_D$ deg.	Sol. in cold H_2O	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-Gal*f* with sidechains in position 6 for about 7% of the units, and an (1→2)-linked α-L-Rha*p* 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 Gal*f*) 19.0 (C6-Me in Rha*p*) peeks around 102 (C1 in Rha*p*)	D_2O/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-Man*p*, monosub. at O4 (10%) or disub. at O2 and O4 (10%) with β-D-Xyl*p*		+25	sol.	105.2 (C1 in β-D-Xyl*p*) 103.0, 100.9, 99.7, 80.1, 17.3	D_2O/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-Man*p* units partly sub. at O2 or O4. Secondly: (1→6)-linked α-D-Gal*f* units, partly 2,4-di-*O*-sub. Terminal units of α-D-Gal*p* or β-D-Gal*f*.	18	+112	sol.	107.9 (C1 in β-D-Gal-*f*) 103.1 (C1 in α-D-Gal*p*) 101.4 (C1 in 1,6-α-D-man*p*) 99.9 (C1 in 1,4,6-α-D-Man*p*) 99.3 (C1 in 1,6-α-D-Gal*p*) 99.0 (C1 in 1,2,4,6-α-D-Gal*p*)	D_2O/25 °C	24

[a] ^1H-NMR shifts for thamnolan: 5.22 (*J* < 2.0 Hz, H1 in Gal*f*), 1.32 (s, C6-Me in Rha*p*).
[b] Not previously published. Measured in D_2O 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 cytocidal 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 active 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 μg/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 μg/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 μg/ml and 1000 μg/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 μg/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→3)-(1→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 etch-viruses (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 *Thamnolia 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→3)-β-glucans. This is perhaps not surprising, as (1→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→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→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.

Acknowledgements

The authors thank S. Baldursdottir M.Sc. for taxonomical assistance in the preparation of Table **1**. A supporting grant from University of Iceland Research Fund, is gratefully acknowledged.

References

[1] Huneck S, Yoshimura I. Identification of Lichen Substances. Berlin: Springer Verlag, 1996
[2] Gorin PAJ, Baron M, Silva MLC, Teixeira AZA, Iacomini M. Lichen carbohydrates. Ciencia e Cultura (Journal of the Brazilian Association for the Advancement of Science) 1993; 45: 27 – 36
[3] Barreto-Bergter E. Chemical Structure, Immunology and Application of Polysaccharides of Fungi and Lichens. In: Atta-ur-Rahman, editor. Studies in Natural Products Chemistry Vol. 5, Structure Elucidation (Part B). Amsterdam: Elsevier, 1989: 275 – 340
[4] Gorin PAJ, Baron M, Iacomini M. Storage Products of Lichens. In: Galun M, editor. CRC Handbook of Lichenology Vol. 3. Florida: CRC Press, 1988: 9 – 23
[5] Gorin PAJ, Barreto-Bergter E. The Chemistry of Polysaccharides of Fungi and Lichens. In: Aspinall GO, editor. The Polysaccharides Vol. 2. New York: Academic Press, 1983: 389 – 94
[6] Olafsdottir ES, Omarsdottir S, Smestad Paulsen B, Jurcic K, Wagner H. Rhamnopyranosylgalactofuranan, a new immunologically

active polysaccharide from *Thamnolia subuliformis*. Phytomedicine 1999; 6 (4): 273 – 9
[7] Iacomini M, Zanin SMW, Fontana JD. Isolation and characterization of β-D-glucan, heteropolysaccharide, and trehalose components of the Basidiomycetous lichen *Cora pavonia*. Carbohydrate Research 1987; 168: 55 – 65
[8] Common RS. The distribution and taxonomic significance of lichenan and isolichenan in the Parmeliaceae (lichenized Ascomycotina), as determined by iodine reactions. I. Introduction and methods. II. The genus *Alectoria* and associated taxa. Mycotaxon 1991; XLI: 67 – 112
[9] Teixeira AZA, Iacomini M, Gorin PAJ. Chemotypes of mannose-containing polysaccharides of lichen mycobionts: a possible aid in classification and identification. Carbohydrate Research 1995; 266: 309 – 14
[10] Takahashi K, Takeda T, Shibata S. Polysaccharides of lichen symbionts. Chemical & Pharmaceutical Bulletin 1979; 27 (1): 238 – 41
[11] Konig J, Pevelung E. Cell wall of the phycobiont *Trebouxia* and *Pseudotrebouxia:* Constituents and their localization. Lichenologist 1984; 16: 129 – 44
[12] Stone BA, Clarke AE. Chemistry and Biology of (1-3)-β-Glucans. Victoria, Australia: La Trobe University Press, 1992: 60 – 75, 283 – 358
[13] Vartia KO. Antibiotics from Lichens. In: Ahmadjian V, Hale MS, editors. The Lichens. New York: Academic Press, 1973: 547
[14] Ingólfsdóttir K. Bioactive Compounds in Iceland Moss. In: Smestad-Paulsen B, editor. Bioactive Carbohydrate Polymers, Proceedings Phytochemical Society of Europe. Dordrecht: Kluwer Academic Publishers, 2000: 25 – 36
[15] Baron M, Iacomini M, Fanta E, Gorin PAJ. Galactomannan, lichenan and isolichenan from the polysaccharide-rich lichen *Newropogon aurantiago-ater*. Phytochemistry 1991; 30: 3125 – 6
[16] Smith AL. Lichens. Cambridge University Press, 1921
[17] Takeda T, Funatsu M, Shibata S, Fukuoka F. Polysaccharides of lichens and fungi V. Antitumor active polysaccharides of *Evernia*, *pacroscyphus* and *Alectoria* species. Chemical & Pharmaceutical Bulletin 1972; 20: 2445 – 9
[18] Chanda NB, Hirst EL, Manners DJ. A comparison of isolichenin and lichenin from Iceland moss (*Cetraria islandica*). Journal of Chemical Society 1957; 1951 – 8
[19] Peat S, Whelan WJ, Turvey JR, Morgan K. The structure of isolichenin. Journal of Chemical Society 1961; 623 – 9
[20] Olafsdottir ES, Ingolfsdottir K, Barsett H, Smestad Paulsen B, Jurcic K, Wagner H. Immunologically active (1→3)-(1→4)-α-D-glucan from *Cetraria islandica*. Phytomedicine 1999; 6 (1): 33 – 9
[21] Ulander A, Tollens B. Untersuchungen über die Kohlenhydrate der Flechten. Berichte der deutschen chemischen Gesellschaft 1906; 39: 401
[22] Preston JF, Gander JE. Isolation and partial characterization of the extracellular polysaccharides of *Penicillium charlesii*. Archives of Biochemistry and Biophysics 1968; 124: 504 – 12
[23] Prado SRT, Gorin PAJ, Stuelp PM, Honda NK, Iacomini M. An unusual juxtaposition of polysaccharide components of *Collema leptosporum*. Carbohydrate Polymers 1999; 40: 271 – 6
[24] Ingolfsdottir K, Jurcic K, Fischer B, Wagner H. Immunologically active polysaccharide from *Cetraria islandica*. Planta Medica 1994; 60: 527 – 31
[25] Iacomini M, Schneider CL, Gorin PAJ. Comparative studies on the polysaccharides of *Cladonia alphestris* (Reindeer moss), *Cladonia confusa* and *Cladonia amaurocraea*. Carbohydrate Research 1985; 142: 237 – 51
[26] Shibata S. Polysaccharides of lichens. Journal of the National Science Council of Sri Lanka 1973; 1: 183 – 8
[27] Woranovicz-Barreira SM, Gorin PAJ, Sassaki GL, Tischer CA, Ahti T, Iacomini M. Chemotyping glucans from lichens of the genus *Cladonia*. Phytochemistry 1999; 52: 1069 – 74
[28] Nishikawa Y, Ohki K, Takahashi K, Kurono G, Fukuoka F, Emori M. Studies on the water soluble constituents of lichens. II. Antitumor polysaccharides of *Lasallia*, *Usnea* and *Cladonia* Species. Chemical & Pharmaceutical Bulletin 1974; 22: 2692 – 702
[29] Woranovicz-Barreira SM, Pinto BM, Gorin PAJ, Iacomini M. Novel structures in galactomannans of the lichens *Cladonia substellata* and *Cladonia ibitipocae*: Significance as chemotypes. Phytochemistry 1999; 51: 395 – 402
[30] Gorshkova RP, Nazarenko EL, Zubkov VA, Stepanenko LS, Isakov VV. Structural study of polysaccharides from *Cetraria cucullata* and *Cetraria islandica* lichens. Russian Journal of Bioorganic Chemistry 1997; 23 (2): 122 – 5
[31] Gorin PA, Iacomini M. Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. Carbohydr. Res. 1984; 128: 119 – 32
[32] Mittal OP, Neelakantan S, Seshadri TR. Chemical investigations of Indian lichens. Part XIV. Journal of Scientific and Industrial Research (India) 1952; 11B: 386 – 7
[33] Yokota I, Shibata S, Saito H. A ^{13}C-n.m.r. analysis of linkages in lichen polysaccharides: an approach to chemical taxonomy of lichens. Carbohydrate Research 1979; 69: 252 – 8
[34] Teixeira AZA, Iacomini M, Gorin PAJ. An unusual glucomannan from *Tornabenia incarnata*. Phytochemistry 1992; 31: 3467 – 70
[35] Micovic VM, Hranisavljevic-Jakovljevic M, Miljkovic-Stojanovic J. Structural study of polysaccharides from the oak lichen *Evernia prunastri* (L.). Ach. Carbohydrate Research 1969; 10: 525 – 33
[36] Iacomini M, Gorin PAJ, Baron M, Tulloch AP, Mazurek M. Novel D-glucans obtained by dimethyl sulphoxide extraction of the lichens *Letharia vulpina*, *Actinogyra muehlenbergii* and an *Usnea* sp. Carbohydrate Research 1988; 176: 117 – 26
[37] Gorin PAJ, Iacomini M. Structural diversity of galactomannan components isolated from lichens having Ascomycetous mycosymbionts. Carbohydrate Research 1985; 142: 253 – 67
[38] Takeda T, Nishikawa Y, Shibata S. A new α-glucan from the lichen *Parmelia caperata* (L.). Ach. Chemical & Pharmaceutical Bulletin 1970; 18 (5): 1074 – 5
[39] Smriga M, Saito H, Shibata S, Narui T, Okuyama T, Nishiyama N. PC-2, linear homoglucan with α-linkages, peripherally enhanches the hippocampal long-term potentiation. Pharmaceutical Research 1996; 13 (9): 1322 – 6
[40] Olafsdottir ES. Unpublished results.
[41] Nishikawa Y, Takeda T, Shibata S, Fukuoka F. Polysaccharides in lichens and fungi III. Further investigation on the structures and the antitumor activity of the polysaccharides from *Gyrophora esculenta* Miyoshi and *Lasailla papulosa* (Ach.) Llano. Chemical & Pharmaceutical Bulletin 1969; 17: 1910 – 6
[42] Periera EC, Nascimento SC, Lima RC, Silva NH, Oliveira AF, Bandeira E, Boitard M, Beriel H, Vicente C, Legaz ME. Analysis of *Usnea fasciata* crude extracts with antineoplastic activity. Tokai Journal of Experimental and Clinical Medicine 1994; 19: 47 – 52
[43] Mittal OP, Seshadri TR. Chemical investigation of Indian lichens XVI. Purification and composition of lichenin and isolichenin from Indian lichens. Journal of Scientific and Industrial Research (India) 1954; 13B: 244 – 5
[44] Shibata S, Nishikawa Y, Takeda T, Tanaka M, Fukuoka F, Nakanishi M. Studies on the chemical structures of the new glucans isolated from *Gyrophora esculenta* Miyoshi and *Lasailla papulosa* (Ach.) Llano and their inhibiting effect on implanted sarcoma 180 in mice. Chemical & Pharmaceutical Bulletin 1968; 16: 1639 – 41
[45] Nishikawa Y, Tanaka M, Shibata S, Fukooka F. Polysaccharides of lichens and fungi IV. Antitumor active *O*-acetylated pustulan-type glucans from lichens of *Umbilicaria* species. Chemical & Pharmaceutical Bulletin 1970; 18: 1431 – 4
[46] Lindberg B, McPherson J. Studies on the chemistry of lichens VI. The structure of pustulan. Acta Chemica Scandinavica 1954; 8: 985

[47] Kjölberg O, Kvernheim AL. Studies on the polysaccharides III. The structure of alkali-soluble polysaccharides in *Umbilicaria pustulata* (L.) Hoffm. and *Umbilicaria spodochroa* (Arch.) Hoffm. Acta Chemica Scandinavica 1989; 43: 280 – 5
[48] Stuelp PM, Carneiro Leao AMA, Gorin PAJ, Iacomini M. The glucans of *Ramalina celastri*: Relation with chemotypes of other lichens. Carbohydrate Research 1999; 40: 101 – 6
[49] Miceno AM, Gorin PAJ, Iacomini M. Galactomannan and isolichenan components of the carbohydrate-rich lichen *Ramalina ecklonii*. Agricultural and Biological Chemistry 1991; 55: 1391 – 2
[50] Takahashi K, Kon K, Yokota I, Shibata S. Chemotaxonomic studies on the polysaccharides of lichens. Polysaccharides of Stereocaulaceous lichens. Carbohydrate Research 1981; 89: 166 – 73
[51] Yokota I, Shibata S. A polysaccharide of the lichen *Stereocaulon japonicum*. Chemical & Pharmaceutical Bulletin 1978; 26: 2668 – 70
[52] Hauan E, Kjölberg O. Studies on the polysaccharides of lichens. I. The structure of a water-soluble polysaccharide in *Stereocaulon paschale* (L.) Fr. Acta Chemica Scandinavica 1971; 25: 2622 – 8
[53] Baron M, Gorin PAJ, Iacomini M. Structural studies on the galactomannan isolated from the lichen *Stereocaulon ramulosum*. Agricultural and Biological Chemistry 1989; 53: 1751 – 8
[54] Baron M, Gorin PAJ, Iacomini M. Isolation and identification of a linear (1→3)-linked β-D-glucan and other carbohydrate components of the lichen *Stereocaulon ramulosum* (Sw.) Rausch. Carbohydrate Research 1988; 177: 235 – 9
[55] Corradi da Silva MDL, Iacomini M, Jablonski E, Gorin PAJ. Carbohydrate, glycopeptide and protein components of the lichen *Sticta* sp. and effect of storage. Phytochemistry 1993; 33: 547 – 52
[56] Krämer P, Wincierz U, Grüber G, Tschakert J, Voelter W, Mayer H. Rational approach to fractionation, isolation and characterization of poylsaccharides from the lichen *Cetraria islandica*. Arzneimittel – Forschung/Drug Research 1995; 45 (I): 726 – 31
[57] Whistler RL, Bushway AA, Singh PP. Noncytotoxic, antitumour polysaccharides. Advances in Carbohydrate Chemistry and Biochemistry 1976; 32: 235 – 75
[58] Wagner H. Immunomodulatory agents from plants. Progress in Inflammation Research. Parnham M, series editor. Basel: Birkhauser Verlag, 1999
[59] Franz G. Polysaccharides in pharmacy: Current applications and future concepts. Planta Medica 1989; 55: 493 – 7
[60] Fukuoka F. et al. Polysaccharides in lichens and fungi. II. Antitumor activities on sarcoma-180 of the polysaccharide preparations from *Gyrophora esculenta* Miyoshi, *Cetraria islandica* (L.). Ach. var. *Orientalis* Asahina, and some other lichens. GANN Japanese Journal of Cancer Research 1968; 59: 421 – 32
[61] Nishikawa Y, Ohno H. Studies on the water-soluble constituents of lichens. IV. Effect of antitumor lichen-glucans and related derivatives on the phagocytic activity of the reticuloendothelial system in mice. Chemical & Pharmaceutical Bulletin 1981; 29 (11): 3407 – 10
[62] Takahashi K, Takeda T, Shibata S, Inomata M, Fukuoka F. Polysaccharides of lichens and fungi. VI. Antitumour active polysaccharides of lichens of Stictaceae. Chemical & Pharmaceutical Bulletin 1974; 22 (2): 404 – 8
[63] Konopa J, Privett OS, Jenkin HM, Goldin A. Polysaccharides as stimulators of nonspecific immunological response against syngeneic tumors in mice. In: Sigienthaler W, Luthy R, editors. Current Chemotherapy: Proceedings of the 10th International Congress of Chemotherapy. Washington D.C.: American Society for Microbiology, 1978: 1095 – 7
[64] Watanabe M, Iwai K, Shibata S, Takahashi K, Narui T, Tashiro T. Purification and characterization of mouse α_1-acid glycoprotein and its possible role in the antitumor activity of some lichen polysaccharides. Chemical & Pharmaceutical Bulletin 1986; 34 (6): 2532 – 41
[65] Tokuzen R, Nakahara W, Fukuoka F, Shibata S, Nishikawa Y. Liver changes in mice treated with lichen polysaccharides. Toxicology and Applied Pharmacology 1970; 17: 529 – 37
[66] Ingólfsdóttir K, Jurcic K, Wagner H. Immunomodulating polysaccharides from aqueous extracts of Iceland moss. Phytomedicine 1998; 5 (5): 333 – 9
[67] Leao AMAC, Buchi DF, Iacomini M, Gorin PAJ, Oliveira MBM. Cytotoxic effect against HeLA cells of polysaccharides from the lichen *Ramalina celastri*. Journal of Submicroscopic Cytology and Pathology 1997; 29 (4): 503 – 9
[68] Stübler D, Buchenauer H. Antiviral activity of the glucan lichenan (poly-β-(1→3,1→4)-D-anhydroglucose). I. Biological activity in tobacco plants. Journal of Phytopathology 1996; 144: 37 – 43
[69] Stübler D, Buchenauer H. Antiviral activity of the glucan lichenan (poly-β-(1→3,1→4)-D-anhydroglucose). II. Studies on the mode of action. Journal of Phytopathology 1996; 144: 45 – 52
[70] Smriga M, Chen J, Zhang J-T, Narui T, Shibata S, Hirano E, Saito H. Isolichenan, an α-glucan isolated from the lichen *Cetrariella islandica*, repairs impaired learning behaviour and facilitates hippocampal synaptic plasticity. Proceedings Japan Academie 1999; 75, Ser. B: 219 – 23
[71] Smriga M, Saito H. Effect of selected thallophytic glucans on learning behaviour and short-term potentiation. Phytotherapy Research 2000; 14 (3): 153 – 5
[72] Nishikawa Y. et al. Studies on the water-soluble constituents of lichens. III. Changes in antitumor effect caused by modifications of pustulan- and lichenan-type glucans. Chemical & Pharmaceutical Bulletin 1979; 27 (9): 2065 – 72
[73] Hirabayashi K, Iwata S, Ito M, Shigeta S, Narui T, Mori T, Shibata S. Inhibitory effect of a lichen polysaccharide sulfate, GE-3-S, on the replication of human immuno-deficiency virus (HIV) *in vitro*. Chemical & Pharmaceutical Bulletin 1989; 37 (9): 2410 – 12
[74] Bohn JA, BeMiller JN. (1→3)-β-D-Glucans as biological response modifiers. Carbohydrate Polymers 1995; 28: 3 – 14
[75] Maeda YY, Chihara G. Lentinan and other antitumoural polysaccharides. In: Wagner H, editor. Immunomodulatory Agents from Plants. Basel: Birkhauser Verlag, 1999: 203 – 23

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