© NIAB 2005
ISSN 1479-2621

Plant Genetic Resources 3(2); 273–287
DOI: 10.1079/PGR200572

Lichens—a promising source of bioactive secondary metabolites

Joël Boustie[1]* and Martin Grube[2]

[1]*Institut de Chimie de Rennes, Equipe: Substances Lichéniques et Photoprotection, UFR Pharmacie, 2 av du Pr. Léon Bernard (CS 34317), 35043 Rennes Cédex, France and*
[2]*Institute of Plant Sciences, Karl-Franzens-University Graz, Holteigasse 6, 8010 Graz, Austria*

Received 3 February 2005; Accepted 25 April 2005

Abstract

Lichen-forming fungi are unique organisms, producing biologically active metabolites with a great variety of effects, including antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic activities. However, only very limited numbers of lichen substances have been screened for their biological activities and their therapeutic potential in medicine. This is certainly due to the difficulties encountered in identification of the species, collection of bulk quantities, and the isolation of pure substances for structure determination and testing activity. Recently, possibilities for bypassing some of these former difficulties have arisen by the introduction of new techniques. This includes axenic cultivation for production of the genuine compounds or new ones, extraction of focused compounds, or synthesis of natural products or their derivatives for testing. Utilizing these new opportunities, the discovery of novel active metabolites, which could serve as lead compounds, is significantly facilitated. At the same time, the evolution of secondary metabolite patterns is studied using phylogenetic approaches. Yet, the genetic background of the complex chemical patterns is poorly understood. The scattered occurrence of some compounds suggests that their production evolved either in parallel or that ancient biosynthetic pathways are abandoned in many lineages. At least, studies on polyketide synthase genes from different lichen groups suggest a high level of gene paralogy. In this context, clades of orthologous polyketide synthase genes, which are often shared with distantly related non-lichenized fungi, can roughly be identified by their sequence similarity and their similar patterns of substitution rates. The functional assignment of paralogs is nevertheless difficult and reasonable only in a few cases. A global approach of the lichen metabolomic features appears to be essential in developing new and viable biotechnological processes which could afford suitable amounts of unique lichen compounds.

Keywords: bioactivity; biosynthesis; drugs; lichens; metabolites, phylogeny; phytochemistry

Introduction

Lichens are characterized as a stable and self-supporting association between fungi—the mycobionts—and photoautotrophic, algal partners—the photobionts. Since the mycobiont is unique in the symbiotic association and usually dominates the association, lichens are traditionally classified as a life-form of fungi. As an apparently successful form of fungal symbioses, the lichen-forming habit is maintained by one-fifth of all fungi, which includes more than *ca* 40% of ascomycetes, but only a few basidiomycetes. About 18,500 different lichen species have been described all over the world. They may grow under rather diverse and sometimes extreme ecological conditions. Lichens can be found in very cold and dry

* Corresponding author. E-mail: joel.boustie@univ-rennes1.fr

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

environments, either at polar latitudes or at extreme altitudes (up to 7400 m). Perhaps the other extreme are lichens which have adapted to tropical rainforest habitats, and may even grow on the surface of living leaves. Despite this extreme range of ecological adaptations, most lichens are sensitive to changes of their preferred ecological conditions and can hardly grow in non-native habitats.

Wherever lichens grow abundantly in nature, they often add a colourful aspect to their habitat. The distinct colours of many lichens are due to the massive accumulation of diverse secondary compounds, the 'lichen substances'. These represent comparatively small, but also chemically complex molecules. Beside the externally visible crystallized and non-crystallized pigments that are deposited in the upper surface layers of the lichen's vegetative body, also colourless substances are common, which are predominantly found in internal parts of the thalli.

The first reports on chemical differences in lichens stem back to the middle of the 19th century, yet, the initial contributions to lichen chemistry were clearly provided by the studies of Wilhelm Zopf, a German chemist (Zopf, 1895; and subsequent publications). A further milestone in lichen chemistry is attributable to the works of Japanese researchers Yasuhiko Asahina and Shoii Shibata, who—together with their co-authors—elucidated the chemical structures of numerous compounds, described their synthesis and introduced the identification of lichen substances by micro-recrystallization (e.g. Asahina, 1936; Shibata, 1958, 2000; and citations therein). In the second half of the 20th century, thin-layer chromatography became a routine technology, and was broadly applied to study the presence of compounds in diverse lichens. As a consequence, secondary compounds were extensibly used as characters at different hierarchic levels in the classification, in an approach called chemotaxonomy. At its rise in lichenology, a compilation of data for about 430 substances, including their occurrences in lichen species, was published as three influential books (Culberson, 1969, 1970; Culberson *et al.*, 1977). The number of known substances from lichens increased to more than 800 in the last major compilation (Huneck and Yoshimura, 1996; Huneck, 2001), while many more still need to be characterized (J. A. Elix, personal communication).

Diversity of secondary compounds in lichens

Structures of some common and unusual compounds in lichens are presented in Fig. 1.

According to their chemical structures, most lichen substances are phenolic compounds (orcinol and β-orcinol derivatives), dibenzofuranes and usnic acids (e.g. usnic acid), depsides (e.g. barbatic acid), depsidones (e.g. salazinic acid), depsones (e.g. picrolichenic acid), lactones (e.g. protolichesterinic acid, nephrosterinic acid), quinones (e.g. parietin), and pulvinic acid derivatives (e.g. vulpinic acid). Lichens apparently evolved diverse biosynthetic pathways to produce this diversity of compounds: mainly polymalonate, shikimic acid and mevalonic acid pathways.

Apart from compounds derived from these common pathways, which are found throughout all major lichen groups, there are also some unusual compound classes among these organisms; for example, arthogalin, a cyclic depsipeptide (Huneck and Himmelreich, 1995), and other amino acid-derived compounds such as the cytotoxic scabrosin esters isolated from *Xanthoparmelia scabrosa* (Ernst-Russell *et al.*, 1999b). Uncommon features are also detected in residues of common substance classes, in the form of other intramolecular arrangements, or in the binding with other compounds such as sugars. Recently, Rezanka *et al.* described many unusual compounds as brominated depsidones (Rezanka and Gushina, 1999), brominated acetylenic fatty acids (Rezanka and Dembitsky, 1999) and monotetrahydrofuranic acetogenin derivatives (Rezanka *et al.*, 2004). Series of γ-lactonic aliphatic acid glycosides were also identified (Rezanka and Gushina, 2000, 2001a, b), some of them forming a macrolactone ring (gobienins: Rezanka and Gushina, 2001c). Glycosides are not frequently encountered in lichens, yet, recent reports revealed the presence of xanthone glucosides (umbilicaxanthosides: Rezanka *et al.*, 2003) and the mycosporine collemin A from *Collema cristatum* (Torres *et al.*, 2004).

Evolutionary patterns of secondary chemistry in lichenized Ascomycotina

The presence of secondary compounds in cultured mycobionts and their absence in algae show that secondary products of lichens are generally formed by the fungus (e.g. Ahmadjian and Reynolds, 1961; Culberson and Armaleo, 1992). Exceptions are strains of lichen-associated cyanobacteria, which may produce nostoclides (Yang *et al.*, 1993), oligopeptides such as microcystins (Oksanen *et al.*, 2004) or derivatives of amino acids (Cox *et al.*, 2005). According to phylogenetic studies, the capacity of ascomycetous fungi to produce diverse and chemically complex compounds seems to have evolved during a principal evolutionary radiation, which gave rise to several major groups: Eurotiomycetes (including highly important and chemically diverse mould species), Lecanoromycetes, Leotiomycetes and Sordariomycetes (Lutzoni *et al.*, 2004). Interestingly, this basal evolutionary radiation includes also all major

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

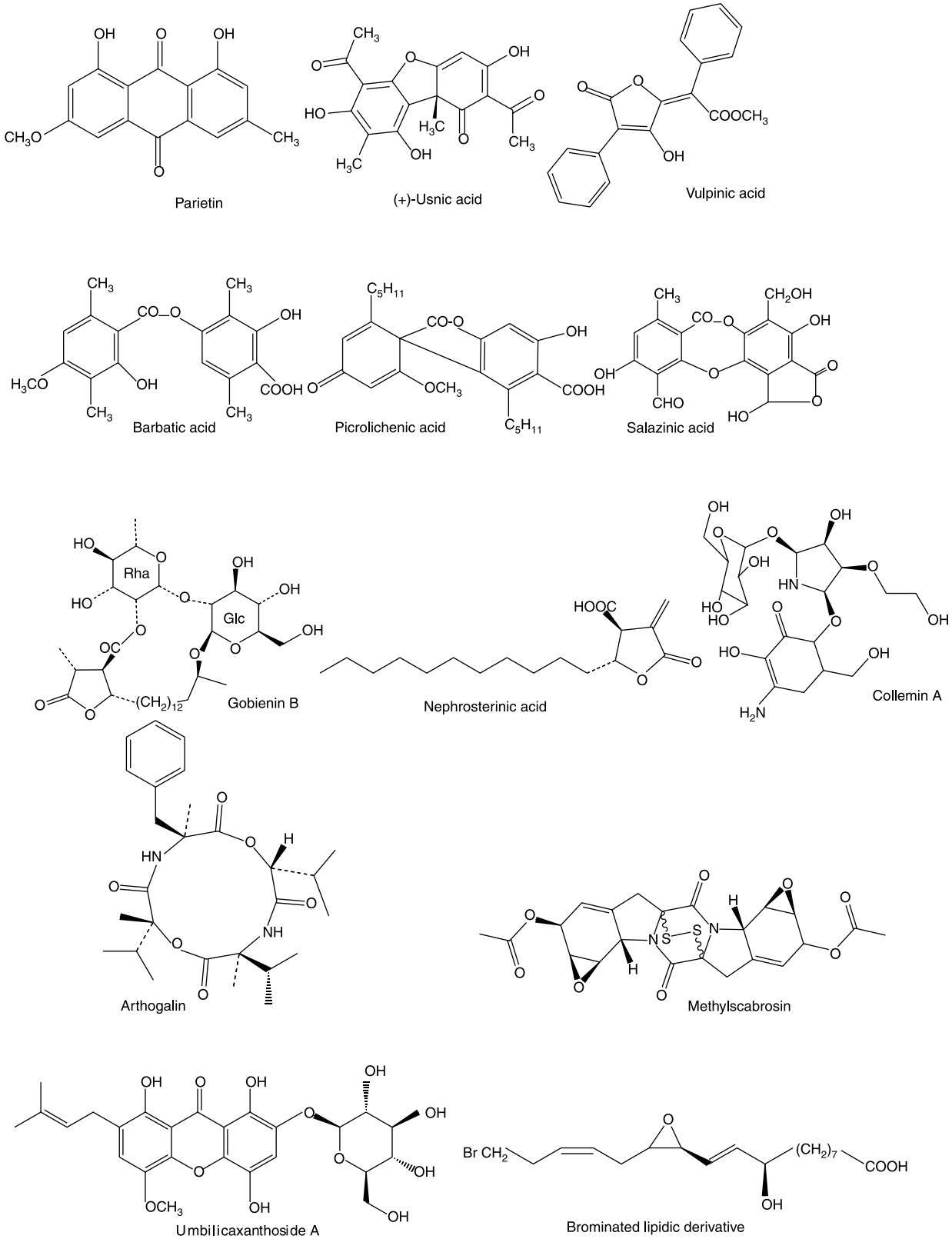


Fig. 1. Some usual and unusual lichen compounds.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

groups of ascomycetous lichens. As a consequence, it was suggested that the potential to form lichen symbioses and richness in secondary compounds could be shared ancestral features of these ascomycetous lineages. While certain groups may have 'secondarily' lost a lichen habit during evolution, it was suggested that the genetic potential for secondary metabolite production is maintained (e.g. Eurotiomycetes, and groups in Chaetothyriomycetes; see Lutzoni *et al*., 2001). The two large lineages of predominantly lichenized fungi—Lecanoromycetes and Arthoniomycetes—emerged independently, and while each of these groups contains some unique compounds, not found in the other, some other compounds are shared (and in certain cases also by other non-lichenized fungal groups). This suggests two possible evolutionary scenarios: either the corresponding biosynthetic pathways evolved prior to the divergence of Lecanoromycetes and Arthoniomycetes, or the shared compounds are the result of biosynthetic convergency. For the metabolites that are unique in evolutionary lineages, it may be hypothesized that pathways are lost or newly gained, either as a whole or by rearrangement of existing genetic material.

Differences in the presence of certain substance classes are sometimes observed among related lichen groups (see a compilation by Poelt and Leuckert, 1993). For example, *Xanthoparmelia* and *Neofuscelia*, two closely related genera of the Parmeliaceae, differ primarily in the pigments of their upper cortex: while *Xanthoparmelia* contains crystallized usnic acid, the closely related genus *Neofuscelia* is characterized by melanin-like cortical pigments. Moreover, other members of the Parmeliaceae may exclusively contain atranorin in the cortex. Such compound replacements are also observed within genera, e.g. in *Rhizocarpon*. There, the crystallized extracellular pulvinic acid derivative rhizocarpic acid, a bright yellow compound, is repeatedly lost in several phylogenetic lineages of this genus (Ihlen and Ekman, 2002), and replaced by melanin-like cell-wall pigments. It has not been clarified whether the production of rhizocarpic acid is suppressed in the latter species, whether the corresponding genes degenerated by mutation, or were lost, or whether the dark wall pigments could result from a polymerization of certain shared precursors. On the other hand, compounds which characteristically occur in large evolutionary lineages—such as anthraquinones throughout the yellow-coloured Teloschistaceae—may have a comparatively dispersed occurrence in other lineages.

As is apparent from the examples above, it is not possible to use chemical differences pragmatically to assess relationships of fungi without respect to other characters. This is clearly found at all hierarchic levels of relationships. Especially within certain species complexes, chemosyndromatic variation (i.e. the optional presence of biogenetically close compounds) has caused much debate about 'proper' classification. Usually, morphologically similar lichens that differ in the presence of compounds of the same chemosyndrome are taxonomically interpreted as varieties or informally as chemotypes of the same species. However, as earlier studies of ribosomal DNA by DePriest (1994) show, chemotypes are not necessarily linked with particular genotypes in a species.

Differential expression of lichen compounds *in vivo* and *in vitro*

The lichen thallus, i.e. the characteristic vegetative body, has in many species a strict organization with different functional layers. This typically includes a surface layer (the upper cortex, usually containing only the mycobiont), an algal layer (containing the photobiont) and a medullary layer (containing the mycobiont). Lichen substances are often expressed differentially in these layers, and typical cortical substances can be distinguished from compounds usually found only in the medulla. This could be linked with their biological function: the cortical compounds are regarded as a kind of light-filter, which is apparently not a function of compounds below the algal layers. In addition to the differences within a lichen thallus, the presence of substances in individuals of a species can also differ according to geographic, altitudinal or microhabitat conditions. For example, a North–South gradient was observed in *Lecanora rupicola* (Leuckert and Poelt, 1978), where chemical strains that contain certain xanthones are more common in the Mediterranean range of the entire geographic distribution of this species. It is not known, however, whether this pattern is due to genetic differentiation of populations, to climatic reasons or to both.

Compound production may fluctuate with the seasons (Huneck *et al*., 2004; Gauslaa and McEvoy, 2005), but is affected also by microclimatic fluctuations, as pale thalli are often observed under suboptimal light conditions. The phenolic content and distribution is modified according to direct UV radiations (mainly accumulation in the outer layers of the medulla) but chemical signals are also involved for a tuned adaptation of these organisms to drastic UV climate changes (Buffoni Hall *et al*., 2002). In a series of papers, Solhaug and co-workers have successfully used acetone-rinsed lichen thalli as a tool to investigate resynthesis of secondary compounds. They showed that lichens start resynthesizing parietin and melanins as a response to hydration and UV-B radiation (Solhaug *et al*., 2003). They have also shown that the parietin resynthesis in *Xanthoria elegans* increased with decreasing latitude (Nybakken *et al*.,

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

2004). Other research groups have looked upon the effects of UV-B radiation in the field and the potential damaging effects of the ozone depletion-related enhancements of shortwave radiation. In particular, the UV-screening roles of usnic acid have been given much focus, and results regarding its UV protection role are equivocal (BeGora and Fahselt, 2001; Bjerke *et al.*, 2002; Buffoni Hall *et al.*, 2002; Rancan *et al.*, 2002). The only long-term studies have shown that lichen activity can be seriously reduced by UV-B exposure (Solheim *et al.*, 2002), but that depside synthesis is more affected by warming than by UV-B radiation (Bjerke *et al.*, 2003).

Lichens synthesize significant amounts of substances only in permissive physiological stages. As a consequence, the production in axenic cultures can differ substantially from that in nature. A chemotype of the natural lichen *Lecanora dispersa* contains 2,7-dichlorolichexanthone as the major secondary compound, but cultured spore isolates, growing without the alga, produced pannarin and related depsidones instead (Leuckert *et al.*, 1990). Pannarin could not be confirmed in voucher materials of the natural source lichen, but the biosynthetic potential for this depsidone was proven for the species by a herbarium survey. Another unexpected result is the production of atranorin in *Usnea hirta*, when cultured in a modified LB medium (Kinoshita *et al.*, 1993), since this compound is not present throughout the entire genus *Usnea*. Moreover, Kon *et al.* (1997) found only very low amounts of the typical metabolites, i.e. usnic acid, salazinic acid, in cultures of *Usnea orientalis*, while these rather produced the dibenzofuranes hypostrepsilic acid and isostrepsilic acid. The list of cases where metabolites are found in culture but not present in lichens can be extended further (Mosbach, 1967; Fox and Huneck, 1969; Ejiri *et al.*, 1975; Hamada and Ueno, 1987; Miyagawa *et al.*, 1994; Yamamoto *et al.*, 1996; Tanahashi *et al.*, 1997; Stocker-Wörgötter, 2002a). Interestingly, if the mycobionts were co-cultured with their original photobionts, the natural metabolite pattern was restored. These findings indicate that a wider variation of metabolites can be induced in culture than found in natural lichen thalli.

An influence of agar concentration on the efficiency of usnic acid production in cultured *Usnea hirta* was indicated by Kinoshita *et al.* (1993). Several other studies indicated that osmotically stressed mycobiont cultures seem to induce the biosynthesis of certain secondary metabolites (Yamamoto *et al.*, 1985; Hamada, 1988, 1989, 1993; Miyagawa *et al.*, 1993), and this includes also the synthesis of new compounds, which do not occur in the natural lichen (e.g. Amano *et al.*, 2000). The effects of osmotic stress on the efficiency of metabolite production under natural conditions also deserve further studies, as Solhaug and Gauslaa (2004) showed that resynthesis of secondary metabolites in acetone-soaked thalli is promoted by exposure to osmolytes.

By using a modified Sabouraud medium, Stocker-Wörgötter (2001) could achieve a higher degree of morphology differentiation in the cultured mycobiont, and, as a consequence, the expression of species-characteristic secondary metabolites in the *Cladonia verticillaris* complex. A study by Stocker-Wörgötter and Elix (2004) suggests that the biosynthesis of compounds may be determined by stage and organization-specific factors. They showed that mycobiont cultures derived from spores and thallus fragments differ in their secondary metabolite patterns. While spore-derived cultures of *Bunodophoron patagonicum* are able to produce both dibenzofuranes and depsides, for unknown reasons, cultures of thallus fragments produced only depsides. For selected further species, Stocker-Wörgötter (2002b) described further how culture conditions can modify the expression of lichen compounds. It is apparent that in most cases high light conditions are promoting similar compound expression patterns of cultured mycobionts and natural lichen samples.

Genes involved in production of secondary metabolites

As far as we know today, the biosynthesis of fungal secondary products is encoded by clusters of sequentially arranged genes (e.g. Keller and Hohn, 1997). For the production of polyketides the first essential step is the concatenation of C2-units by polyketide synthases (PKS). Hence, these enzymes synthesize the basic structure of a secondary metabolite. Fungal PKS genes encode multifunctional proteins (fungal type I PKSs) with only a single, reiteratively used ketoacyl synthesis domain that sequentially condensates C2-units. The gene fragment encoding this domain can be targeted by PCR primers (Bingle *et al.*, 1999; Lee *et al.*, 2001; Miao *et al.*, 2001; Nicholson *et al.*, 2001; Sauer *et al.*, 2002; Grube and Blaha, 2003; Schmitt *et al.*, 2005) to establish a phylogeny. Grube and Blaha (2003), using a heterologous primer approach, found that a gene from *Xanthoria parietina* was related to genes that encode for the production of aflatoxins or similar mycotoxins. Since anthraquinones are precursors of these mycotoxins and due to the presence of the crystallized anthraquinone parietin in the upper cortex of *Xanthoria*, it was reasonable to suggest that this gene represents an anthraquinone-producing polyketide synthase.

In Fig. 2, a preliminary Bayesian phylogenetic tree is presented for predicted amino acid sequences of ketoacyl synthase domains, which are part of fungal PKSs that

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

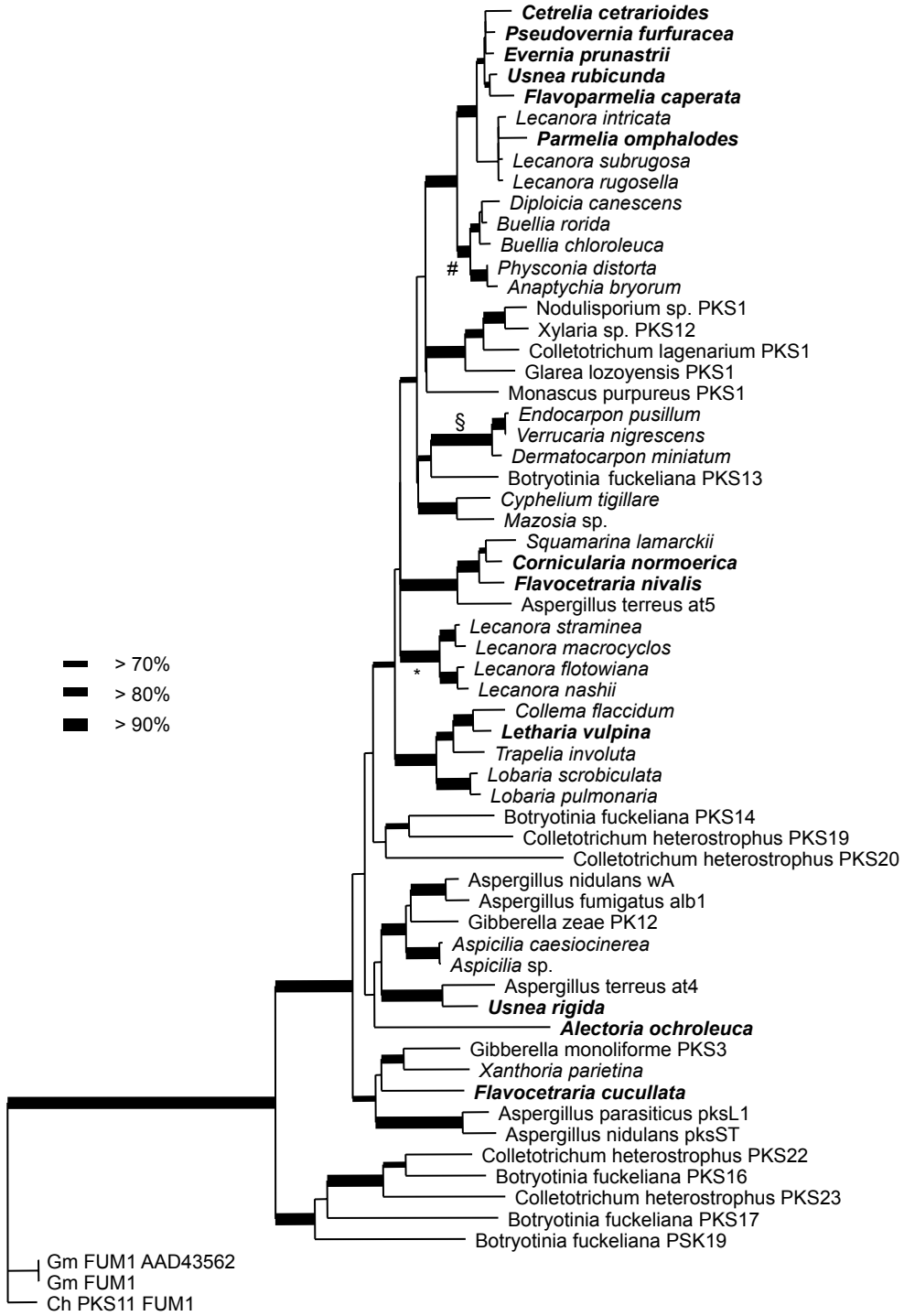


Fig. 2. Phylogenetic tree of amino acid sequence data from ketoacyl synthase domains in lichens. Results from a Bayesian analysis using the program MrBayes (Huelsenbeck and Ronquist, 2001). Lichen fungi are in italics, members of Parmeliaceae are in boldface and clades of other lichen taxa, as mentioned in the text, are marked: #, Members of Physciaceae, §, members of Verrucariales and *, members of the genus *Lecanora*. Bayesian posterior probabilities of branches are indicated by different thickness of branches (details about specimens and the analysis available on request from the authors).

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

putatively produce non-reduced polyketides. Unique PCR products were obtained from total DNA extracts of lichens with the primers LC1-LC2 (Bingle *et al.*, 1999). While the represented genes from the lichen families Physciaceae (# in Fig. 2) and Verrucariaceae (§ in Fig. 2) form monophyletic lineages and correspond well with the known relationships among the taxa within these orders, the relationship of sequences in *Lecanora* (Lecanoraceae; *in Fig. 2) does not reflect the species relationships within that genus and likely represent paralogous genes. Another paralogous gene from *Lecanora* groups more closely with a gene from *Parmelia* (Parmeliaceae), whereas other members of Parmeliaceae (in boldface in Fig. 2) contain further paralogous genes that are widely dispersed in the tree, and often group with paralogs of non-lichenized fungi. This pattern certainly represents just the peak of an iceberg and it is rather possible that numerous paralogous PKS genes evolved by complex patterns of gene duplications in lichen mycobiont genomes. Meanwhile, bioinformatic approaches are used to explore completely sequenced genomes of non-lichenized fungal model organisms for polyketide synthases (Kroken *et al.*, 2003; Varga *et al.*, 2003). As lichen fungal genomes have so far not been sequenced, the results from non-lichenized fungi are taken as a framework for more detailed investigations of paralogy and functional divergence of polyketide synthases also in lichens. The heterologous primers for PKS genes known so far will preferentially amplify one gene or a few genes from a subset of paralogs in lichen genomes, therefore the discovery of additional PKS genes can follow three approaches: (i) cloning of PCR products obtained with primers of low specificity for different paralogs (Schmitt *et al.*, 2005), (ii) amplification with more specific primers for particular paralog lineages (M. Opanowicz and M. Grube, unpublished), and (iii) screening of genomic libraries of lichen mycobionts using PKS gene probes (Miao *et al.*, 2001). Perhaps due to the slow growth in culture, genomic libraries of lichen mycobionts are rare. However, they will be extremely useful for characterizing full-length PKS genes and additional genes that form biosynthetic pathway gene clusters in lichens.

While these approaches will clearly increase our knowledge about the diversity of polyketide synthesis in lichen mycobionts, there is still a long way to go until we begin to understand the function of these genes and their contribution to the production of secondary metabolites. This will definitely also involve further experimental studies, such as the analysis of transcribed PKS paralogs, and perhaps also their expression in heterologous hosts.

Yet, studies of full-length genes from lichens and their use in biotechnological approaches were accomplished only once. Orotidine 5′-monophosphatase—responsible for the decarboxylation of orotidylic acid to uridylic acid—was recovered from a phage library of the lichen *Solorina crocea* with hybridization methods (Sinnemann *et al.*, 2000). Heterologous expression in *Aspergillus nidulans* indicated temperature sensitivity in the absence of uridine, suggesting an adaptation to lower temperature of the lichen enzyme. Because *Solorina crocea* is a lichen that grows at higher altitudes, this result fits well with the ecology of the species. Many other lichens can grow under rather hostile environmental conditions, therefore similar studies could reveal a number of other enzymes with interesting physiological adaptations.

Bioactive compounds and potential medicinal use

The wide variety of biological activities of lichens is generally correlated to their special ecological circumstances (e.g. Lawrey, 1986, 1995; Rikkinen, 1995), with production of metabolites involved in antimicrobial actions and deterrent properties (sometimes highly toxic to certain animals). Some review articles recently underlined the pharmaceutical potential of these substances (e.g. Huneck, 1999; Yamamoto, 2000; Muller, 2001). For centuries, lichens have also been used in folk medicine (Richardson, 1988; Schindler, 1988; Hawksworth, 2003). Commercial preparations based on the presence of usnic acid are available in some countries for local antiseptic activity and many properties have been described for this compound (for a review see Ingólfsdóttir, 2002). Usnic acid, like many other lichen compounds, causes allergic reactions, but a recent incident of some hepatotoxicity was attributed to usnic acid due to its inadequate use in nutraceuticals (about 500 mg a day taken orally to lose weight; Durazo *et al.*, 2004; Neff *et al.*, 2004).

Lichens are still part of folk and alternative medicines in Asian countries (e.g. Benalu teh®) with various indications (Saklani and Upreti, 1992), and in the *European Pharmacopoiea* (2005), Iceland moss (= *Cetraria islandica*) is included and indicated uses correspond to a cough remedy and to a *tonicum amarum*. *Lobaria pulmonaria* has also been used as a cough remedy based on its lung-shaped appearance, and is still used in homeopathy (as *Sticta pulmonaria*). Together with recent studies on the antifungal activities of some Indian lichens (Shahi *et al.*, 2000, 2001, 2003), considerable information is available on ethnobotanical utilization of lichens by the different ethnic groups in India (Brij Lal and Upreti, 1985; Brij Lal *et al.*, 1985; Brij Lal, 1988; Singh *et al.*, 2000; Kumar and Upreti, 2001). The pharmacological and other biological activities of lichens and lichen substances can be divided into the following categories based on current knowledge.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

Antibiotic activity

A number of lichen extracts and compounds were screened for antibacterial activity in the 1950s and in many cases activity was confirmed against mycobacteria and Gram-positive organisms (Stoll *et al.*, 1950). A review of the work performed during this period is presented in Vartia (1973). More recent reports include a study describing the antimicrobial screening of lichen extracts and subsequent isolation of compounds with a broad spectrum of activity against yeast as well as Gram-positive and -negative bacteria (Ingólfsdóttir *et al.*, 1985). The *in vitro* activity of usnic acid against pathogenic Gram-positive organisms has been confirmed (Ghione *et al.*, 1988) and it has been used in topical preparations, mouthwash and toothpaste. In addition, the compound was reported active against anaerobic bacteria, e.g. *Bacteroides* species and *Clostridium perfringens* (Lauterwein *et al.*, 1995). In light of the use of Iceland moss for the treatment of gastric and duodenal ulcers, it is noteworthy that a constituent of this lichen, protolichesterinic acid, has been shown to exhibit *in vitro* activity against *Helicobacter pylori* (Ingólfsdóttir *et al.*, 1997). The emergence of drug-resistant bacterial strains, e.g. in the case of tubercular mycobacteria, has currently led to severe clinical complications. Hence, new antimicrobial agents are urgently needed and as the search is being extended to new sources, lichens are clearly an interesting source of compounds.

Antitumour and antimutagenic activity

Some lichen substances have been partially investigated for antitumour and antimutagenic activity: (−)-usnic acid, a dibenzofuran with an antitumoral effect against Lewis Lung carcinoma and P388 leukaemia (Kupchan and Kopperman, 1975; Takai *et al.*, 1979) was found to be involved in mitosis inhibition (Huovinen and Lampero, 1989; Cardarelli *et al.*, 1997) and apoptotic induction (Bézivin *et al.*, 2004). The butyrolactone, protolichesterinic acid, was also found active as an antiproliferative against leukaemia cells K-562 ($IC_{50} = 20\,\mu g/ml$) and against Ehrlich solid tumour, while nephrosteranic acid derivatives have a poor activity (Hirayama *et al.*, 1980). Polyporic acid (a terphenylquinone) and derivatives (Cain, 1961, 1966), a depsidone, physodalic acid (Shibamoto and Wei, 1984), lichen glucans (Nishikawa et al., 1969, 1979; Hirayama *et al.*, 1980; Nishikawa and Ohno, 1981; Hirabayashi *et al.*, 1989) including lichenin derivatives (Demleitner *et al.*, 1991) have also been investigated in this way. A review was recently published on structure features and bioactivities of lichen polysaccharides (Ólafsdóttir and Ingólfsdóttir, 2001). Concerning new lichen secondary metabolites that exhibit relevant cytotoxic activities against cancer cell lines, original N-containing complex structures (scabrosin esters) isolated from *Xanthoparmelia scabrosa* ($IC_{50} = 0.27\,\mu M$ p-815), an unusual indenone naphthopyrone euplectin from *Flavoparmelia euplecta* ($IC_{50} = 0.58\,\mu M$, P-815), and a naphthazarin-derived dimer (hybocarpone) isolated from a mycobiont culture of *Lecanora hybocarpa* ($IC_{50} = 1\,nM$, MCF-7), have been recently described (Ernst-Russell *et al.*, 1999a, b).

Extracts from lichens of various countries were evaluated in several predictive tests (including *in vitro* antiproliferation of cancer cells, Brine shrimp test and cancer chemopreventive assays; Nascimento *et al.*, 1994; Esimone *et al.*, 1999; Ingólfsdóttir *et al.*, 2000, Bézivin *et al.*, 2003). Most of them were found active with $IC_{50} < 20\,\mu g/ml$. Depsidone and depside series such as pannarin, 1′-chloropannarin and sphaerophorin tested in cell cultures of lymphocytes were shown to have a higher cytotoxic effect than colchicine (Correché *et al.*, 2002). The depsidones salazinic acid, stictic acid and psoromic acid were the most apoptotic active derivatives among 15 lichen compounds evaluated on primary cultures of rat hepatocytes (Correché *et al.*, 2004).

Antiviral activity

Some anthraquinonic compounds which are also encountered in higher plants (emodin and derivatives) have shown *in vitro* antiviral activity considered of interest against Human Cytomegalovirus (Wood *et al.*, 1990). Seventeen depsides and depsidones have been examined for their inhibitory activity against HIV integrase and pharmacophores derived from virensic acid allowed selection of various potent inhibitors (Neamati *et al.*, 1997). The butyrolactone, protolichesterinic acid, isolated from *Cetraria islandica*, was also found to inhibit HIV-RT (Pengsuparp *et al.*, 1995). Four depsides and above all (+)-usnic acid were found active against EBV activation and could therefore constitute valuable candidates as antitumour promoters (Yamamoto *et al.*, 1995).

Concerning high molecular weight compounds, a sulphate (GE-3-S) prepared by chlorosulphonic acid treatment of GE-3, a partially acetylated (1 → 6) glucan from the lichen *Umbilicaria esculenta*, inhibited the cytopathic effect of HIV *in vitro* (Hirabayashi *et al.*, 1989).

Enzyme inhibitory activities

Lichen acids, mainly atranorin, evernic, physodic and usnic acids, are powerful inhibitors of some metabolic

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

enzymes, interestingly related to polyamine metabolism, such as arginase, arginine decarboxylase, ornithine decarboxylase, etc. (Legaz *et al.*, 1983, 2001; Planelles and Legaz, 1987; Okuyama *et al.*, 1991; Matsubara *et al.*, 1997, 1998; Kinoshita *et al.*, 2002).

Inhibition of lipoxygenase has also been demonstrated for some lichen extracts and compounds (Ingólfsdóttir *et al.*, 1985, 1994, 2002; Ögmundsdóttir *et al.*, 1998). The inhibition of prostaglandin biosynthesis (Sankawa *et al.*, 1982) as well as the inhibition of leukotriene B4 biosynthesis depicted by Kumar and Muller (1999) can be related to the anti-inflammatory activity, analgesic and antipyretic activities (Okuyama *et al.*, 1995) and local anaesthesic effect (Correia Da Silva, 1981) of some lichen compounds.

Inhibition of xanthine oxidase (a key enzyme in hyperuricaemia) and of tyrosinase (involved in melanin biosynthesis) by extracts of natural lichens (Behera *et al.*, 2004) or cultured lichen tissue (Higuchi *et al.*, 1993; Behera and Makhija, 2002) was found in cases to be superior to the assay standards used. Tyrosinase inhibitors such as divarinol or diphenylmethane derivatives have been isolated (Matsubara *et al.*, 1997) and more potent derivatives obtained by synthesis (Matsubara *et al.*, 1998).

Due to the phenolic nature of most of the lichen compounds, many antioxidant activities (Hidalgo *et al.*, 1993, 1994; Jayaprakasha and Rao, 2000) and superoxide scavenging properties (Behera *et al.*, 2004) have been described. Such properties, as well as the metal-chelating ability and UV-filtering performances of a number of these compounds (Stepanenko *et al.*, 2002), should lead to various applications of these or their derivatives for protective or environmental purposes.

Future prospects: new and hemisynthetic lichen compounds and chimeric genes

Total synthesis of some lichen compounds has been achieved (Huneck *et al.*, 1993; Huneck, 2001; Nicolaou and Gray, 2004). Pharmacomodulation of active lichen compounds has been initiated with usnic acid coupled to various amines and polyamines using an unusual chemical way (Ouzzine *et al.*, 2001) but also performed using solid–phase organic synthesis (SPOS, MultiPins® Chiron; Ouzzine *et al.*, 2001).

In the Vicente and Legaz Laboratory (Blanch *et al.*, 2001, 2002; Fontaniella *et al.*, 2000), detection of free and conjugated polyamines to lichen phenolics has been extensively studied in several lichen taxa (*Evernia*, *Pseudevernia* and *Xanthoria*). In addition, the use of bioreactors consisting of both cells and enzymes immobilized in several matrices (alginate, kaolinite, polyacrylamide) is applied to elucidate the nature of enzymes involved in the production of depsides and depsidones by *Evernia* and *Pseudevernia* species. Affinity binding with such enzymes is also useful for characterization and purification of lichen compounds (Molina and Vicente, 2000; Legaz *et al.*, 2001).

Purification and identification of lichen compounds is a challenge of importance generally managed by crystallization or chromatographic techniques, and some special techniques such as supercritical CO_2 should be more developed (Lisickov *et al.*, 2002). As many secondary metabolites are currently found in various lichens, the use and development of standardized methods is crucial for a dereplicative approach. Thin-layer chromatography (Culberson and Kristinsson, 1970; Culberson, 1972; BeGora and Fahselt, 2000) and high-performance liquid chromatography (Huovinen *et al.*, 1985; Huovinen, 1987; Feige *et al.*, 1993; Yoshimura *et al.*, 1994) procedures have been developed. To deal with small amounts of material more refined techniques such as thermochemolysis–gas chromatography (MacGillivray and Helleur, 2001), solid-phase microextraction–gas chromatography–mass spectrometry (De Angelis *et al.*, 2003) or Fourier-transform Raman (Edwards *et al.*, 2003) can be used. Thus, a focus on minor compounds, generally complicated by the relatively high abundance of some lichen compounds should allow findings of more specific and interesting compounds.

Genetic technology can now also be used to design chimeric genes with novel functions. An interesting case has been demonstrated in *Aspergillus*, where a novel hexaketide naphthalene was synthesized by a polyketide synthase that was derived from the combination of gene fragments of pentaketide and heptaketide synthases (Watanabe and Ebizuka, 2002). Such pioneering experiments can contribute to combinatorial approaches for the production of novel bioactive compounds. With their diversity of metabolites, lichenized fungi could provide an interesting set of genes for such future prospects.

Conclusions

The above-mentioned activities clearly indicate the potential of lichen compounds for pharmaceutical purposes, but some properties of lichens still need consideration. Their slow growth, both in nature and in axenic cultures, may be regarded as a major problem for the production of metabolites. However, culture conditions of lichen mycobionts can be optimized in order to support the synthesis of interesting secondary compounds. Much progress has been achieved recently by specifically addressing these issues (Stocker-Wörgötter, 1998, 2001, 2002a, b; Stocker-Wörgötter and Elix, 2004).

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

On the other hand, the large amount of compounds in a lichen thallus (extraction yields often between 5 and 25% of dried lichen material) may be sufficient both for testing, even though many cases require special solubilization efforts (Kristmundsdóttir *et al.*, 2002), and for direct crystallizations. This may be of interest for pharmacological purposes, as direct crystallizations facilitate the isolation and characterization of compounds and will also allow their derivatization and hemisynthetic studies. In parallel, progress in the investigation of the rich genetic treasure of polyketide synthase genes may revolutionize their use in future biotechnological approaches, including heterologous expression in fast-growing hosts and the design of chimeric molecules.

New compounds are to be described from poorly studied lichens, and even in species that are considered chemically well known, new chemical strains are still detected (e.g. Stocker-Wörgötter *et al.*, 2004). Particularly in tropical habitats, many lichens are known which contain structurally unknown lichen products. Here we are at the entrance door of a vast and interesting field of research.

Acknowledgements

COST and members of the D28/005/03 Working Group (K. Ingólfsdóttir, Reykjavik; E. Stöcker-Worgötter, Salzburg; M. E. Legaz, Madrid) are gratefully acknowledged for their input in lichen bioactive compounds research and contribution in reviewing some parts of the manuscript. M.G. thanks the Austrian Science Foundation (FWF) for financial support (P17638, P14610), as well as B. Fetz, J. Blaha (both Graz) and M. Opanowicz (Wroclaw) for help in the laboratory.

References

Ahmadjian V and Reynolds JT (1961) Production of biologically active compounds by isolated lichenized fungi. *Science* 133: 700–701.

Amano T, Miyagawa H, Ueno T and Hamada N (2000) Production of 5,7-dihydroxy-6-hydroxymethyl-2-methoxy-1,4-naphthoquinone by the cultured lichen mycobiont of *Opegrapha* sp. no. 9771836. *Zeitschrift für Naturforschung* 55b: 667–669.

Asahina Y (1936) Microchemischer Nachweis der Flechtenstoffe. I. *Journal of Japanese Botany* 12: 516–525.

BeGora MD and Fahselt D (2000) An alternative method for the quantification of lichen secondary products. *The Bryologist* 103: 563–567.

BeGora MD and Fahselt D (2001) Usnic acid and atranorin concentrations in lichens in relation to bands of UV irradiance. *The Bryologist* 104: 134–140.

Behera BC and Makhija U (2002) Inhibition of tyrosinase and xanthine oxidase by lichen species *Bulbothrix setschwanensis*. *Current Science* 82: 61–66.

Behera BC, Adawwadkar B and Makhija U (2004) Capacity of some Graphidaceous lichens to scavenge superoxide and inhibition of tyrosinase and xanthine oxidase activities. *Research Communications* 87: 83–87.

Bézivin C, Tomasi S, Lohézic-Le Devehat F and Boustie J (2003) Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* 10: 499–503.

Bézivin C, Tomasi S, Rouaud I, Delcros JG and Boustie J (2004) Cytotoxic activity of compounds from the lichen: *Cladonia convoluta*. *Planta Medica* 70: 874–877.

Bingle LEH, Simpson TJ and Lazarus CM (1999) Ketosynthese domain probes identify two subclasses of fungal polyketide synthese genes. *Fungal Genetics and Biology* 26: 209–223.

Bjerke JW, Lerfall K and Elvebakk A (2002) Effects of ultraviolet radiation and PAR on the content of usnic and divaricatic acids in two arctic-alpine lichens. *Photochemical and Photobiological Sciences* 1: 678–685.

Bjerke JW, Zielke M and Solheim B (2003) Long-term impacts of simulated climatic change on secondary metabolism, thallus structure and nitrogen fixation activity in two cyanolichens from the Arctic. *New Phytologist* 159: 361–367.

Blanch M, Blanco Y, Fontaniella B, Legaz ME and Vicente C (2001) Production of phenolics by immobilized cells of the lichen *Pseudevernia furfuracea*: the role of epiphytic bacteria. *International Microbiology* 4: 89–92.

Blanco Y, Blanch M, Fontaniella B, Legaz ME, Millanes AM, Pereira EC and Vicente C (2002) Bioproduction of lichen phenolics by immobilized cells with emphasis on the role of epiphytic bacteria. *Journal of the Hattori Botanical Laboratory* 92: 245–260.

Brij Lal (1988) Traditional remedies for bone fracture among the tribals of Madhya Pradesh, India. *Aryavaidyan* 1: 190–195.

Brij Lal and Upreti DK (1995) Ethnobotanical notes on three Indian lichens. *Lichenologist* 27: 77–79.

Brij Lal, Upreti DK and Kalakoti BS (1985) Ethnobotanical utilization of lichens by the tribals of Madhya Pradesh. *Journal of Economic and Taxonomic Botany* 7: 203–204.

Buffoni Hall RS, Bornman JF and Björn LO (2002) UV-induced changes in pigment content and light penetration in the fruticose lichen *Cladonia arbuscula* ssp. *mitis*. *Journal of Photochemistry and Photobiology B (Biology)* 66: 13–20.

Cain BF (1961) Potential antitumour agents. Part I. Polyporic acid series. *Journal of the Chemical Society* Abstracts: 936–940.

Cain BF (1966) Potential anti-tumour agents. Part IV. Polyporic acid series. *Journal of the Chemical Society* Section C: Organic: 1041–1045.

Cardarelli M, Serino G, Campanella L, Ercole P, De Cicco Nardone F, Alesiani O and Rossiello F (1997) Antimitotic effects of usnic acid on different biological systems. *Cellular and Molecular Life Sciences* 53: 667–672.

Correché ER, Carrasco M, Giannini F, Piovano M, Garbarino J and Enriz D (2002) Cytotoxic screening activity of secondary lichen metabolites. *Acta Farmaceutica Bonaerense* 21: 273–278.

Correché ER, Enriz RD, Piovano M, Garbarino J and Gomez-Lechon MJ (2004) Cytotoxic and apoptotic effects on hepatocytes of secondary metabolites obtained from lichens. *Alternatives to Laboratory Animals* 32: 605–615.

Correia Da Silva J (1981) Acçao anestésica local de produtos extraidos de liquens. *Revista Portuguesa Farmacia* 31: 69–72.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA and Bergman B (2005) Diverse taxa of cyanobacteria produce β-*N*-methylamino-L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences, USA* 102: 5074–5078.

Culberson CF (1969) *Chemical and Botanical Guide to Lichen Products*. Durham: University of North Carolina.

Culberson CF (1970) Supplement to 'Chemical and Botanical Guide to Lichen Products'. *The Bryologist* 73: 177–377.

Culberson CF (1972) Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* 72: 113–125.

Culberson CF and Armaleo D (1992) Induction of a complete secondary-product pathway in cultures of a lichen fungus. *Experimental Mycology* 16: 52–63.

Culberson CF and Kristinsson H (1970) A standardized method for the identification of lichen products. *Journal of Chromatography* 46: 85–93.

Culberson CF, Culberson WL and Johnson A (1977) *Second Supplement to 'Chemical and Botanical Guide to Lichen Products'*. St. Louis: American Bryological and Lichenological Society, Missouri Botanical Garden.

De Angelis F, Ceci R, Quaresima R, Reale S and Di Tullio A (2003) Investigation by solid phase microextraction and gas chromatography/mass spectrometry of secondary metabolites in lichens deposited on stone monuments. *Rapid Communication in Mass Spectrometry* 17: 526–531.

Demleitner S, Kraus J and Franz G (1991) Synthese und Antitumoraktivitat von Licheninderivaten. *Pharmazie in unserer Zeit* 20: 120.

DePriest PT (1994) Variation in the *Cladonia chlorophaea* complex II: ribosomal DNA variation in a Southern Appalachian population. *The Bryologist* 97: 117–126.

Durazo FA, Lassman C, Han SHB, Saab S, Lee NP, Kawano M, Saggi B, Gordon S, Farmer DG, Yersiz H, Goldstein RLI, Ghobrial M and Busuttil RW (2004) Fulminant liver failure due to usnic acid for weight loss. *American Journal of Gastroenterology* 99: 950–952.

Edwards HGM, Newton EM, Wynn-Williams DD and Lewis-Smith RI (2003) Non-destructive analysis of pigments and other organic compounds in lichens using Fourier-transform Raman spectroscopy: a study of Antartic epilithic lichens. *Spectrochimica Acta Part A* 59: 2301–2309.

Ejiri H, Sankawa U and Shibata S (1975) Graciliformin and its acetates in *Cladonia graciliformis*. *Phytochemistry* 14: 277–279.

Ernst-Russell MA, Chai CLL, Hurne AM, Waring P, Hockless DCR and Elix JA (1999a) Revision and cytotoxic activity of the scabrosin esters, epidithiopiperazinediones from the lichen *Xanthoparmelia scabrosa*. *Australian Journal of Chemistry* 52: 279–283.

Ernst-Russell MA, Elix JA, Chai CLL, Willis AC, Hamada N and Nash TH (1999b) Hybocarpone, a novel cytotoxic naphthazarin derivative from mycobiont cultures of the lichen *Lecanora hybocarpa*. *Tetrahedron Letters* 40: 6321–6324.

Esimone CO, Adikwu MU, Ebi GC, Anaga A and Njoku C (1999) Preliminary physicochemical evaluations and bioactive properties of column fractions from the lichen. *Bollettino Chimico Farmaceutico* 138: 169–175.

European Pharmacopeia (2005) Iceland moss—Lichen islandicus. *European Pharmacopeia*, 5th edn (Vol. 5.0). Strasbourg: Council of Europe.

Feige GB, Lumbsch HT, Huneck S and Elix JA (1993) The identification of lichen products by a standardized high-performance liquid chromatographic method. *Journal of Chromatography* 646: 417–427.

Fontaniella B, Legaz ME, Pereira EC, Sebastian B and Vicente C (2000) Requirements to produce fumarprotocetraric acid using alginate-immobilized cells of *Cladonia verticillaris*. *Biotechnology Letters* 22: 813–817.

Fox CH and Huneck S (1969) The formation of roccellic acid, eugenitol, eugenetin, and rupicolon by the mycobiont *Lecanora rupicola*. *Phytochemistry* 8: 1301–1304.

Gauslaa Y and McEvoy M (2005) Seasonal variation in solar radiation drive acclimation of the sun-screening compound parietin in the lichen *Xanthoria parietina*. *Basic and Applied Ecology* 6: 75–82.

Ghione M, Parrello D and Grasso L (1988) Usnic acid revisited, its activity on oral flora. *Chemiotherapia* 7: 302–305.

Grube M and Blaha J (2003) On the phylogeny of some polyketide synthase genes in the lichenized genus *Lecanora*. *Mycological Research* 107: 1419–1426.

Hamada N (1988) Depside from isolated lichen mycobiont II. *Lichenologist* 10: 294–295.

Hamada N (1989) The effect of various culture conditions on depside production by an isolated mycobiont. *The Bryologist* 92: 310–313.

Hamada N (1993) Effects of osmotic culture conditions on cultured mycobionts. *The Bryologist* 96: 569–572.

Hamada N and Ueno T (1987) Depside from an isolated lichen mycobiont. *Agricultural and Biological Chemistry* 51: 1705–1706.

Hawksworth DL (2003) Hallucinogenic and toxic lichens. *International Lichenological Newsletter* 36: 33–35.

Hidalgo ME, Fernandez E, Quilhot W and Lissi EA (1993) Photohaemolytic activity of lichen metabolites. *Journal of Photochemistry and Photobiology B* 21: 37–40.

Hidalgo ME, Fernandez E, Quilhot W and Lissi E (1994) Antioxidant activity of depsides and depsidones. *Phytochemistry* 37: 1585–1587.

Higuchi M, Miura Y, Boohene J, Kinoshita Y, Yamamoto Y, Yoshimura I and Yamada Y (1993) Inhibition of tyrosinase activity by cultured lichen tissues and bionts. *Planta Medica* 59: 253–255.

Hirabayashi K, Iwata S, Ito M, Shigeta S, Narui T, Mori T and Shibata S (1989) Inhibitory effect of a lichen polysaccharide sulfate, GE-3-S, on the replication of human immunodeficiency virus (HIV) in vitro. *Chemical and Pharmaceutical Bulletin* 37: 2410–2412.

Hirayama T, Fujikawa F, Kasahara T, Otsuka M, Nishida N and Mizuno D (1980) Anti-tumor activities of some lichen products and their degradation products. *Yakugaku Zasshi* 100: 755–759.

Huelsenbeck JP and Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Huneck S (1999) The significance of lichens and their metabolites. *Naturwissenschaften* 86: 559–570.

Huneck S (2001) Progress *in the Chemistry of Organic Natural Products*, Vol. 81. *New Results on the Chemistry of Lichen Substances*. New York: Springer.

Huneck S and Himmelreich U (1995) Arthogalin, a cyclic depsipeptide from the lichen *Arthothelium galapagoense*. *Zeitschrift für Naturforschung, B: Chemical Sciences* 50: 1101–1103.

Huneck S and Yoshimura I (1996) *Identification of Lichen Substances*. Berlin: Springer.

Huneck S, Porzel A and Schmidt J (1993) Structure and synthesis of arthonin, a lichen metabolite from *Arthonia endlicheri*. *Tetrahedron: Asymmetry* 4: 303–311.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

Huneck S, Lumbsch HT, Porzel A and Schmidt J (2004) Die Verteilung von Flechteninhaltsstoffen in *Lecanora muralis* und *Lecidea inops* und die Abhängigkeit der Usninsäure-Konzentration vom Substrat und von den Jahreszeiten bei *Lecanora muralis*. *Bibliotheca Lichenologica* 88: 211–221.

Huovinen K (1987) A standard HPLC method for the analysis of aromatic lichen in *Progress and Problems in Lichenology in the Eighties*. *Bibliotheca Lichenologica* 25: 457–466.

Huovinen K and Lampero M (1989) Usnic acid as a mitotic inhibitor in the allium test. *Planta Medica* 55: 98.

Huovinen K, Hiltunen R and Von Schahtz, M (1985) A high performance liquid chromatographic method for the analysis of lichen compounds from the genera *Cladina* and *Cladonia*. *Acta Pharmaceutica Fennica* 94: 99–112.

Ihlen PG and Ekman S (2002) Outline of phylogeny and character evolution in *Rhizocarpon* (Rhizocarpaceae, lichenized Ascomycota) based on nuclear ITS and mitochondrial SSU ribosomal DNA sequences. *Biological Journal of the Linnean Society* 77: 535–546.

Ingólfsdóttir K (2002) Molecules of interest: usnic acid. *Phytochemistry* 61: 729–736.

Ingólfsdóttir K, Bloomfield SF and Hylands PJ (1985) In vitro evaluation of the antimicrobial activity of lichen metabolites as potential preservatives. *Antimicrobial Agents and Chemotherapy* 28: 289–292.

Ingólfsdóttir K, Breu W, Huneck S, Gudjonsdottir GA and Wagner H (1994) *In vitro* inhibition of 5-lipoxygenase by protolichesterinic acid from *Cetraria islandica*. *Phytomedicine* 1: 187–191.

Ingólfsdóttir K, Hjálmarsdóttir MÁ, Guðjónsdóttir GA, Brynjolfsdóttir A, Sigurðsson A and Steingrímsson Ó (1997) In vitro susceptibility of *Helicobacter pylori* to protolichesterinic acid from *Cetraria islandica*. *Antimicrobial Agents and Chemotherapy* 41: 215–217.

Ingólfsdóttir K, Lee SK, Bhat KPL, Lee K, Chai HB, Kristinsson H, Song LL, Gills J, Gudmundsdottir JT, Mata-Greenwood E, Jang MS and Pezzuto JM (2000) Evaluation of selected lichens from Iceland for cancer chemopreventive and cytotoxic activity. *Pharmaceutical Biology* 38: 313–317.

Ingólfsdóttir K, Gudmundsdóttir GF, Ogmundsdóttir HM, Paulus K, Haraldsdóttir K, Kristinsson H and Bauer R (2002) Effect of tenuiorin and metyl orsellinate from the lichen *Peltigera leucophebia* on 5-/15-lipoxygenases and proliferation of malignant cell lines *in vitro*. *Phytomedicine* 9: 654–658.

Jayaprakasha GK and Rao LJ (2000) Phenolic constituents from the lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activities. *Zeitschrift für Naturforschung* 55c: 1018–1022.

Keller NP and Hohn TM (1997) Metabolic pathway gene clusters in filamentous fungi. *Fungal Genetics and Biology* 21: 17–21.

Kinoshita Y, Yamamoto Y, Yoshimura I, Kurokawa T and Yamada Y (1993) Production of usnic acid in cultured *Usnea hirta*. *Bibliotheca Lichenologica* 53: 137–146.

Kinoshita K, Saito D, Koyama K, Takahashi K, Sato Y, Okuyama E, Fujimoto H and Yamazaki M (2002) Monoamino oxidase inhibitory effects of some lichen compounds and their synthetic analogues. *Journal of the Hattori Botanical Laboratory* 92: 277–284.

Kon Y, Iwashina T, Kashiwadani H, Wardlaw JD and Elix J (1997) A new dibenzofuran, isostrepsilic acid, produced by cultured mycobiont of the lichenized ascomycete *Usnea orientalis*. *Journal of Japanese Botany* 72: 67–71.

Kristmundsdóttir T, Aradóttir HA, Ingólfsdóttir K and Ogmundsdóttir HM (2002) Solubilization of the lichen metabolite (+)-usnic acid for testing in tissue culture. *Journal of Pharmacy and Pharmacology* 54: 1447–1452.

Kroken S, Glass NL, Taylor JW, Yoder OC and Turgeon BG (2003) Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proceedings of the National Academy of Sciences, USA* 100: 15670–15675.

Kumar K and Upreti DK (2001) *Parmelia* spp. (lichen)—in ancient medicinal plant lore of India. *Economic Botany* 55: 458–459.

Kumar S and Muller K (1999) Lichen metabolites. I. Inhibitory action against leukotriene B4 biosynthesis by a non-redox mechanism. *Journal of Natural Products* 62: 817–820.

Kupchan SM and Kopperman HL (1975) L-Usnic acid: tumor inhibitor isolated from lichens. *Experientia* 31: 625.

Lauterwein M, Oethinger M, Belsner K, Peters T and Marre R (1995) *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid and (−)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrobial Agents and Chemotherapy* 39: 2541–2543.

Lawrey JD (1986) Biological role of lichen substances. *The Bryologist* 89: 11–122.

Lawrey JD (1995) The chemical ecology of lichen mycoparasites. *Canadian Journal of Botany* 73(Suppl. 1): 603–608.

Lee T, Yun S-H, Hodge KT, Humber RA, Krasnoff SB, Turgeon GB, Yoder OC and Gibson DM (2001) Polyketide synthase genes in insect- and nematode-associated fungi. *Applied Microbiology and Biotechnology* 56: 181–187.

Legaz ME and Vicente C (1983) Endogenous inactivators of arginase, l-arginine decarboxylase and agmatine amidinohydrolase in *Evernia prunastri* thallus. *Plant Physiology* 71: 300–302.

Legaz ME, Vicente C and Pedrosa MM (2001) Binding of lichen phenolics to purified secreted arginase from the lichen *Evernia prunastri*. *Journal of Biochemistry and Molecular Biology* 34: 194–200.

Leuckert C and Poelt J (1978) Über Nord-Süd-Gradienten von Chemotypen europäischer Flechten. *Plant Systematics and Evolution* 130: 53–77.

Leuckert C, Ahmadjian V, Culberson CF and Johnson A (1990) Xanthones and depsidones of the lichen *Lecanora dispersa* in nature and of its mycobiont in culture. *Mycologia* 82: 370–378.

Lisickov K, Najdenova V and Zoltan D (2002) Application of supercritical CO_2 extraction for separation of natural antibiotics from lichens. *Herba Polonica* 48: 32–39.

Lutzoni F, Pagel M and Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937–940.

Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hall B, Hansen K, Harris RC, Hosaka K, Lim Y-W, Liu Y, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R and Vilgalys R (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446–1480.

MacGillivray T and Helleur R (2001) Analysis of lichens under environmental stress using TMAH thermochemolysis-gas chromatography. *Journal of Analytical and Applied Pyrolysis* 58/59: 465–480.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

Matsubara H, Kinoshita K, Koyama K, Ye Y, Takahashi K, Yoshimura I, Yamamoto Y, Miura Y and Kinoshita Y (1997) Anti-tyrosinase activity of lichen metabolites and their synthetic analogues. *Journal of the Hattori Botanical Laboratory* 83: 179–185.

Matsubara H, Miharu K, Kinoshita K, Koyama K, Ye Y, Takahashi K, Yoshimura I, Yamamoto Y, Miura Y and Kinoshita Y (1998) Inibitory effect of lichen metabolites and their synthetic analogues on melanin biosynthesis in cultured B-16 mouse melanoma cells. *Natural Product Sciences* 4: 161–169.

Miao V, Coeffet-LeGal M-F, Brown D, Sinneman S, Donaldson G and Davies J (2001) Genetic approaches to harvesting lichen products. *Trends in Biotechnology* 19: 349–355.

Miyagawa H, Hamada N and Ueno T (1993) Hypostrepsilic acid, a new dibenzofuran from the cultured lichen mycobiont of *Evernia esorediosa*. *Phytochemistry* 34: 589–591.

Miyagawa H, Hamada N, Sato M and Ueno T (1994) Pigments from the cultured lichen mycobionts of *Graphis scripta* and *G. desquamescens*. *Phytochemistry* 36: 1319–1322.

Molina MC and Vicente C (2000) Purification and characterization of two isolectins with arginase activity from the lichen *Xanthoria parietina*. *Journal of Biochemistry and Molecular Biology* 33: 300–307.

Mosbach K (1967) On the biosynthesis of lichen substances. Part 4. The formation of pulvinic acid derivatives by isolated lichen fungi. *Acta Chemica Scandinavica* 21: 2331–2334.

Müller K (2001) Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology* 56: 9–16.

Nascimento SC, Pereira EC, Oliveira AFM, Da Silva NH, Boitard M and Beriel H (1994) Screening de atividade citotoxica de extratos liquenicos: Cladoniaceae. *Acta Botanicae Brasiliensis* 8: 97–107.

Neamati N, Hong H, Mazumder A, Wang S, Sunder S, Nicklaus MC, Milne GW, Proksa B and Pommier Y (1997) Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. *Journal of Medical Chemistry* 40: 942–951.

Neff GW, Reddy KR, Durazo FA, Meyer D, Marrero R and Kaplowitz N (2004) Severe hepatotoxicity associated with the use of weight loss diet supplements containing ma huang or usnic acid. *Journal of Hepatology* 41: 1062–1064.

Nicholson TD, Rudd BAM, Dawson M, Lazarus CM, Simpson TJ and Cox RJ (2001) Design and utility of oligonucleotide gene probes for fungal polyketide synthases. *Chemistry and Biology* 8: 157–178.

Nicolaou KC and Gray DLF (2004) Total synthesis of hybocarpone and analogues thereof. A facile dimerization of naphtazarins to pentacyclic systems. *Journal of the American Chemical Society* 126: 607–612.

Nishikawa Y and Ohno H (1981) Studies on the water-soluble constituents of lichens. IV. Effect of the antitumor lichenglucans and related derivatives on the phagocytic activity of the reticuloendothelial system in mice. *Chemical and Pharmaceutical Bulletin* 29: 3407–3410.

Nishikawa Y, Takeda T, Shibata S and Fukuoka F (1969) Polysaccharides in lichens and fungi. III. Further investigation on the structures and the antitumor activity of the polysaccharides from *Gyrophora esculenta* Miyoshi and *Lasallia papulosa* Llano. *Chemical and Pharmaceutical Bulletin* 17: 1910–1916.

Nishikawa Y, Yoshimoto K, Horiuchi R, Michishita K, Okabe M and Fukuoka F (1979) Studies on the water-soluble constituents of lichens. III. Changes in antitumor effect caused by modifications of pustulan- and lichenan-type glucans. *Chemical and Pharmaceutical Bulletin* 27: 2065–2072.

Nybakken L, Aubert S and Bilger W (2004) Epidermal UV-screening of arctic and alpine plants along a latitudinal gradient in Europe. *Polar Biology* 27: 391–398.

Ögmundsdóttir H, Zoëga GM, Gissurarson SR and Ingólfsdóttir K (1998) Anti-proliferative effects of lichen-derived inhibitors of 5-lipoxygenase on malignant cell lines and mitogen-stimulated lymphocytes. *Journal of Pharmacy and Pharmacology* 50: 107–115.

Oksanen I, Jokela J, Fewer DP, Wahlsten M, Rikkinen J and Sivonen K (2004) Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I. *Applied and Environmental Microbiology* 70: 5756–5763.

Okuyama E, Hossain CF and Yamazaki M (1991) Monoamino oxidase inhibitor from a lichen, *Solorina crocea* (L.) Ach. *Shouyakugakuzasshi* 45: 159–162.

Okuyama E, Umeyama K, Yamazaki M, Kinoshita Y and Yamamoto Y (1995) Usnic acid and diffractaic acid as analgesic and antipyretic components of *Usnea diffracta*. *Planta Medica* 61: 113–115.

Ólafsdóttir ES and Ingólfsdóttir K (2001) Polysaccharides from lichens: structural characteristics and biological activity. A review. *Planta Medica* 67: 199–208.

Ouzzine H, Bazin MA, Le Devehat-Lohezic F, Tomasi S, Corbel JC, Boustie J and Uriac Ph (2001) Synthèse de conjugués(+) acide usnique-polyamines. Xèmes Conf. européennes du Groupement des Pharmacochimistes de l'ARC Atlantique GP2A, Nantes, 29–30 October.

Pengsuparp T, Cai L, Constant H, Fong HHS, Lin LZ, Kinghorn AD, Pezzuto JM, Cordell GA, Ingólfsdóttir K, Wagner H and Hughes SH (1995) Mechanistic evaluation of new plant-derived compounds that inhibits HIV-1 reverse transcriptase. *Journal of Natural Products* 58: 1024–1031.

Planelles V and Legaz ME (1987) Purification and some properties of the secreted arginase of the lichen *Evernia prunastri* and its regulation by usnic acid. *Plant Science* 51: 9–16.

Poelt J and Leuckert C (1993) Substitution and supplementary addition of secondary products in the evolution of lichenized Ascomycotina. *Bibliotheca Lichenologica* 53: 201–215.

Rancan F, Rosan S, Boehm K, Fernández E, Hidalgo ME, Quilhot W, Rubio C, Boehm F, Piazena H and Oltmanns U (2002) Protection against UVB irradiation by natural filters extracted from lichens. *Journal of Photochemistry and Photobiology B, Biology* 68: 133–139.

Rezanka T and Dembitsky V (1999) Novel brominated lipidic compounds from lichens of central Asia. *Phytochemistry* 51: 963–968.

Rezanka T and Gushina IA (1999) Brominated depsidones from *Acarospora gobiensis*, a lichen of Central Asia. *Journal of Natural Products* 62: 1675–1677.

Rezanka T and Gushina IA (2000) Glycosidic compounds of murolic, protocontipatic and allo-murolic acids from lichens of Central Asia. *Phytochemistry* 54: 635–645.

Rezanka T and Gushina IA (2001a) Glycosides esters from lichens of Central Asia. *Phytochemistry* 58: 509–516.

Rezanka T and Gushina IA (2001b) Further glucosides of lichens' acids from Central Asian lichens. *Phytochemistry* 56: 181–188.

Rezanka T and Gushina IA (2001c) Macrolactones glycosides of three lichen acids from *Acarospora gobiensis*, a lichen of Central Asia. *Phytochemistry* 58: 1281–1287.

Rezanka T, Jachymova J and Dembitsky VM (2003) Prenylated xanthone glucosides from Ural's lichen *Umbilicaria proboscidea*. *Phytochemistry* 62: 607–612.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

Rezanka T, Temina M, Hanus L and Dembitsky VM (2004) The tornabeatins, four tetrahydro-2-furanone derivatives from the lichenized ascomycete *Tornabea scutellifera* (With.) J.R. Laundon. *Phytochemistry* 65: 2605–2612.

Richardson DHS (1988) Medicinal and other aspects of lichens. In: Galun M (ed.) *CRC Handbook of Lichenology*, Vol. III. Boca Raton, FL: CRC Press, pp. 93–108.

Rikkinen J (1995) What's behind the pretty colours? A study on the photobiology of lichens. *Bryobrothera* 4: 1–239.

Saklani A and Upreti DK (1992) Folk uses of some lichens in Sikkim. *Journal of Ethnopharmacology* 27: 229–233.

Sankawa U, Shibuya M, Ebizuka Y, Noguchi H, Kinoshita T, Iitaka Y, Endo A and Kitahara N (1982) Depside as potent inhibitor of prostaglandin biosynthesis: a new active site model for fatty acid cyclooxygenase. *Prostaglandins* 24: 21–34.

Sauer M, Lu P, Sangari R, Kennedy S, Polishook J, Bills G and An Z (2002) Estimating polyketide metabolic potential among non-sporulating fungal endophytes of *Vaccinium macrocarpum*. *Mycological Research* 106: 460–470.

Schindler H (1988) Zur Geschichte der Anwendung von Flechten (Lichenes) in der Medizin. *Carolinea* 46: 31.

Schmitt I, Martin M, Kautz S and Lumbsch Th (2005) Diversity and evolution of secondary metabolite encoding genes in the Pertusariaceae. *Phytochemistry* (in press).

Shahi SK, Shukla AC, Uperti DK and Dikshit A (2000) Use of lichens as antifungal drugs against superficial fungal infections. *Journal of Medicinal and Aromatic Plant Sciences* 22(4A)/23(1A): 169–172.

Shahi SK, Shukla AC, Dikshit A and Upreti DK (2001) Broad spectrum antifungal properties of the lichen. *Heterodermia leuocomela*. *Lichenologist* 33: 177–179.

Shahi SK, Patra M, Dikshit A and Upreti DK (2003) *Parmelia cirrhatum*: a potential source of broad spectrum natural antifungal. *Phytotherapy Research* 17: 399–400.

Shibamoto T and Wei CI (1984) Mutagenicity of lichen constituents. *Environmental Mutagenesis* 6: 757–762.

Shibata S (1958) Especial compounds of lichens. *Handbuch der Pflanzenphysiologie* 10: 560–623.

Shibata S (2000) Great discoveries in bryology and lichenology—Yasuhiko Asahina (1880–1975) and his studies on lichenology and chemistry of lichen metabolites. *Bryologist* 103: 710–719.

Singh KK, Upreti DK and Kaushal K (2000) Ethnobotanical note on some less known Himalaya lichens. *Bulletin of the British Lichen Society* 86: 36–37.

Sinnemann SJ, Andrésson OS, Brown DW and Miao VPW (2000) Cloning and heterologous expression of *Solorina crocea* pyrG. *Current Genetics* 37: 333–338.

Solhaug KA and Gauslaa Y (2004) Photosynthates stimulate the UV-B induced fungal anthraquinone synthesis in the foliose lichen *Xanthoria parietina*. *Plant, Cell & Environment* 27: 167–176.

Solhaug KA, Gauslaa Y, Nybakken L and Bilger W (2003) UV-induction of sun-screening pigments in lichens. *New Phytologist* 158: 91–100.

Solheim B, Johanson U, Callaghan TV, Lee JA, Gwynn-Jones D and Björn LO (2002) The nitrogen fixation potential of arctic cryptogram (*sic*) species is influenced by enhanced UV-B radiation. *Oecologia* 133: 90–93.

Stepanenko LS, Krivoshchekova OE and Skirina IF (2002) Functions of phenolic secondary metabolites in lichens from far east Russia. *Symbiosis* 32: 119–131.

Stocker-Wörgötter E (1998) Culture methods and culture of selected mycobionts and photobionts as exemplified by South American lichens. In: Marcelli MP and Seaward MRD (eds) *Lichenology in Latin Ameria: History, Current Knowledge and Applications*. São Paulo: CETESB, pp. 143–155.

Stocker-Wörgötter E (2001) Experimental lichenology and microbiology of lichens: culture experiments, secondary chemistry of cultured mycobionts, resynthesis and thallus morphogenesis. *The Bryologist* 104: 576–581.

Stocker Wörgötter E (2002a) Laboratory cultures of selected lichen fungi from Brazil and Chile. *Mitteilungen des Instituts für Allgemeine Botanik, Hamburg* 30: 253–270.

Stocker-Wörgötter E (2002b) Analysis of secondary compounds in cultured mycobionts. In: Kranner I, Beckett RP and Varma AK (eds) *Protocols in Lichenology, Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring*. *Springer Lab Manual*. Berlin: Springer, pp. 296–306.

Stocker-Wörgötter E and Elix JA (2004) Experimental studies of lichenized fungi: formation of rare depsides and dibenzofuranes by the culture mycobiont of *Bunodophoron patagonicum* (Sphaerophoraceae, lichenized Ascomycota). *Bibliotheca Lichenologica* 88: 659–669.

Stocker-Wörgötter E, Elix JA and Grube M (2004) Secondary chemistry of lichen-forming fungi: chemosyndromic variation and DNA-analyses of cultures and chemotypes in the *Ramalina farinacea* complex. *The Bryologist* 107: 152–162.

Stoll A, Brack A and Renz J (1950) Die Wirkung von Flechtenstoffen auf Tuberkelbakterien und auf einige andere Mikroorganismen. *Schweizer Zeitschrift für Allgemeine Pathologie und Bakteriologie* 13: 729–751.

Takai M, Uehara Y and Beisler JA (1979) Usnic acid derivatives as potential antineoplastic agents. *Journal of Medicinal Chemistry* 22: 1380–1384.

Tanahashi K, Kuroishi M, Kuwahara A, Nagakura N and Hamada N (1997) Four phenolics from the cultured lichen mycobiont of *Graphis scripta* var *pulverulenta*. *Chemical and Pharmaceutical Bulletin* 45: 1183–1185.

Torres A, Hochberg M, Pergament I, Smoum R, Niddam V, Dembitsky VM, Temina M, Dor I, Lev O, Srebnik O and Enk CD (2004) A new UV-B absorbing mycosporine with photoprotective activity from the lichenized ascomycete *Collema cristatum*. *European Journal of Biochemistry* 271: 780–784.

Varga J, Rigo K, Kocsube S, Farkas B and Pal K (2003) Diversity of polyketide synthase gene sequences in *Aspergillus* species. *Research in Microbiology* 154: 593–600.

Vartia KO (1973) Antibiotics in lichens. In: Ahmadjian V and Hale ME (eds) *The Lichens*. New York: Academic Press, pp. 547–561.

Watanabe A and Ebizuka Y (2002) A novel hexaketide naphthalene synthesized by a chimeric polyketide synthase composed of fungal pentaketide and heptaketide synthases. *Tetrahedron Letters* 43: 843–846.

Wood S, Huffman J, Weber N, Andersen D, North J, Murray B, Sidwell R and Hughes B (1990) Antiviral activity of naturally occurring anthraquinones and anthraquinone derivatives. *Planta Medica* 56: 651–652.

Yamamoto Y (2000) Screening of biological activities and isolation of biological-active compounds from lichens. *Shokubutsu no Kagaku Chosetu* 35: 169–179.

Yamamoto Y, Mizuguchi R and Yamada Y (1985) Tissue culture of *Usnea rubescens* and *Ramalina yasudae* and production of usnic acid in their cultures. *Agricultural and Biological Chemistry* 49: 3347–3348.

Yamamoto Y, Miura Y, Kinoshita Y, Higuchi M, Yamada Y, Murakami A, Ohigashi H and Koshimizu K (1995) Screening of tissue cultures and thalli of lichens and some of

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press

their active constituents for inhibition of tumor promoter-induced Epstein-Barr virus activation. *Chemical and Pharmaceutical Bulletin* 43: 1388–1390.

Yamamoto Y, Matsubara H, Kinoshita Y, Kinoshita K, Koyama K, Takahashi K, Ahmadjian V, Kurokawa T and Yoshimura I (1996) Naphthazarin derivatives from cultures of the lichen *Cladonia cristatella*. *Phytochemistry* 43: 1239–1242.

Yang X, Shimizu Y, Steiner JR and Clardy J (1993) Nostoclide I and II, extracellular metabolites from a symbiotic cyanobacterium, *Nostoc* sp., from the lichen *Peltigera canina*. *Tetrahedron Letters* 34: 761–764.

Yoshimura I, Kinoshita Y, Yamamoto Y, Huneck S and Yamada Y (1994) Analysis of secondary metabolites from lichen by high performance liquid chromatography with a photodiode array detector. *Phytochemical Analysis* 5: 197–205.

Zopf W (1895) Zur Kenntnis der Flechtenstoffe. *Annalen der Chemie* 284: 107–132.

https://doi.org/10.1079/PGR200572 Published online by Cambridge University Press