Lichen Metabolites: An Overview of Some Secondary Metabolites and Their Biological Potential

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Abstract

Lichens present a symbiotic association between two or more organisms. These unique organisms produce many chemical compounds, known as secondary metabolites or lichen acids. Most of them are localized in the cortex and form specific crystals on the surface of the fungal hyphae. Approximately 1000 secondary metabolites were discovered so far and most of them are specific for lichens. Lichen secondary metabolites showed many pharmaceutical activities, including antimicrobial, antiproliferative, antioxidant, antiviral, anti-inflammatory, and further allelopathic, antiherbivore, photoprotective activities. Lichens are important source of bioactive compounds, and despite a lot of studies dealing with activity of lichen secondary metabolites, their production in lichens and their role is still very enigmatic. In this chapter, we demonstrated all three main pathways of how secondary compounds originate and chose most characteristic acids with their proposed biological and ecological activities. This chapter gives a basic overview of lichens, secondary metabolites, and their properties.

Keywords

Symbiosis · Lichens · Biosynthetic pathways · Secondary metabolites · Pharmaceutical activities

1 Introduction

Colonization of land by phototrophic organisms started in Silurian era around 450 million years ago [1]. The environment was not friendly for these organisms because they needed to counter a low content of mineral nutrition, harmful UV-radiation from the sun, high oscillation of temperature, as well as a lower content of water or even its absence. All of these abiotic factors played important role in adaptations to the terrestrial environment. Living forms, which would like to stand in these adaptations, needed phosphorus to create nucleic acids and ATP (adenosine triphosphate). One example of how to solve problem with phosphorus uptake was that first colonizing organisms formed associations with mycorrhizal fungi [2, 3]. It needs to be mentioned that early organisms were forced to establish a form of mutualism which means the interaction between at least two different species of the individuals. Mutualism provided various adaptations for terrestrial plants and played a crucial part for settling on soil as well as in the evolution of land phototrophs [4].

Lichens (lichen-forming fungi) represent nearly one-fifth of all known fungal species so far [5]. They are typical examples of mutualistic symbiosis, where both partners need each other to benefit. The total number of lichens is still not known, but around 18,500 species were already described around the world [6]. Lichens are the dominant vegetation of approximately 8% of terrestrial ecosystems [7] and are typically found in environments subjected to extremes such as temperature, desiccation, and nutrient status.

This symbiotic partnership consists of fungal partner (called also mycobiont) and one or more photoautotrophic partners (called photobiont or phycobiont) [8]. For almost 150 years, lichens had been the model organisms of symbiosis on the lands until the researchers uncovered an unexpected third partner in the lichen cortex – yeast [9].

Lichens as fossils are scarce. Fossil evidence for the interactions of fungi with other organisms, including phototrophs, has been found originating from an era approximately 400 million years ago, in the area of Rhynie chert in Scotland. However, the discovery of lichen-like fossils preserved in marine phosphorite of the Doushantuo Formation (approximately 600 million years old) at Weng'an in southern China indicates that lichenization could have arisen even before the evolution of vascular plants [10]. In addition, recent molecular data suggest that lichen symbioses arose repeatedly during the evolution of fungi [11] and had a very important role in the evolution of Ascomycota [12].

2 Lichen Symbiotic Partners

Based on the most definitions, the lichen is the organism that represents the symbiotic association between the fungus (mycobiont) and the photosynthetic partner (photobiont). Photobiont coexistence with the lichen mycobiont brings many benefits that none of the organisms itself cannot achieve [13]. Although the dual nature of most lichens is now widely established, it is less commonly known that some lichens are symbioses involving three or more partners [8]. It has been suggested that they are mainly bacteria involved in the formation of complete lichen thalli [14].

2.1 Mycobiont

Most of the fungal partners belong to Ascomycota [15, 16], but we can also find species belonging to the Basidiomycota and anamorphic fungi. Mycobiont (Fig. 1a) is the dominant component of the lichen thallus. Separated "biont" cells in most cases are in direct contact, where fungal hyphae try to penetrate to cells of photobiont. There are known some cases where mycobiont is in contact not only with one type of photobiont but with two or even more. This leads to the creation of specific structure, cephalodium (e.g., *Peltigera aphthosa*). Because mycobiont is unable to produce the organic substances necessary for its growth, hence it must acquire them from a symbiosis. Heterotrophic mycobiont acquires fixed carbon in symbiosis from an autotrophic green algae or cyanobacteria. These are photosynthesis products (ribitol, sorbitol, glucose). In the lichen symbiosis, mycobiont ensures the intake of water and minerals for lichen thallus. It creates morphology and structures that are involved in both sexual and nonsexual reproduction. One of the most important roles

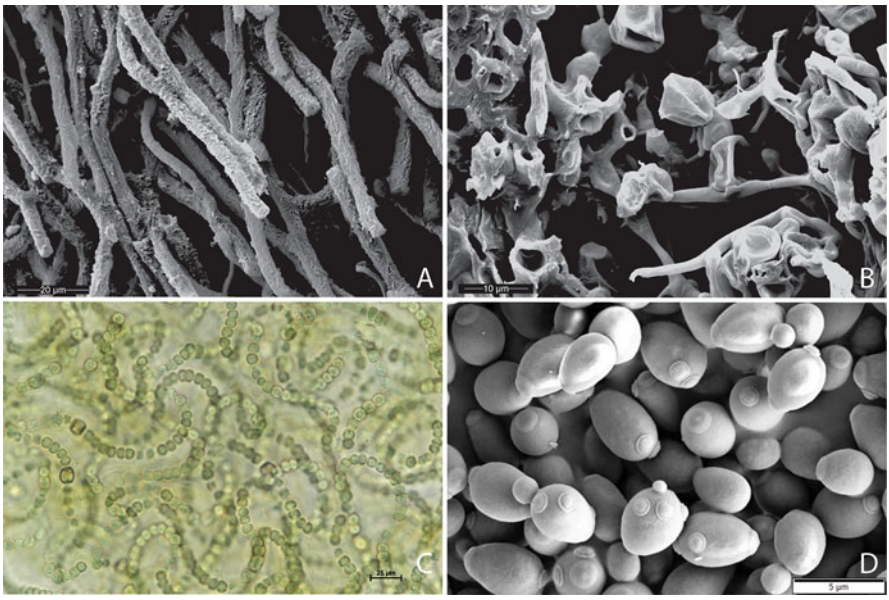


Fig. 1 (**a**) Mycobionts cells consisting of hyphae, (**b**) photobiont cells consisting of green algae, (**c**) photobiont cells consisting of cyanobacteria, (**d**) third symbiotic partner cells consisting of yeasts

of mycobiont is protection of photobiont from exposure to intense sunlight and desiccation by production of secondary metabolites. There is a predominant view that mycobiont has a higher tolerance to various environmental factors [17].

2.2 Photobiont

The role of photobionts in the lichen thallus can play nearly 40 genera of algae and cyanobacteria [18, 19]. The vast majority are eukaryotic photobionts, which belong to green algae (*Chlorophyta*) (Fig. 1b). They have a large number of common cytological features and their pigmentation, such as the presence of chlorophylls *a* and *b*, whose presence is common with higher plants [20, 21]. In only a small percentage of lichens, the photobionts are represented by prokaryotic cyanobacteria (Fig. 1c), sometimes called "cyanobionts." There are also known examples in which both groups of obligatory photobionts were observed simultaneously in the lichen thalli. The most frequent photobionts are represented by the genera *Trebouxia*, *Trentepohlia*, and *Nostoc*. Unlike green algae, cyanobacteria are diazotrophic because they can fix atmospheric nitrogen. The diversity of these photosynthetic partners is related to the variety of substrates that individual species are able to colonize within the genus. Main role of autotrophic photobiont is to synthesize organic compounds from carbon dioxide. Transfer of metabolites from photobiont to the mycobiont depends on the type of autotrophic photobiont involved [8].

2.3 Third Symbiont "Yeast"

In the study of Spribille et al. [9] is stated that many common lichens consist of a known ascomycete, the autotrophic photosynthesizing partner, and unexpectedly specific basidiomycete yeast (Fig. 1d). These yeasts are anchored in the cortex of lichen thallus and their abundance correlates with previously unexplained variations of the phenotype.

3 Anatomy and Morphology

Lichen morphology and anatomy is highly adapted to environmental restrictions; the mycobiont forms the exhabitant and the photobiont is the inhabitant [22]. The lichen "body" is called thallus. In the cross section (Fig. 2c), the lichen thallus usually consists of the upper cortex, a photosynthetic layer, the medulla, and the lower cortex. Some species also developed a central cord which has a support function (Fig. 2a, b). The thickness of the layers can vary in different species which is a response to the different environmental conditions.

Symbiosis is a source of dynamic evolution which is reflected by the different growth forms of thalli [23]. Many different thalli structures are known [24, 25], but they can be divided into three morphological types: (Fig. 3a) fruticose, (Fig. 3b) crustose, and (Fig. 3c) foliose. Other types can be included into these main three types. For example, *Cladonia macilenta* is lichen with squamulose bases and fruticose fruiting structures which are called podetia (Fig. 3d). *Lepraria* species have leprose, crustose lichen thalli with a powdery or granular surface. Genus *Collema*, *Leptogium*, or *Lathagrium* are characteristic of their gelatinous foliose thallus (Fig. 3e).

Crustose (Fig. 3a) lichens are tightly attached to the substrate by whole thalli, and it is very hard to remove them without any damage. These lichens usually grow on rocks or barks and colonize extreme habitats, including metal rich substrates. Unfortunately, the physiological studies of these lichens are very poor due to the complicated removal from substrate and low biomass production for routine analysis [26]. Extreme examples are endolithic species which penetrate a rock surface and only fruiting bodies are exposed (Fig. 3f).

Foliose lichens (Fig. 3b) are known as leafy-like. They are partially attached to the substrate or in one single point. The thallus is usually divided into lobes (*Parmelia sp.*) with various degrees of branching, but in some species (*Umbilicaria sp.*) the thallus is from one single unbranched lobe or a "multilobe" with limited branching [8]. According to their biomass and easy collection, they are used in biochemical and ecophysiological studies [26].

Fruticose lichens (Fig. 3c, d) are known as hair-like or strap-shaped. The lobes are usually flat or cylindrical. The thallus can grow horizontally or vertically (*Cladonia sp.*) or even hanging (*Usnea sp.*). The branching of lobes may be different within the systematic groups or even a single genus. Fruticose lichens are growing usually on tree barks but also on the ground. As with the foliose types, lichenologists prefer for experiments fruticose growth forms of thalli due to the easy removal from surface.

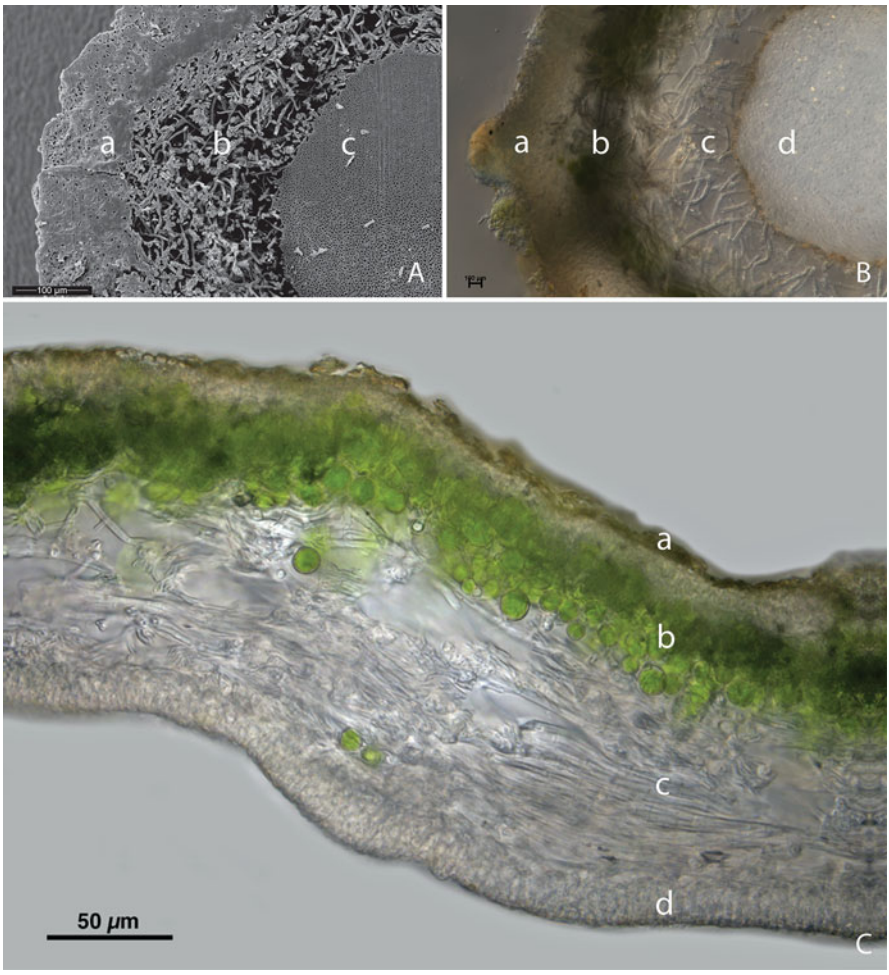


Fig. 2 (**a**) SEM photo of lichen *Usnea* sp. (a) upper cortex, (b) medulla, (c) central cord, (**b**) light microscopy (LM) photo of lichen *Usnea* sp. (a) upper cortex, (b) photosynthetic layer consisting of green algae cortical metabolites, (c) medulla, (d) central cord, (**c**) cross section of lichen thallus (*Xanthoria parietina*) in LM consisting of (a) upper cortex, (b) photosynthetic layer, (c) medulla, (d) lower cortex

4 Lichen Secondary Metabolites

Lichens present pioneer organisms, which can live in extreme habitats. These symbiotic organisms can deal with very specific conditions of environment because they produce secondary metabolites, which provide them with a good protection against various negative physical and biological influences [27]. As Lawrey [28] described, lichens produce two main groups of metabolites: primary (intracellular)

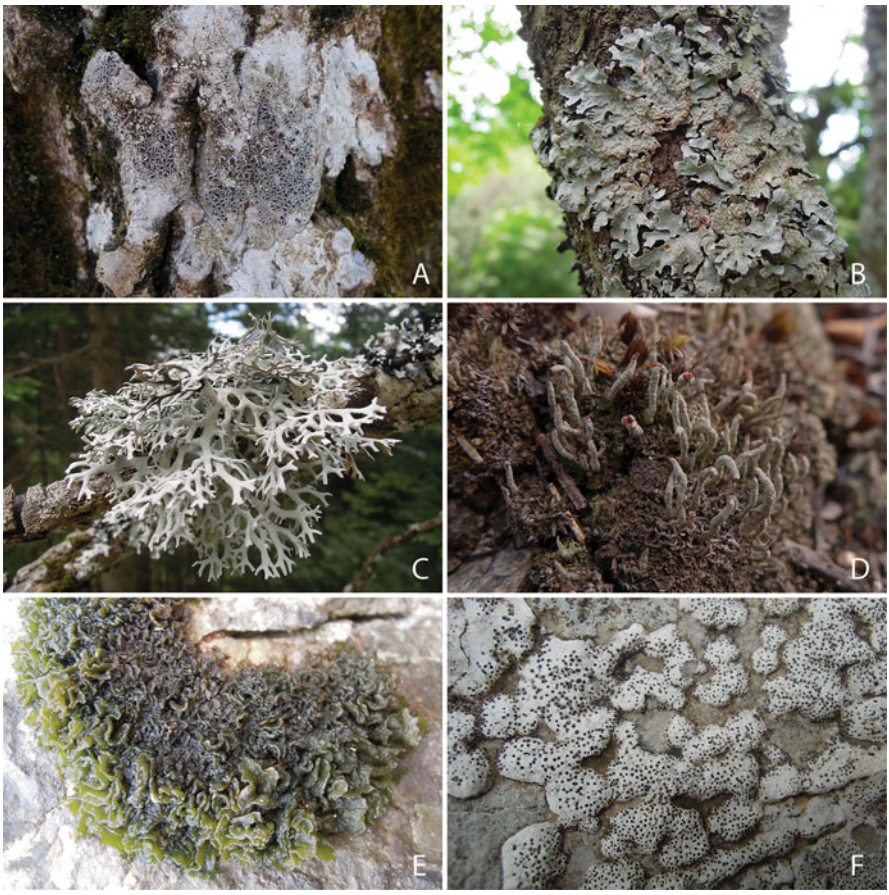


Fig. 3 Morphology of lichen thallus: (**a**) crustose thallus (*Lecanora argentata*), (**b**) foliose thallus (*Parmelia sulcata*), (**c**) fruticose thallus (*Pseudevernia furfuracea*), (**d**) bipartite thallus (*Cladonia macilenta*), (**e**) gelatinous thallus (*Lathagrium* sp.), (**f**) endolithic thallus (*Bagliettoa* sp.)

and secondary (extracellular). More than 1000 lichen substances are already known. The isolation, identification, and structures are described in the handbook of Huneck and Yoshimura [29].

Into the products of primary metabolism, we can include amino acids, amines, peptides, proteins, polyols, saccharides (mono-, oligo-, poly-) carotenoids, and vitamins, which are bound in the cell walls and the protoplasts. Most of them are soluble in water and can be extracted with boiling water [30]. Some of the primary metabolites are produced by fungal and some of them by photosynthetic partner. Many of these primary metabolites are not specific only for lichens and can be easily found in free-living fungi, algae, as well as higher plants [31]. Lichens dispose a similar amount of free amino acids as do the other plants. Lichen thallus present from 1.6% to 11.4% dry weight of nitrogen compounds [31], 1.5 to 24 mg/g dry weight of carotenoids, and 3–5% dry weight of polysaccharides [32].

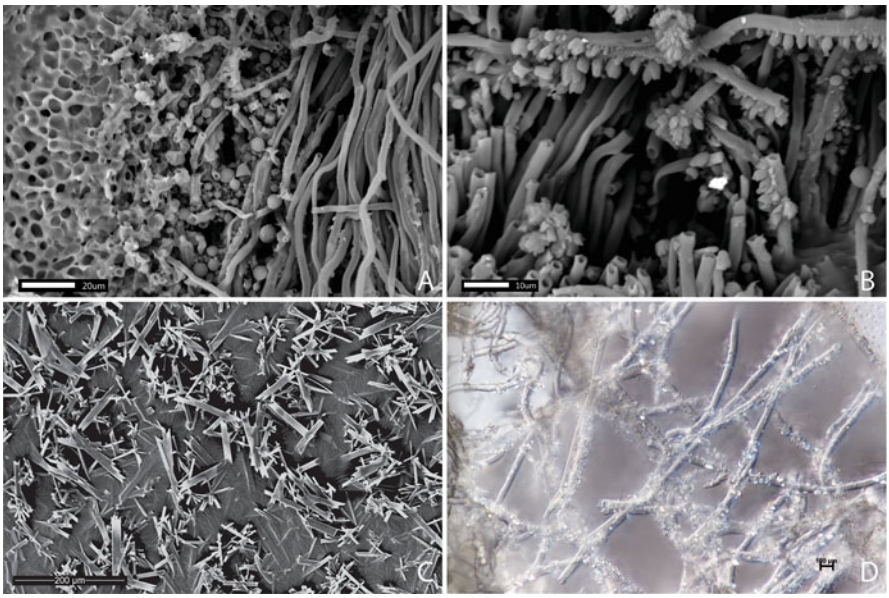


Fig. 4 (**a**) Various shape of lichen crystals, (**b**) secondary metabolites as crystals on hyphae, (**c**) crystals of usnic acid after recrystallization (SEM), (**d**) lichen crystals attached on mycobiont hyphae (LM)

The major group of these organic compounds which are found in lichens are products of secondary metabolism. The amount of secondary metabolites varies usually between 0.1% to 10% of dry weight of thallus but sometimes up to 30% [33–35]. All of the secondary metabolites (Fig. 4) of lichens are of fungal origin [6]. These lichen substances can be found in crystal form deposited on the surface of the hyphae of mycobiont. The solubility in water is very poor and mostly organic solvents can be used for their isolation.

Crystals of secondary metabolites are very stable, once they are formed, which was confirmed in several studies. Herbarium specimens of the lichens showed no significant decrease in concentrations of secondary metabolites [36].

Production of secondary metabolites in lichens is influenced by environmental factors including a light, UV-exposure, elevation, temperature fluctuations, and seasonality [6]. The age of lichens also plays significant role in production of lichen compounds and their location in lichen thallus as well.

4.1 Lichen Biosynthetic Pathways

Lichen secondary metabolites are classified by Culberson and Elix [37] according to their biosynthetic origins and chemical structures [38]. Three chemical pathways are

known in lichens: acetyl-malonate pathway, shikimate pathway, and mevalonate pathway.

4.1.1 Acetyl-Malonate Pathway

The formation of the polyketide chain could be envisaged as a series of Claisen reactions between the starting acetyl CoA and various number of malonyl CoA since every step ends by decarboxylation reaction. Orsellinic acid, the main intermediate in the biosynthesis of depsides and depsidones, is formed by intramolecular aldol reaction of the polyketide containing four keto groups and subsequent enolization and hydrolysis. Esterification of two orsellinic acid molecules affords lecanoric acid as member of depsides class. The most known orcinol-type depsidones have an α- or a β-keto group in the side chain of the first ring. It is well known that this functional group has a strong effect upon the ester linkage between the two rings since enol lactones form readily. Oxidative cyclization of depsides to depsidones usually joins the 2-hydroxyl of ring A and the 5-position of ring B.

C-methylation, Claisen reaction, and subsequent aromatization of the same polyketide leads to methylphloracetophenone. Radical coupling of two radicals derived from this intermediate affords bis dienone from which usnic acid is formed.

By Claisen reaction, aromatization and subsequent cyclization reactions of the polyketide containing five keto groups 5,7-dihydroxy-2-methylchromone are formed as key intermediate for synthesis of chromones and xanthones.

Polyketide containing eight keto groups undergoes several aldol reactions followed by reactions such as enolization, oxidation, decarboxylation, and selective methylation to give parietin, member of the anthraquinone group. Classes of lichen substances, which are derived by acetyl-malonate pathway, are depsides, depsidones, dibenzofurans, anthraquinones, chromones, and xanthones (Fig. 5).

Depsides

Polyphenolic compounds consisting of two or more monocyclic aromatic units linked by an ester bond are called depsides. The most common are products of intermolecular esterification of similar or identical units. Second esterification leads to tridepsides.

Evernic Acid

Evernic acid showed strong antioxidant, antimicrobial, and anticancer activities (Fig. 6). Antiherbicidal activity was also reported. Kosanić et al. [39] found varying antioxidant activity of evernic acid in free radical scavenging, superoxide anion radical scavenging. Strong antibacterial activity was reported against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus megaterium*) [28]. Antitumor activity of evernic acid against HeLa cancer cell lines was also reported [40]. Evernic acid acts also as photosystem II inhibitor [41].

Lecanoric Acid

The antitumor, antioxidant, antibacterial, and antifungal activities of lichen compound lecanoric acid were confirmed (Fig. 7). Bogo et al. [42] tested cytotoxicity of

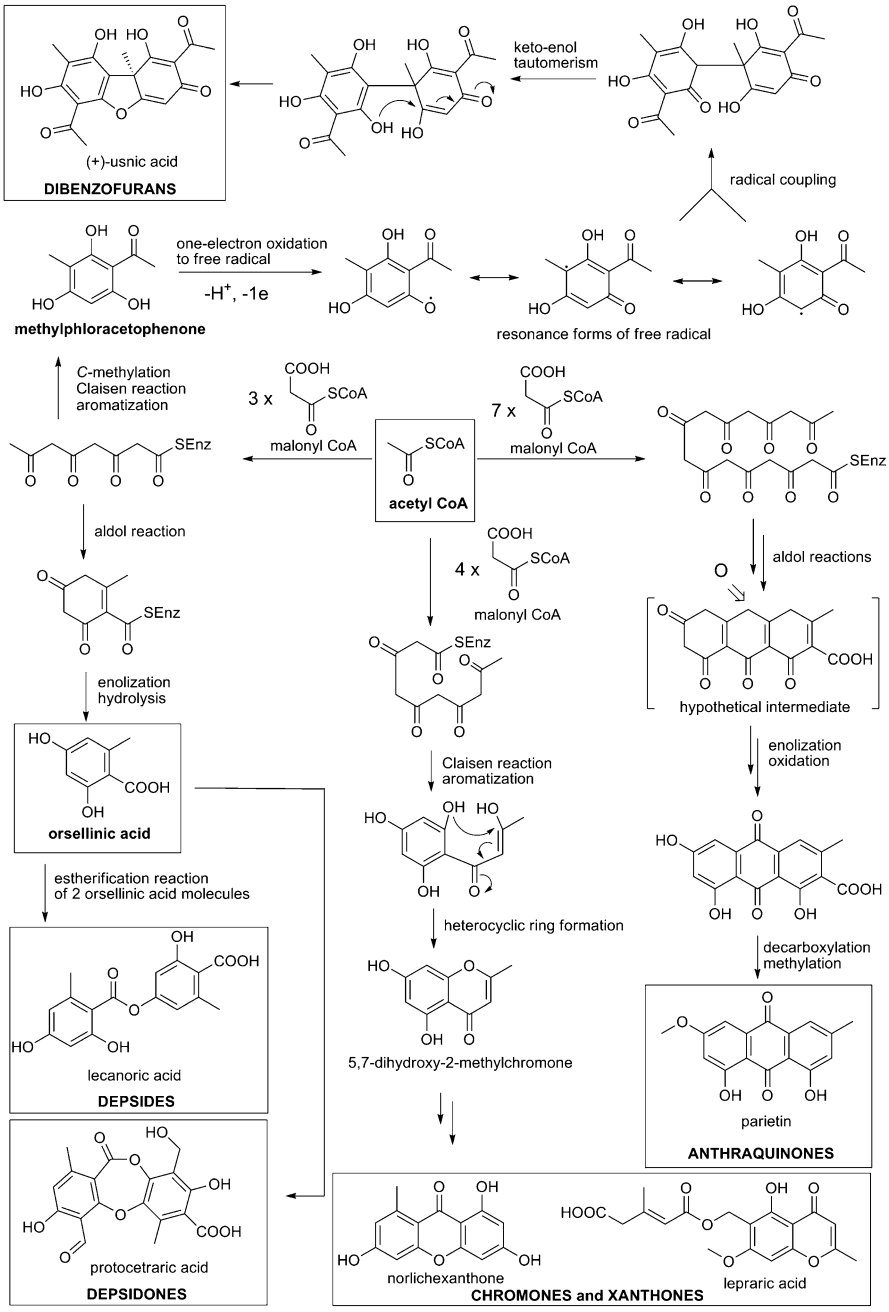


Fig. 5 Classes of lichen substances which are derived by acetyl-malonate pathway

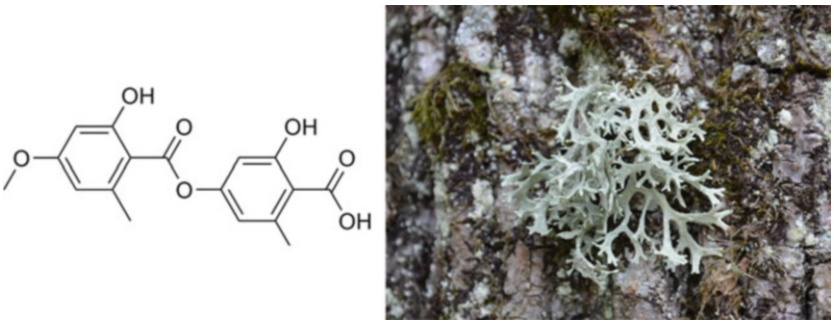


Fig. 6 Evernic acid structure (*Evernia prunastri*)

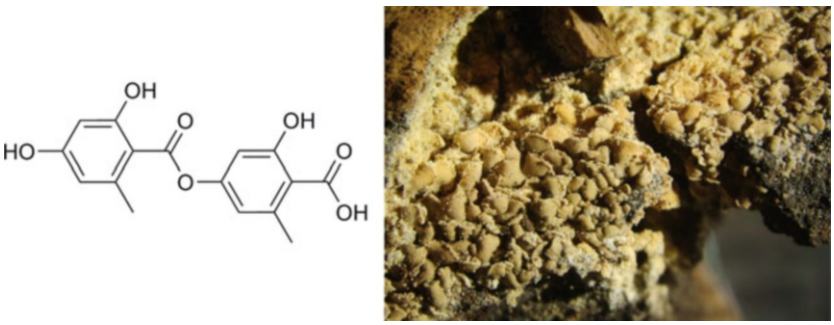


Fig. 7 Lecanoric acid structure (*Hypocenomyce scalaris*)

lecanoric acid and its orsellinate derivates against cancer cell lines (HEP-2, MCF-7, 786-0, and murine melanoma cell) and structural modifications increased activity. Promising antioxidant activity of lecanoric acid in SOR (superoxide radical) was demonstrated [43]. This compound showed relatively strong antimicrobial effects against 6 bacteria and 10 fungi containing human, animal, and plant pathogens, mycotoxin producers, as well as food-spoilage organisms [44, 45]. Lecanoric acid was also reported as a potent fungitoxic compound, which was tested against fungus *Cladosporium sphaerospermum* [46].

Gyrophoric Acid

Gyrophoric acid demonstrated antioxidant, antibacterial, cytotoxic, and antitumor activities (Fig. 8). This lichen compound is a common metabolite in *Umbilicaria* lichen species. Antioxidant activity of lichen members in family *Umbilicariaceae* was demonstrated [47]. Antibacterial activity of gyrophoric acid was showed against some foodborne bacteria and fungi [48]. Gyrophoric acid was highly effective against cancer cell lines (HL-60, A2780, Jurkat), where cytotoxicity and pro-apoptosis activity were confirmed [49].

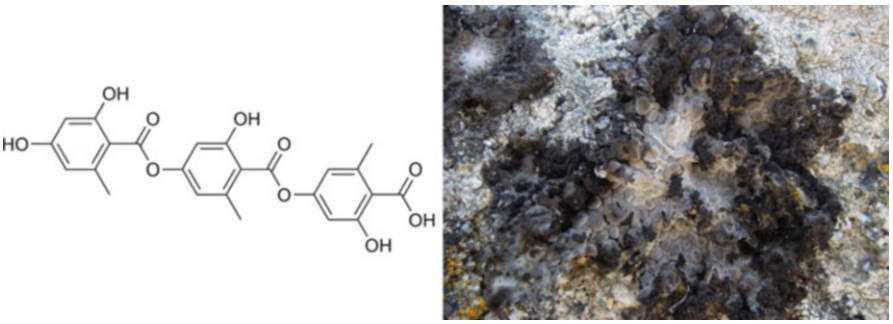


Fig. 8 Gyrophoric acid structure (*Lasallia pustulata*)

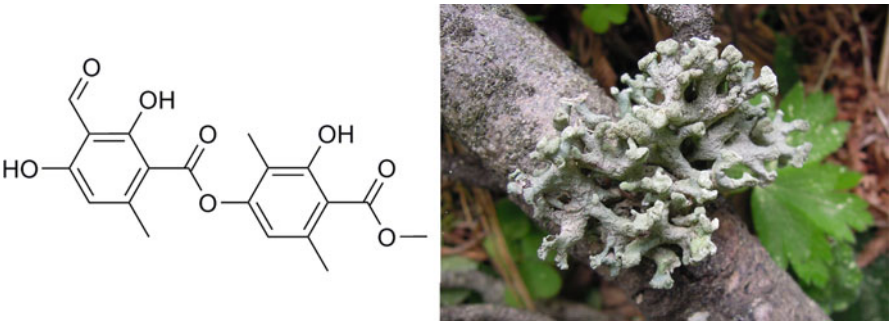


Fig. 9 Atranorin structure (*Hypogymnia tubulosa*)

Atranorin

Atranorin has strong antioxidant and antitumor properties (Fig. 9). This lichen compound has one of the largest free radical scavenging activities from lichen substances tested and the most effective reducing power and superoxide radical scavenging so far [50]. Another property of atranorin is anticancer activity against cancer cell lines (A2780 and HT-29) which was demonstrated by Bačkorová et al. [51]. This depside demonstrated strong pro-apoptic action and inhibition of cancer cell proliferation. Atranorin is counted also as a potential anticancer agent in hepatocytes from rat [52]. Antibacterial activities of this metabolite were also tested [44, 45].

Thamnolic Acid

In the study of Cankılıç et al. [53], thamnolic acid showed potential antibacterial, antituberculosis, and antifungal activities (Fig. 10). Strong effect of this compound was determined also against bacteria and yeasts. This compound can be used as potential antimicrobial agent in food industry and for the purpose of controlling different diseases.

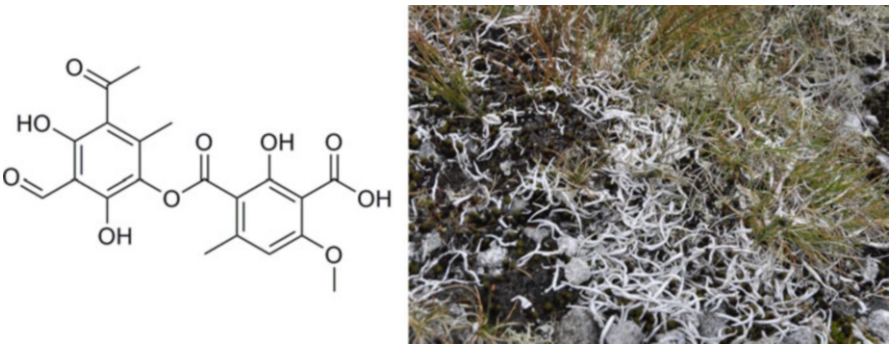


Fig. 10 Thamnolic acid structure (*Thamnolia vermicularis*)

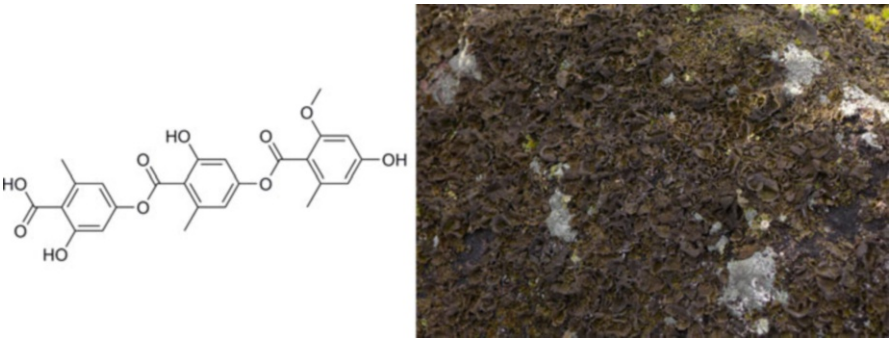


Fig. 11 Umbilicaric acid structure (*Umbilicaria polyphylla*)

Umbilicaric Acid

Umbilicaric acid is a common lichen substance in family *Umbilicariaceae* (Fig. 11). Antioxidant and antimicrobial activities of this metabolite were demonstrated. Umbilicaric acid was tested for potential antioxidant ability and showed the highest antioxidant activity with 68.14% inhibition among all tested metabolites [47]. Inhibitory effect on three Gram-positive bacteria and two yeasts, which are known as foodborne microorganisms and lead to infections in humans, was observed.

Depsidones

Orcinol-type depsidones have keto-group in the side chain of the first ring. It is well known that this functional group has a strong effect upon the ester linkage between the two rings, since enol lactones form readily. Oxidative cyclization of depsides to depsidones usually joins the 2-hydroxyl of ring A and the 5-position of ring B.

Protocetraric Acid

Antibacterial, antifungal, antioxidant and anticancer potential was found in protocetraric acid (Fig. 12). Antibacterial activity of protocetraric acid against

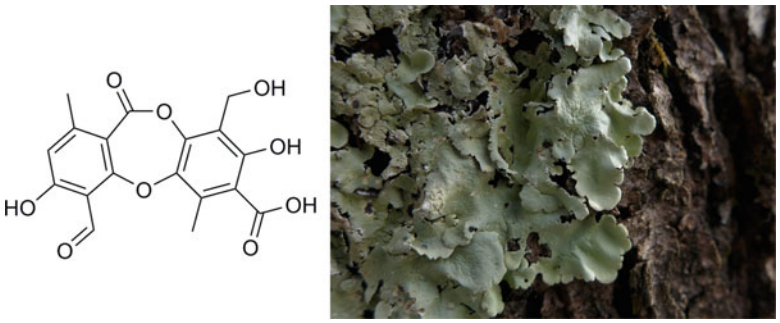


Fig. 12 Protocetraric acid structure (*Flavoparmelia caperata*)

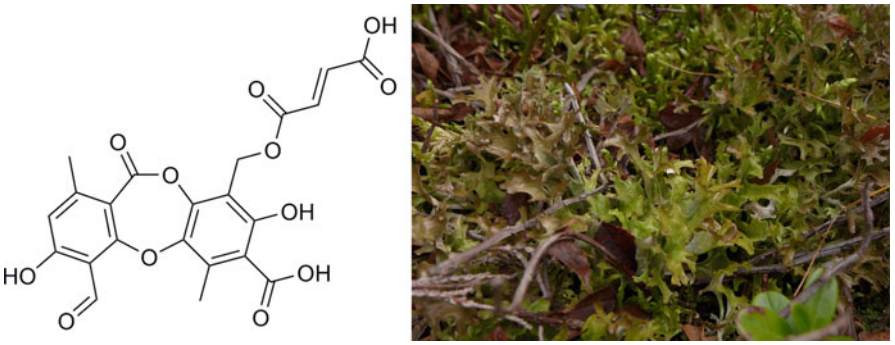


Fig. 13 Fumarprotocetraric acid structure (*Cetraria islandica*)

Salmonella typhi (0.5 μg/mL) and significant antifungal effect against *Tripthyton rubrum* (1 μg/1 mL) were reported. Protocetraric acid can be used as potential antimicrobial drug against human pathogenic microbes [54]. Antitubercular activity of several lichen substances was also tested. Protocetraric acid (MIC value 125 μg/mL, 334 μM) showed moderate inhibitory activity [55]. Antiproliferative activity of protocetraric acid against FemX (human melanoma) and LS174 (human colon carcinoma) cell lines with IC_{50} values from 35.67 to 60.18 μg/mL was confirmed.

Fumarprotocetraric Acid

Fumarprotocetraric acid as one of the bioactive compounds of lichen (Fig. 13) was tested as expectorant and for its antioxidant activities (Fig. 13). Orally administered compound (25 and 50 mg/kg) showed significantly greater dose-dependent phenol red activity in the bronchoalveolar lavage and expectorant activity ($p < 0.05$). Lipid peroxidation was also reduced by 50% in the lung tissue [56]. The growth inhibition of bacteria (*Bacillus cereus, Bacillus subtilis, Listeria monocytogenes*) and yeasts (*Candida albicans, Candida glabrata*) was observed after use of fumarprotocetraric acid (MIC 4.6 μg/mL, 0.33 mM for bacteria, and 18.7 μg/mL and 1.32 mM for yeasts) [57].

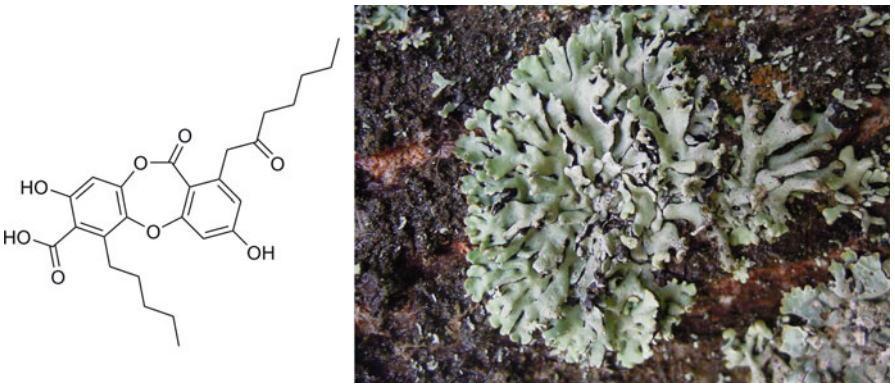


Fig. 14 Physodic acid structure (*Hypogymnia physodes*)

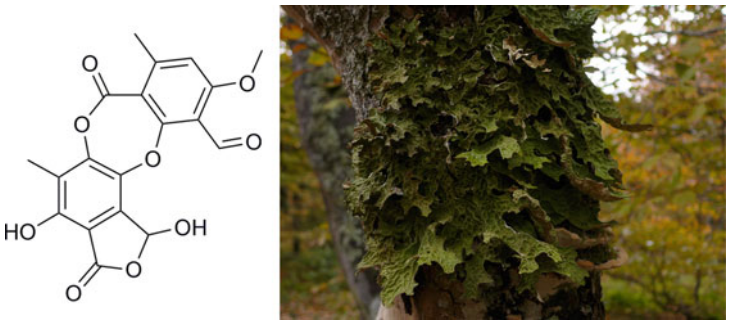


Fig. 15 Stictic acid structure (*Lobaria pulmonaria*)

Physodic Acid

Depsidone physodic acid was tested for anticancer activity (Fig. 14). This compound activated an apoptic process on A375 cells in the concentration of 6.25–50 μM. It probably involves the reduction of Hsp70 expression [58]. Another cytotoxic activity of physodic acid was tested on tumorigenic (MDA-MB-231, MCF-7, and T-47D) and nontumorigenic (MCF-10A) cell lines. Strong activity was observed against tumoric cell lines (IC_{50} = 46–94 μM) and inactivity of compound against nontumoric cell line (IC_{50} > 100 μM). In study of antimicrobial activity, physodic acid was active against the same bacteria or yeasts and inactive against all of the filamentous fungi, which were tested [59].

Stictic Acid

Stictic acid (Fig. 15) showed neuroprotection through the antioxidant activity against U373MG cell line (5 and 10 μg/mL) by decreasing productivity of ROS induced by hydrogen peroxide [60]. Antioxidant activity of concentration range 0.012–0.015 mg/mL was observed according to radical scavenging Co (II) EDTA-induced luminol plateau chemiluminescence assay [61]. Growth inhibition of cancer cell lines HT-29

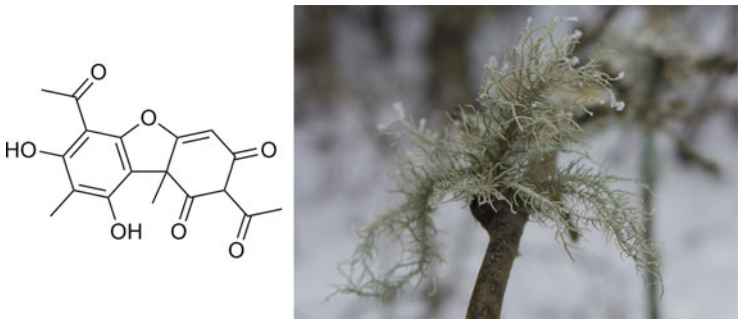


Fig. 16 Usnic acid structure (*Usnea* sp.)

and MCF-7 was tested as potential anticancer activity of stictic acid. Results showed strong potential of this compound as anticancer agent (IC_{50} = 29,29 μg/mL). For comparison was tested normal cell line MRC-5 with IC_{50} = 2478.40 μg/mL [62].

Dibenzofurans

Dibenzofurans are heterocyclic aromatic organic compounds with two benzene rings fused to a central furan ring. As secondary metabolites, the phenolic units are derived by the orsellinic acid-type cyclization. The dibenzofurans appear to form by carbon–carbon coupling and cyclodehydration of two such acetate–polymalonate-derived phenolic acid units.

Usnic Acid

One of the most studied secondary metabolite of lichen is usnic acid (Fig. 16). Based on the wide biological and ecological activities, it is used in cosmetics, deodorants, toothpastes, and medical creams. It also exhibits antimitotic, anti-inflammatory, analgesic, antiviral, antiprotozoal activities, as well as preserving properties, anti-growth, and antiherbivore activity [63]. Usnic acid serves as a repellent against insect feeding. Larvae of *Cleorodes lichenaria* were affected by retarded growth, increased mortality, and enhanced concentrations of usnic acid in the animal tissue [64]. Usnic acid is an effective UV-absorbing compound, which is also one of the known roles of secondary metabolites, and protects algal layer from intense light levels [65]. This compound also decreased the proliferation of human breast cancer cells and human lung cancer cells without any DNA damage [66]. Strong hepato-toxic activity was also observed against monogastric murine hepatocytes, with inhibition of the electron transport chain in the mitochondria and induction of oxidative stress in cells [67]. Usnic acid plays also important role as an allelopathic agent in competition between lichens and mosses. Growth inhibition of protonemata and reduced development of gametophores was observed. Usnic acid has a strong effect on cell division in protonemata [68]. The level of ploidy in mosses is also influenced by presence of usnic acid and can be counted as a physiological change after stress [69].

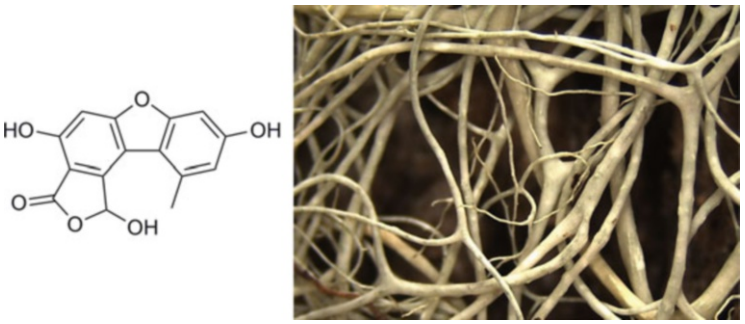


Fig. 17 Alectosarmentin structure (*Alectoria sarmentosa*)

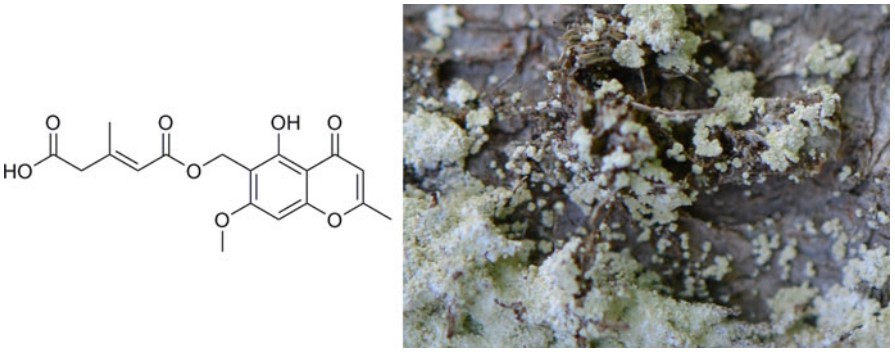


Fig. 18 Lepraric acid structure (*Lepraria* sp.)

Alectosarmentin

Alectosarmentin is a relatively newly discovered compound identified in lichen *Alectoria sarmentosa* (Fig. 17). This compound has antibacterial activity including microorganisms *Staphylococcus aureus* and *Mycobacterium smegmatis* [70].

Chromones

Chromone, parent compound of the chromones group, is derivative of benzopyran with substituted keto group on the pyran ring. Chromones are probably formed by internal cyclization of a single, folded polyketide chain and are often identical or analogous to products of nonlichen-forming fungi or higher plants.

Lepraric Acid

Lepraric acid (Fig. 18) can be used as chemotaxonomic marker in *Hypoxylon aeruginosum, Chlorostroma subcubisporum*, and *Chlorostroma cyaninum* [71].

Xanthones

Xanthones are known in free-living fungi and recent studies indicate that they are rather common in lichens too. Unlike the fungal xanthones, many lichen xanthones

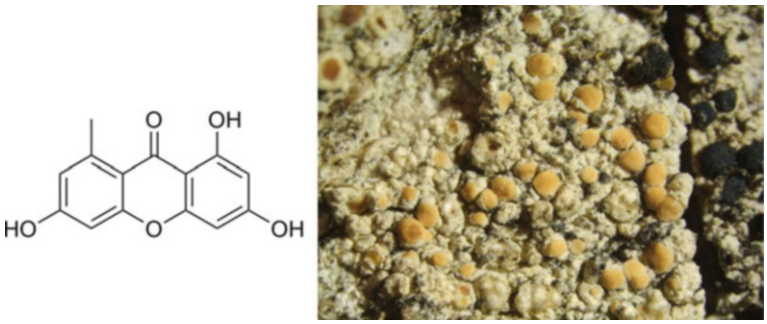


Fig. 19 Norlichexanthone structure (*Lecanora symmicta*)

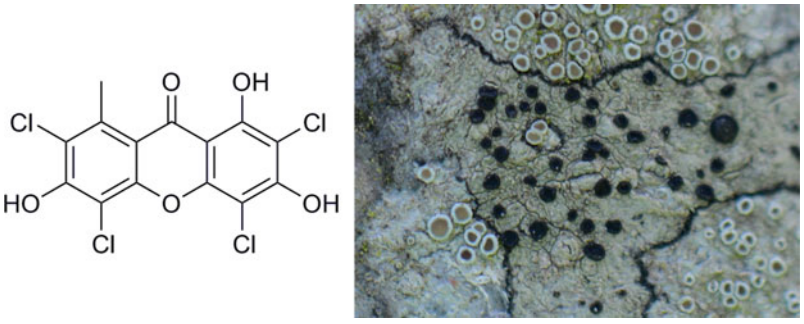


Fig. 20 Thiophanic acid structure (*Lecidella elaeochroma*)

have one or more nuclear chlorine substituents. The fundamental structure of the known lichen xanthones could be derived directly by linear condensation of seven acetate and malonate units with one orsellinic acid-type cyclization. The two rings are joined by a ketonic carbon and by an ether-oxygen arising from cyclodehydration.

Norlichexanthone

Norlichexanthone (Fig. 19) is lichen compound that fully inhibits $p56^{lck}$ tyrosine kinase at 200 μg/mL [72] and inhibits the activity of the protein kinases aurora-B, PIM1, and VEGF-R2, where IC_{50} values from 0.3 to 12 μM [73].

Thiophanic Acid

Allelopathic effect of thiophanic acid (Fig. 20) on wide number of higher plants was demonstrated [74]. Fungicidal activity of thiophanic acid and thiophaninic acid was recorded as well [75].

Anthraquinones

Anthraquinones is a class of phenolic compounds based on the 9,10-anthraquinone skeleton and is probably formed by internal cyclization of a single polyacetyl chain.

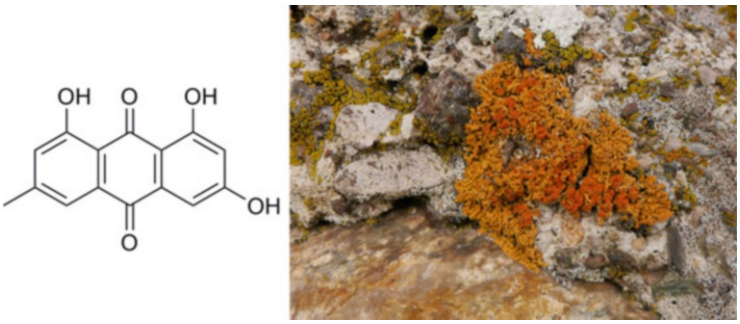


Fig. 21 Emodin structure (*Xanthoria elegans*)

Fig. 22 Structure of parietin (*Xanthoria parietina*)

These substances are typical for members of family *Teloschistaceae* within genera *Caloplaca*, *Teloschistes* and *Xanthoria* [76]. Anthraquinones are produced by lichens, as well as by nonlichenized fungi [77]. Biological activities of various anthraquinones were confirmed in several studies such as antitumoral, anti-inflammatory, and bactericide effects [77–79]. They are all pigmented compounds which also acting as a light filters.

Emodin

Generally, anthraquinones are potential antiviral agents against HIV virus [80]. Emodin (Fig. 21), 7-chloroemodin, and 7-chloro-1-O-methylemodin showed partial inactivation of the herpes simplex virus type 1. With an increasing substitution of chlorine in the anthraquinone nucleus, an antiviral activity increases [81]. Derivatives of emodin revealed anticancer activity against leukemia cells [82].

Parietin

Parietin (Fig. 22) is an orange anthraquinone pigment and it is widespread in lichens, which are characteristic for sun-exposed habitats. Mainly it is localized in the upper cortex of lichen genera *Xanthoria, Teloschistes*, *and Caloplaca*. According to Hill and Woolhouse [83], the content of parietin is positively correlated to intensity of

light in habitat. Since parietin absorbs light, it may help to protect the photosynthetic apparatus of the photobiont against damage by high light levels [36, 84]. Solhaug and Gauslaa [85] reported that UV-B radiation may trigger the resynthesis of this cortical pigment parietin (= physcion) in the lichen *Xanthoria parietina*. Despite the long-term study of parietin, we still cannot claim that this secondary metabolite serves as UV-B or PAR screening pigment [86]. It is possible that parietin also acts as an antioxidant [87].

4.1.2 Mevalonate Pathway

The terpenoids form a large and structurally diverse family of natural products derived from C_5 isoprene units joined in a head-to-tail fashion. Isoprene is produced naturally but is not involved in the formation of these compounds, and the biochemically active isoprene units were identified as the diphosphate esters – dimethylallyl pyrophosphate and isopenthenyl pyrophosphate.

Two molecules of acetyl-coenzyme A combine initially in a Claisen condensation to give acetoacetyl-CoA, and a third is incorporated via a stereospecific aldol addition giving the branched-chain ester. The thioester is then reduced to primary alcohol via hemithioacetal and aldehyde to give mevalonic acid. The six-carbon mevalonic acid is then transformed into the five-carbon phosphorylated isoprene units in a series of reactions, beginning with phosphorylation of the primary alcohol group. Two different ATP-dependent enzymes are involved, resulting in mevalonic acid diphosphate, and decarboxylation/dehydration then follows to give isopentenyl pyrophosphate. Combination of two isoprene units head-to-tail forms monoterpenes. Limonene is formed by cyclization reactions of geranyl pyrophosphate. Diterpenes consist of four isoprene units. Geranyl PP reacts with first isoprene unit to give farnesyl PP which reacts with second isoprene to give geranylgeranyl PP. Phytol is one of the best known diterpenes. Triterpenes are derived from squalene, six isoprene units containing compound. Steroids are then formed by cyclization of squalene. Another well-known class of terpenoids – carotenes – are derivatives of tetraterpene lycopene, which is formed by combination of two geranylgeranyl PP units. Classes of lichen substances that are derived by mevalonate pathway are terpenes, steroids, and carotenoids (Fig. 23).

Terpenes

Terpenes are large and diverse class of organic compounds. Terpenes are formed biosynthetically from units of isopentenyl pyrophosphate, which is the product of mevalonate pathway.

Limonene

In study of Kahriman et al. [88], the antimicrobial and antifungal activity of the essential oil obtained by hydrodistillation from *Evernia prunastri* (L.) Ach. and *Evernia divaricata* (L.) has been analyzed. The major substances in the essential oil of *Evernia prunastri* and *Evernia divaricata* were β-pinene (6.3 and 8.0%), α-pinene (6.6%, 7.2%), limonene (1.6%, 6.3%), α-phellandrene (3.3%, 4.4%), camphene (3.0%, 3.1%), and p-cymene (1.5%, 1.8%), respectively. The antimicrobial and

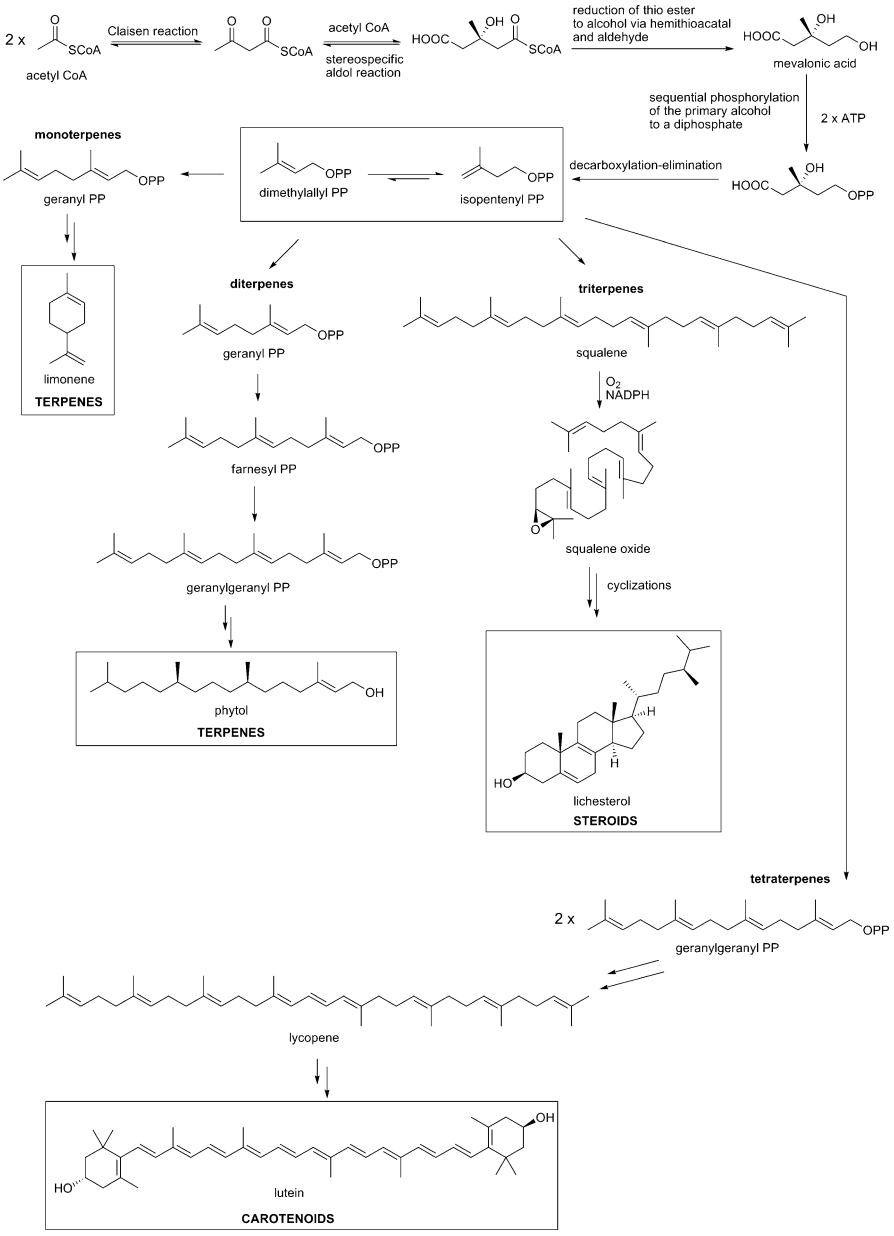


Fig. 23 Classes of lichen substances that are derived by mevalonate pathway

antifungal activities of the essential oil of *Evernia prunastri* and *Evernia divaricata* were tested in vitro against the bacteria *E. coli, Y. pseudotuberculosis, S. aureus, E. faecalis, B. cereus, C. albicans*. *Evernia divaricata* showed antimicrobial activity

Fig. 24 Limonene structure (*Evernia prunastri*)

and antifungal activity. Essential oil of *Evernia prunastri* exhibited only antifungal activity (Fig. 24).

Phytol

This secondary metabolite of terpenoid origin showed mainly antimycobacterial activity. Rajab et al. [89] tested (E)-phytol (Fig. 25) as the principal antimycobacterial constituent against *Mycobacterium tuberculosis* with a minimum inhibitory concentration (MIC) of 2 μg/ml. Inhibitory value was also observed for (3R,5,7R,11R)-phytanol, (Z)-phytol, and a commercially available 2: 1 mixture of (E)- and (Z)-phytol with lower antimycobacterial activity with MIC $>$ 128 μg/ml.

Zeorin

Zeorin (Fig. 26) (6α,22-dihydroxyhopane) is the main triterpene in various species of lichens [90]. In the study of Kosanić et al. [91] has been tested antibacterial and antifungal activity of the lichen *Lecanora frustulosa* and *Parmeliopsis hyperopta* and their zeorin constituents and divaricatic acid. Acetone, methanol, and aqueous extracts of these lichens have been tested in vitro against: *Bacillus mycoides, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloaceae, Escherichia coli, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus fumigatus, Botrytis cinerea, Candida albicans, Fusarium oxysporum, Mucor mucedo, Paecilomyces variotii, Penicillium purpurescens, Penicillium verrucosum*, and *Trichoderma harzianum.* According to this study, zeorin exhibited stronger antibacterial activity than divaricatic acid at a concentration of 0.39 mg/ml which inhibited 4 out of 6 tested bacteria.

Steroids

Steroids are products of the mevalonate pathway and are highly often present in lichens. Steroids are derived from cyclization of the triterpene squalene.

It has been reported that sterol compounds can play an important role in the medicine. They possess different types of pharmacological activities like anti-

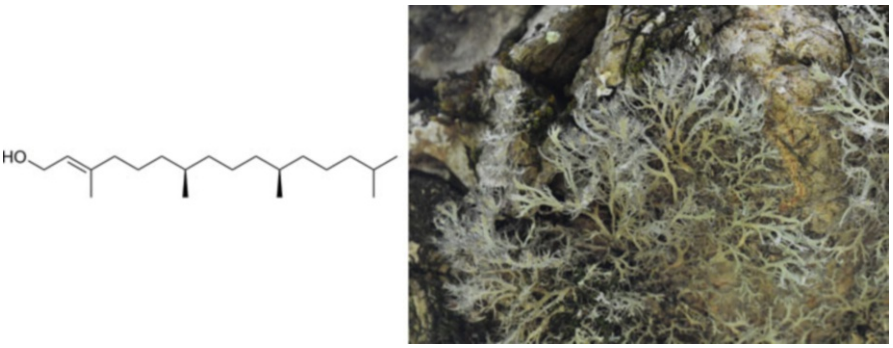


Fig. 25 Phytol structure (*Anaptychia ciliaris*)

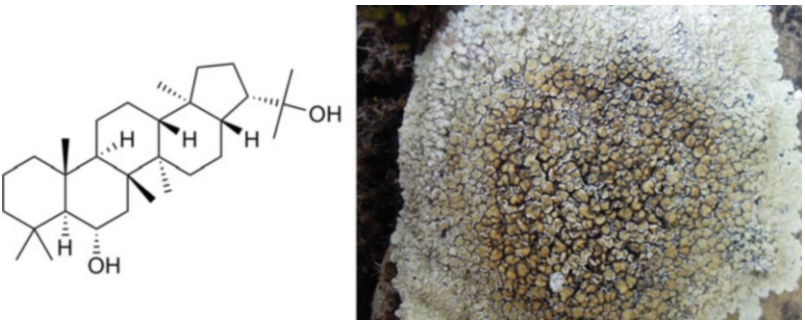


Fig. 26 Zeorin structure (*Protoparmeliopsis muralis*)

inflammatory, antiulcerogenic, antibacterial, antifungal, and antirheumatic activities [90].

Brassicasterol

Brassicasterol (Fig. 27) known in higher plant is also found in the various lichens [90]. In acetone extract of the lichen *Stereocaulon azoreum* were identified several substances by column chromatography. In addition to the main compounds, three sterols such as brassicasterol, ergosterol peroxide, and cerevisterol were obtained [92].

Ergosterol

It has been demonstrated in previous studies that sterols may play a role in the membrane permeability in the lichen thallus. The content of ergosterol (Fig. 28) (Ergosta-5,7,22-trien-3β-ol) in the lichens, which is the major sterol of the fungal plasma membrane, responds rapidly to the presence of xenobiotics in the environment, including the presence of heavy metals. Ergosterol can be considered as marker of the fungal metabolic activity [93].

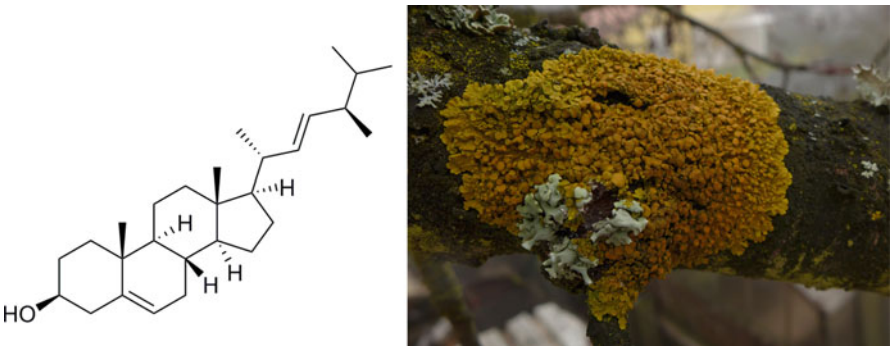


Fig. 27 Brassicasterol (*Xanthoria parietina*)

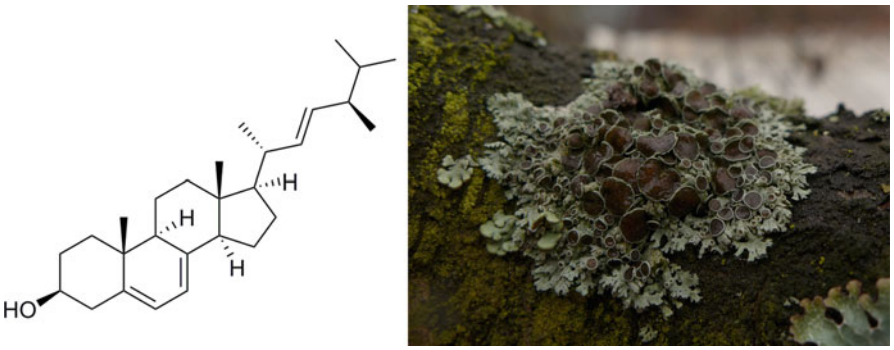


Fig. 28 Ergosterol structure (*Physcia stellaris*)

Lichesterol

Lichesterol (Fig. 29) or Ergosta-5,8,22-trien-3β-ol has been isolated and characterized in lichens *Usnea longissima*, *Lobaria pulmonaria*, *Lobaria scrobiculata* [94], and *Ramalina africana* [95].

Carotenoids

These linear molecules with multiple conjugated double bonds are found in all photosynthetic organisms. They are products of primary (intracellular) metabolism such as proteins, amino acids, polysaccharides, and vitamins. Carotenoids are products of both symbionts – fungi and algae.

In lichens with green algae photobionts following carotenoids are usually present β-carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin [96, 97]. In lichens with cyanobacterial photobionts occur mainly β -carotene, zeaxanthin, canthaxanthin, and echinenone.

β-Carotene

β-Carotene (Fig. 30) is a pigment frequently found in lichen thalli, which has been analyzed using different methods. Czeczuga et al. [98] investigated in ten lichen

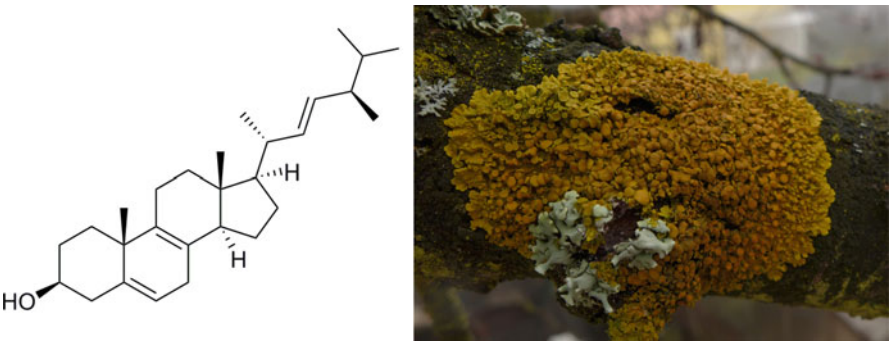


Fig. 29 Lichesterol structure (*Xanthoria parietina*)

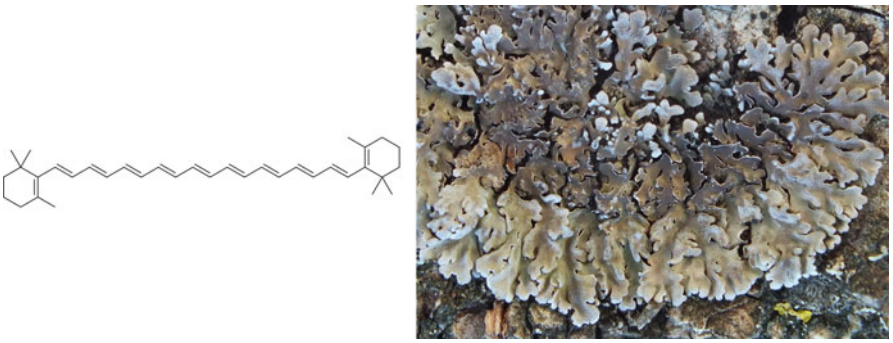


Fig. 30 β-Carotene structure (*Physconia distorta*)

species carotenoids by column and thin-layer chromatography. Lichen encrustations from *Diploschistes scruposus* showed characteristic vibrational spectra using Raman spectroscopy [99]. Nowadays it is a frequent practice to measure total carotenoids spectrophotometrically and using high-performance liquid chromatography (HPLC) techniques which facilitated the separation and identification of plastid pigments.

Lutein

Lutein (Fig. 31) mainly occurs in higher plants but was also found in lichens and algae growing near to shaded habitats, because at sunny sites it is replaced by lutein epoxide. The growth of lichens in poorly lit places is possible due to the mechanism called chromatic adaptation by an increasing of photosynthetically active pigments [100].

Zeaxanthin

The presence of this carotenoid (Fig. 32) together with violaxanthin in plants is influenced by intensity of light in xanthophyll cycle. In case that insolation is intensive, the accumulation of zeaxanthin occurs. When light intensity decreases,

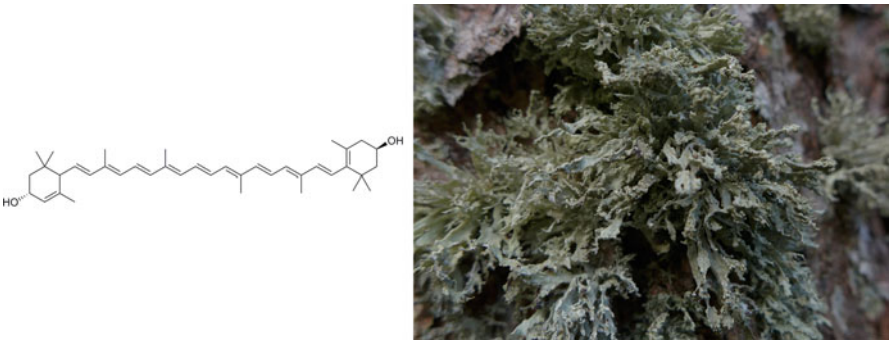


Fig. 31 Lutein structure (*Ramalina farinacea*)

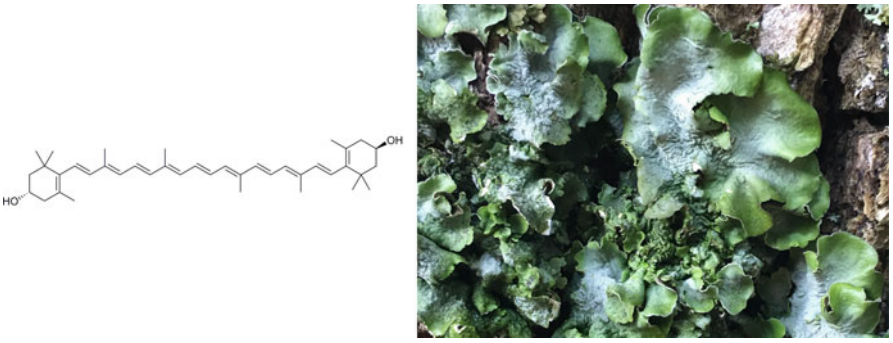


Fig. 32 Zeaxanthin structure (*Pleurosticta acetabulum*)

the zeaxanthin is converted through antheraxanthin into violaxanthin and vice versa [101]. It may be considered that this cycle occurs in the photobionts of lichens.

4.1.3 Shikimate Pathway

The shikimate pathway provides a route to aromatic compounds, particularly the aromatic amino acids and their derivatives. The pathway is employed by microorganisms and plants but not by animals. A central intermediate in the pathway is shikimic acid. The shikimate pathway begins with a coupling of phosphoenolpyruvate (from glycolysis) and D-erythrose 4-phosphate (from the pentose phosphate cycle) by aldol-type reaction. Then by elimination of phosphate and another aldol-type reaction, a cyclic product 3-dehydroquinic acid is formed. Next step involves dehydration and reduction of carbonyl function.

Phosphoenolpyruvate combines with shikimic acid 3-phosphate to an intermediate in which 1,2-elimination of phosphoric acid in side-chain and then 1,4-elimination of phosphoric acid leads to chorismic acid. The reaction transforming chorismic acid to prephenic acid is Claisen rearrangement which transfer the side-chain so that it becomes directly bonded to the carbocycle. Next reaction

steps leading to the C_6-C_3 building block (phenylpyruvic acid, L-phenylalanine) include decarboxylation, aromatization, and dehydroxylation. Generally two of the C_6-C_3 building blocks combine to form terphenylquinones and reaction pathway continues to pulvinic acid derivatives. Classes of lichen substances which are derived by shikimate pathway are therphenylquinones and pulvinic acid derivates (Fig. 33)

Terphenylquinones

Phenylquinones are well-documented examples of lichen secondary products derived by the shikimic acid pathway and are widespread especially among fungi. Terphenylquinones are formed by condensation of two (probably activated) phenylpyruvic acid derivatives. Only two terphenylquinones, polyporic acid and thelephoric acid, are known.

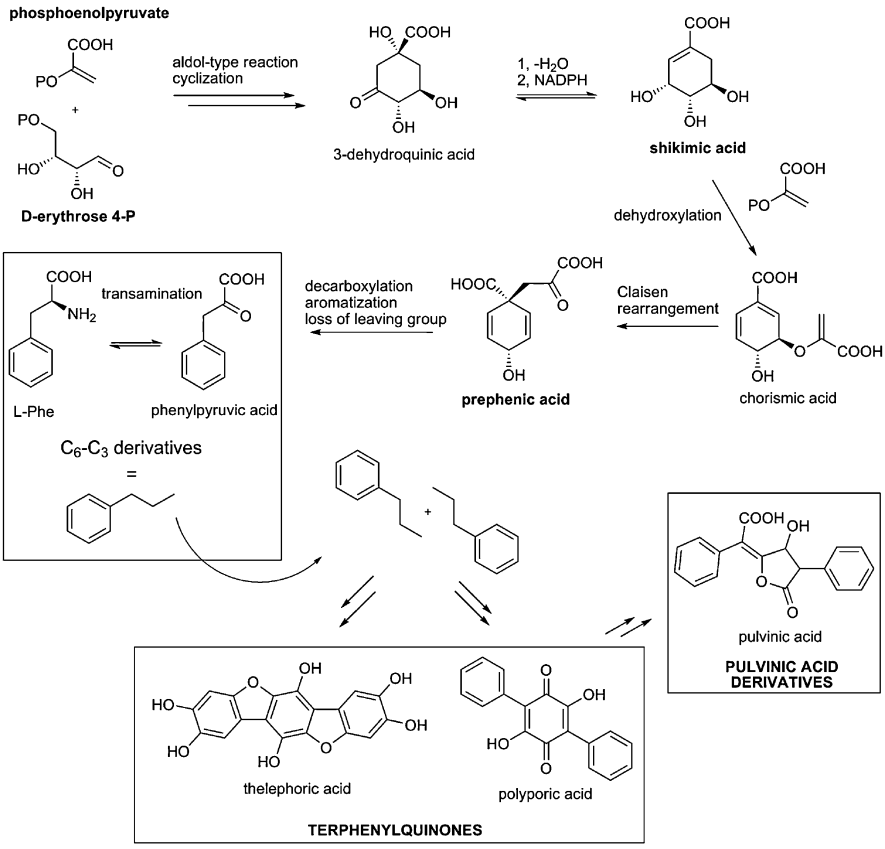


Fig. 33 Classes of lichen substances which are derived by shikimate pathway

Fig. 34 Polyporic acid structure

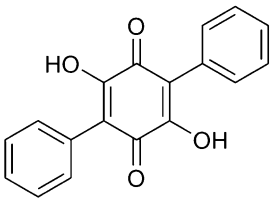
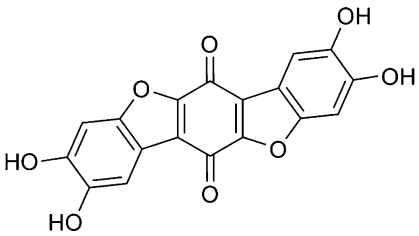


Fig. 35 Thelephoric acid structure



Polyporic Acid

Polyporic acid (Fig. 34) extracted from fungus *Hapalopilus rutilans* decreased activity of DHOdehase enzyme in rats by 20–30% due to its inhibitory effect. DHOdehase enzyme catalyzes reaction of pyrimidine de novo synthesis at the inner mitochondrial membrane. Activity of the human enzyme was not affected [102]. Another study conducted on rats showed strong inhibitory effect of polyporic acid; the rats exhibited reduced locomotor activity, hepatorenal failure, and metabolic acidosis [103]. Burton and Cain [104] showed antileukemic activity of polyporic acid isolated from lichen *Sticta coronata* on mice.

Thelephoric Acid

Thelephoric acid (Fig. 35) from fungus *Polyozellus multiplex* exhibited inhibitory effect against prolyl endopeptidase, in which increased level is involved in the development of Alzheimer's type senile dementia [105]. Antioxidative properties were investigated by Chung et al. [106] where results were conclusive for superoxide anion radical, hydroxyl radical, and DPPH radical. Rao et al. [107] found theleophoric acid in lichen *Lobaria insidiosa* from Western Himalayas. This acid is also found in *Thelephora* spp. and *Hydnum* spp.

Pulvinic Acid Derivatives

Pulvinic acid derivatives are lichen secondary products which are derived by the shikimic acid pathway. Pulvinic acid derivatives are not present in all lichen species that contain blue-green algae. Nitrogen fixing algae present in some lichens are no necessary in species, where pulvinic acid pigments were observed.

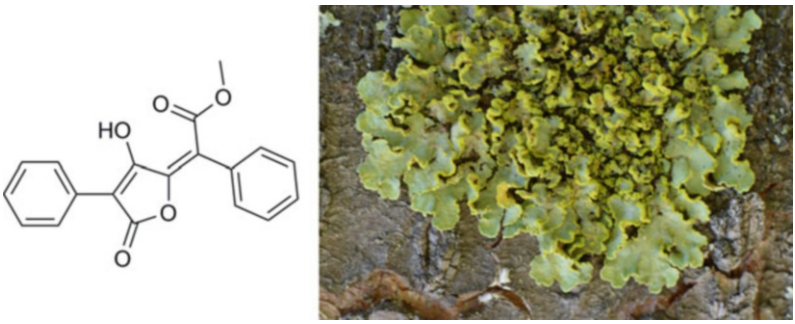


Fig. 36 Vulpinic acid structure (*Vulpicida pinastri*)

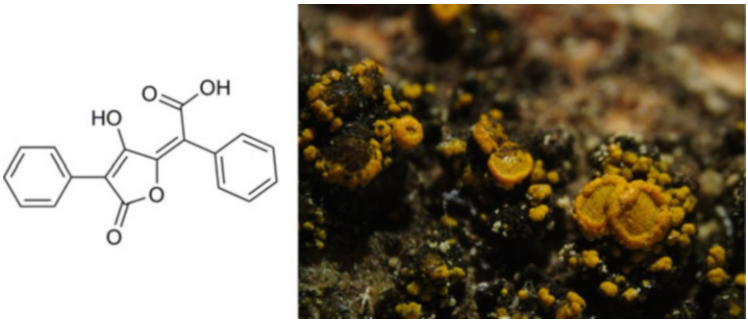


Fig. 37 Pulvinic acid structure (*Candelariella vitellina*)

Vulpinic Acid

Vulpinic acid (Fig. 36) isolated from *Letharia vulpina* induced uncoupling by acting on the inner mitochondrial membrane in mice liver in vitro [108]. Extract from *Vulpicida pinastri* (containing vulpinic acid, pinastric acid, usnic acid) acts as a UV-A and UV-B blocker agent due to its superoxide anion scavenging activity [109, 110]. Application of vulpinic acid strongly influenced growth of lichen photobiont *Trebouxia irregularis* [111]. When used on larvae of the polyphagous insect herbivore *Spodoptera littoralis*, vulpinic acid showed strong mortality and growth retardation in concentration lower than naturally occurring in lichens [112]. Antiproliferative effect of vulpinic acid was tested on HepG2 and NS20Y cancer cell lines, exhibited strong antiangiogenic potential, and showed no toxic effects on noncancerous cells [113].

Pulvinic Acid

Pulvinic acid (Fig. 37) derivate pulvinamide exhibited antioxidant properties [114]. Another set of derivates – atromentic acid, variegatic acid, and xerocomic acid – showed nonspecific inhibitory effects on four cytochrome P450 (CYP) – 1A2, 2C9, 2D6, and 3A4 – probably by reduction of ferryl heme to ferric heme [115].

5 Conclusion

Lichens are very typical symbiotic organisms, which can be found everywhere around the world and dominantly present in 8% of earth's land surface. Due to the fact that they belong to the slowest growing organisms, they are very important and interesting because of their secondary metabolites. One of the first descriptions of uses is from time of early Chinese and Egyptian civilizations.

Since the sixteenth century, lichens have been used in the perfume and cosmetic industries. They are attractive also for their typical color hence used as dyes. Lichen secondary compounds are studied for more than one hundred years for their pharmaceutical, biological, and ecological potential, which was described in many studies.

Based on the pathways how lichen secondary metabolites are produced, the acetate-polymalonate pathway is unique for lichens. Most of bioactive compounds are synthetized by this pathway, and their biological properties are very promising and still the aim of study around the world. In this chapter, we showed all three pathways, which can serve for better understanding of synthesis of lichen compounds. Main groups that belong to the pathways are also described as well as their typical secondary compounds with their pharmaceutical, biological, and ecological uses. It is evident that secondary compounds of lichens have wide area where they can be applied.

Approximately 1000 secondary metabolites of lichens were discovered and described. Most of them are solely present in lichens. Their antiproliferative, antibacterial, antiviral, allelopathic, antiherbivore, UV-protective, antioxidant, anti-inflammatory, analgesic, antipyretic potential is evident. Lichens are still source of many bioactive compounds, which application is still in process of research.

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