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LICHEN SUBSTANCES: BIOCHEMISTRY, ECOLGICAL ROLE AND ECONOMIC USES

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ABSTRACT

Lichens are fungi that live as ecologically obligate biotrophs in symbiosis with algal and/or cyanobacterial photobionts. About 8% of terrestrial ecosystems are dominated by lichens in situations where vascular plants are at their physiological limits. There are three types of lichens, namely crustaceous, foliose and fruticose. Lichens are very slow growing and large plants are relatively old, suggesting a well balanced relationship between the algal and the fungal partners. Lichens produce a wide array of secondary metabolites. The main types are depsides, depsidones, dibenzofurans anthraquinones, xanthones, chromones, pulvinic acid derivatives, terphenylquinones, terpenes and steroids. The majority of lichen substances are deposited in the medulla. There are several ecological, medicinal and other economic roles of lichen substances such as light-screening, chemical withering, biological activity, anti-herbivore defense, and allergic activity. Furthermore they have found use as dyes, perfumes and food sources. The application of chemical discriminators to lichen taxonomy is widely accepted. The secondary metabolites in about 5000 species (33 % of all known species) have been studied and the data are used in the routine identification of lichens. Within a complex of morphologically similar species, three common patterns of chemical variation can be discerned: Replacement compounds, chemosyndromic variation and accessory type compounds. Colour spot tests done on thalli and microchemical methods such as TLC coupled with HPLC, GC and MS are also routinely used in obtaining txonomical details.

1. INTRODUCTION

Lichens are the symbiotic phenotype of nutritionally specialised fungi that live as ecologically obligate biotrophs in symbiosis with algal and/or cyanobacterial photobionts[1]. This relationship between the algal partner and the fungus is now regarded as a symbiosis in which both partners benefit. Despite this, the algal partner is never found in a sexual state, and the fungal partner is dependent on nutrients from photosynthesis or nitrogen fixation of photobiont partner. About 8% of terrestrial ecosystems are dominated by lichens in situations where vascular plants are at their physiological limits.

Most lichens are crustaceous, growing closely appressed to the substratum of rock, wood or occasionally soil. Some are less attched to the substratum and are referred to as foliose or fruticose. Foliose and fruticose lichens form only a minority of species, but they are the most abundant in vegetation. Usually, each lichen thallus consists of a single species of fungus associated with a single species of alga. Over twenty seven apecies of algae have been found in lichens. The commonest species is *Trebouxia* which contains the genus *chlorella*. The 1325 species of lichen-forming fungi constitute about 46% ascomycetes and about one fifth of all known fungi. Lichens are very slow growing plants. Most crustaceous species rarely grow more than a few millimetres a year. Foliose and fruticose lichens grow faster (about one centimetre a year). It follows that large plants are relatively old, suggesting a well-balanced relationship between the algal and the fungal partners.

Primary metabolites of lichens, which are intracellular, are proteins, amino acids, polyols, caratenoids, polysaccharides and vitamins. Lichens produce a wide array of secondary metabolites (intracellular). There are over 700 lichen substances reported to date and many are restricted to the lichenised state[2]. Broadly speaking, there are three types of lichen substances based on their biosynthetic origin.

(a) Depsides, depsidones and dibenzofurans formed by the acetate-malonate pathway. The most important of these are the esters and the oxidative coupling products of simple phenolic units related to orcinol and β-orcinol. Most depsides and depsidones are colorless compounds which occure in the medulla of the lichen. However, usnic acids, yellow cortical compounds formed by the oxidative coupling of methylphloroacetophenone units are found in the cortex of many lichen species.

(b) Anthraquinones, xanthones and chromones, are all pigmented compounds which occur in the cortex. They are also produced by the acetate-malonate pathway, but their biosynthesis results from intramolecular condensation of long, folded polyketide units rather than the coupling of phenolic units.

(c) The shikimic acid biosynthetic pathway produces two major groups of pigmented compounds, which occur in the cortex: pulvinic acid derivatives and terphenylquinones. Although most pulvinic acid derivatives lack nitrogen, they are biosynthesised through phenyl alanine. Nitrogen is strongly limiting to metabolic activities in most lichens, and nitrogen rich metabolites such as alkaloids are unknown among lichen substances.

(d) Terpenes and steroids are produced by the mevalonic acid pathway. These include compounds unique to lichens and many that occur in higher plants as well.

2. ECOLOGICAL ROLE OF LICHEN SUBSTANCES

A significant number of lichen substances are unknown in nonlichanised fungi. Nevertheless, many genera of lichens do not produce any of the characterised products, thus the presence of these compounds are not required to maintain the symbiotic state.

Histologically, lichen substances are deposited in either the cortex or the medulla as crystals on the outer surface of hyphae. The commonest cortical compounds are usnic acid and atranorin. Antraquinoes, pulvinic acid derivatives and xanthones also occur in the cortex to a lesser degree. All these compounds with the exception of atranorin are pigmented. The majority of lichen substances are deposited in the medulla. Concentration of lichen substances are variable and typically it is around 1% but values of 5-6% are not uncommon. Once formed, lichen substances are very stable. In lichens, there is no correlation between growth rates and concentration of lichen substances although high concentrations are found at actively growing lobe or branch-tips as well as soralia and apothecial margins.

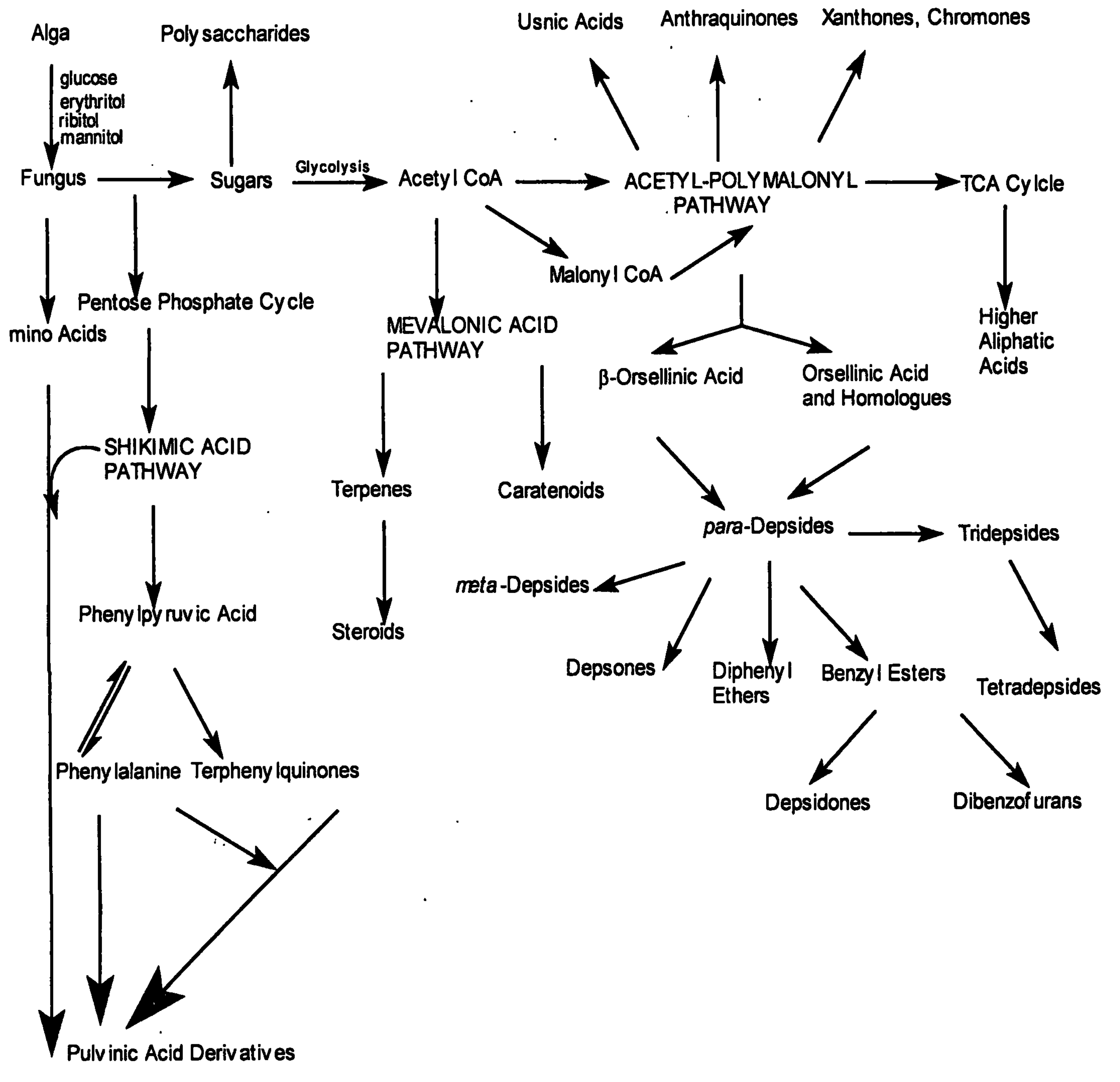


Fig. 1. Biosynthetic Pathways to the Major Groups of Lichen Substances[3].

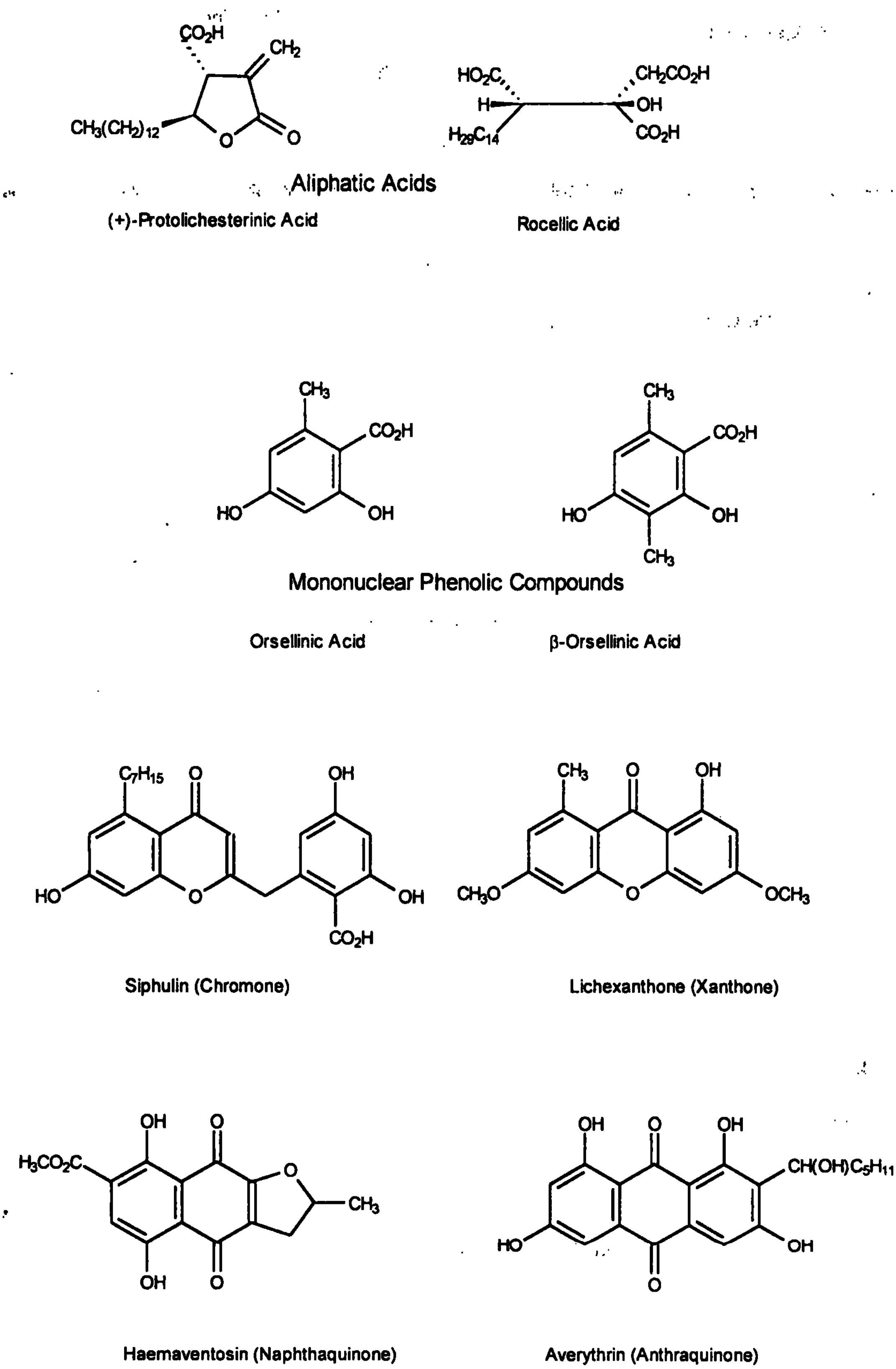
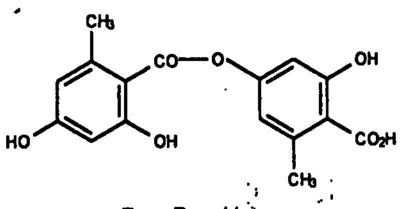
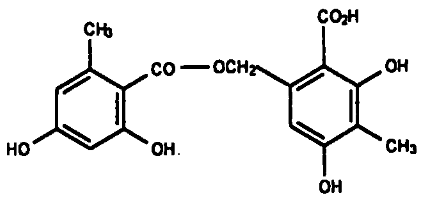


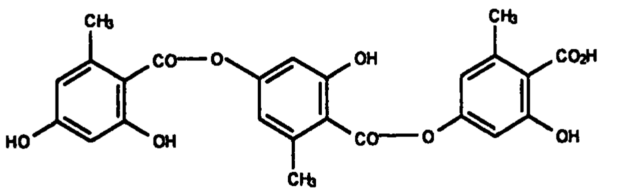
Fig. 2. Structures of typical acetyl-polymalonyl lichen products derived from a single polyketide chain.



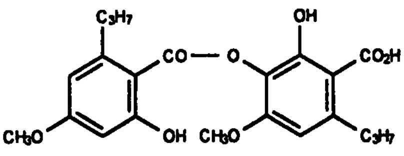
Para-Depside
Lecanoric acid



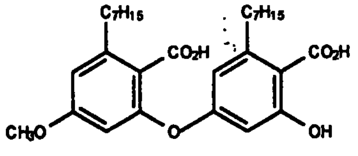
Benzyl Ester
Alectorialic Acid



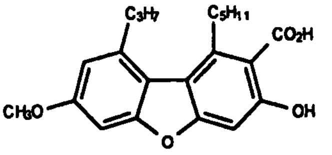
Tridepside
Gyrophoric Acid



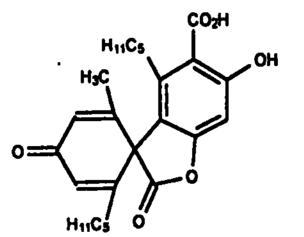
Meta-Depside
Sekikaic Acid



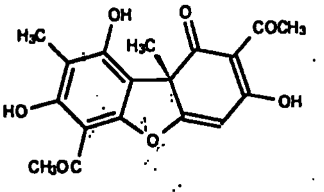
Diphenyl Ether
Micareic Acid



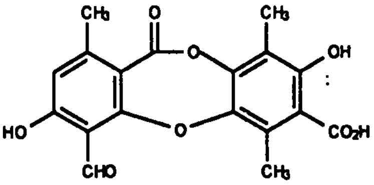
Dibenzofuran
Didymic acid



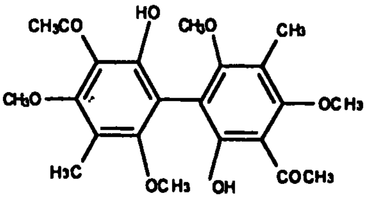
Depsone
Picrolichenic Acid



Usnic Acid

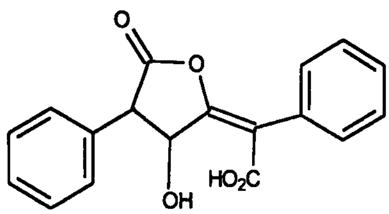


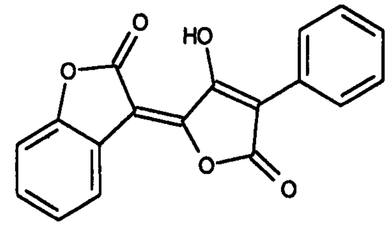
Depsidone
Verensic Acid



Biphenyl
Contortin

Fig. 3. Structures of typical acetyl-polymalonyl lichen products derived from two or more polyketide chains.

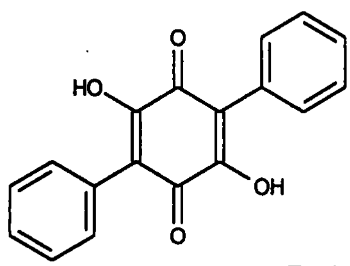


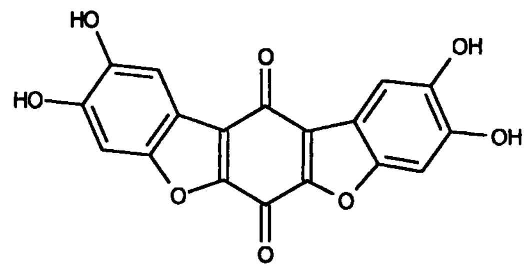


Pulvinic Acid and Derivatives

Pulvinic Acid

Calycin

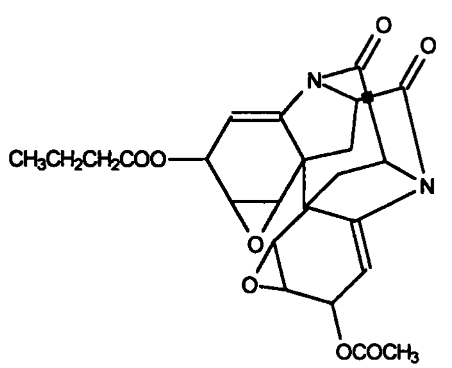


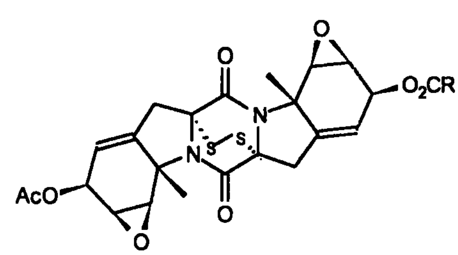


Terphenylquinones

Polyporic Acid

Thelephoric Acid





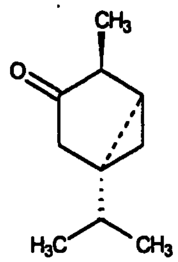
Amino Acid Derivatives

4-Acetyl-4'-butyrylscabrosin

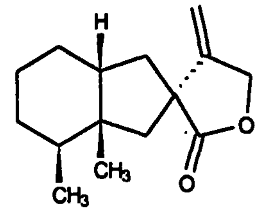
Ambewelamide A 1 R = $-(CH_2)_2CH_3$
Ambewelamide B 2 R = $-(CH_2)_4CH_3$

Fig. 5. Structures of some lichen products derived from the shikimic acid pathway.

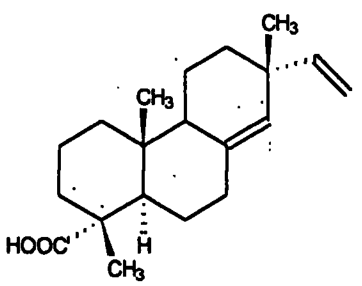
Terpenoids



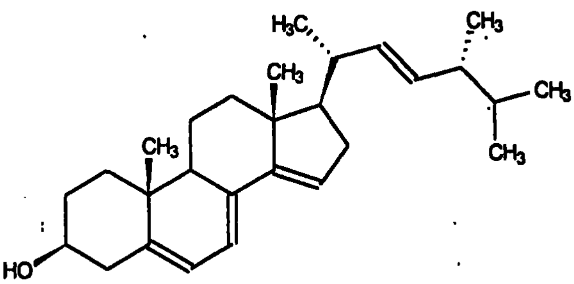
(-)-β-Thujone (Mnonterpenoid)



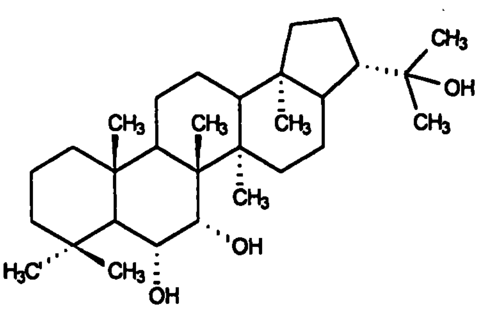
Fukinanolide A (Sesquiterpenoid)



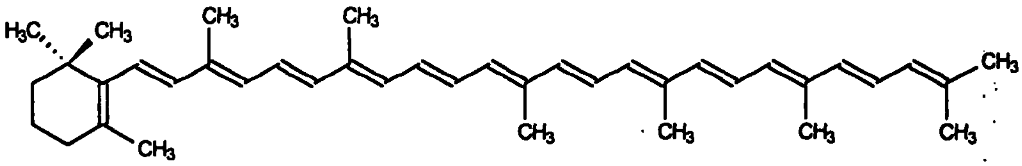
(-)-Sandaracopimaric Acid (Diterpenoid)



Ergosterol (steroid)



Hopan-6α,7α,22-triol (Triterpenoid)



γ-Carotene

Fig. 6. Structures of typical lichen products derived from the mevalonic acid pathway.

There appear to be several ecological, medicinal and other economic roles of lichen substances, which determine the relative ecological success of individual lichen species and the structure and diversity of natural lichen communities:

(a) Light-screen compounds
(b) Chemical withering compounds
(c) Biologically active compounds
(d) Anti-herbivore defense compounds
(e) Allergenic compounds

Light-screen compounds:

Many cortical lichen substances show variation in concentration along light gradients. Evidence suggests that these compounds act as light-screens, regulating the light intensity reaching the algal layer in the upper cortex. Usnic acid, a yellow cortical pigment is the most widespread lichen substance found in thousands of lichens. Lichen populations having usnic acid which are exposed to the sun are yellow, while the shade populations are grey-green, indicating a lower concentration of the depside. *Cladonia subtenuis* and *Parmeliopsis ambigua* show concentrations of usnic acid varying along concentration gradients[4].

Anthraquinones such as parietin (a reddish cortical pigment) appear to act as light filters like usnic acid. This compound is characteristic of species within Teloschistaceae. These species occur in exposed habitats. In extreme desert environments, species of *Caloplaca* may be the only saxicolous lichens present, while *Xanthoria* and *Caloplaca* dominate highly exposed coastal areas. Pigmented xanthones in the outer cortex of many crustose lichens may also play a role in regulating the light intensity reaching the algal layer. These xanthones which are yellow or brown in colour, occur in many species of *Lecanora*, *Pertusaria*, *Melanaria*, *Lecidea* and *Buellia* in habitats with high light intensities. Unlike fungal xanthones, lichen xanthones contain one or more chlorinated substituents, suggesting that the availability of chloride in the environment may affect the production of these compounds[5].

Chemical withering:

Lichen substances play an important role in the withering of rock due to their ability to complex metal ions[6]. This process leads to soil formation. Low but significant amounts of these substances dissolve in water. Solubility is determined by the nature and the number of polar groups present. The metal complexation is advantageous to many crustose and foliose lichens growing in close proximity to rock substrates. Since lichen substances commonly occur as crystals on the outer surfaces of hyphae in the medulla, it puts them in direct contact with the substrate where biochemical withering could aid attachment.

Biological active compounds:

Lichens have been used in medicine by the ancient Chinese and Egyptian[7,8]. Nowadays, the medicinal use of lichens per se is not prevalent, particularly since the atom bomb tests in the atmosphere and the Chernobyl accident in 1986 have increased the concentrations of radionucliedes (^{137}Cs and ^{40}K) by alarming proportions[9]. According to recent investigations, lichens possess a variety of biological activities:

Antimicrobial activity:

Although lichen substances are used as antibiotics, their activity is found to be low compared to those isolated from microorganisms. Furthermore, lichen antibiotics are not very water soluble for therapeutic use. Depsides, depsidones and usnic acid are active against Gram-positive microorganisms[10]. Sodium salt of usnic acid was used as drug a in Russia[11].

Antiseptic creams such as "Usno" and "Evosin" contain usnic acid. Usnic acid acts by uncoupling phosphorylation and is effective because animal cells are far less permeable to usnic acid than the microorganisms. Lichen substances such as usnic acid, atranorin, orsellinic acid and erythrin have shown antifungal activity against *Cladosporium cladosporioides* and several other fungi that are econimocally important in postharvest diseases[12].

Antitumour and antimutagenic acivity:

(-)-Usnic acid[13], protolichesterinic and nephrosteranic acids[14], physodalic acid[15], lichen glucans[16], and lichenin derivative[17] have shown antitumour and antimutagenic activity. Lichen polysaccharides, the class of compounds to wich the latter two belong are particularly interesting due to their antitumour activity. The mechanism of action of these lichen polysaccharides are not completely known, but appears to be host mediated: such compounds are believed to generate lymphoid cells, plama cells, macrophages in the vicinity of the grafted tumour[18].

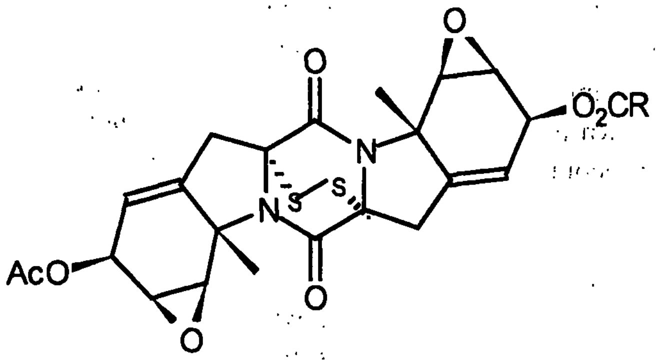
In Sri Lanka, lichens had remained completely unexplored (both chemically and taxonomically) until 1996, when we initiated a systematic study to harness their biological and medicinal value at the Department of Chemistry, University of Peradeniya[19].

It was found that extracts of the lichen *Usnea* sp., collected in Ambewela, Sri Lanka, exhibited potent in vitro cytotoxicity. Bioassay guided fractionation of the *Usnea* sp. extracts led to the isolation of ambewalamides A(1) and B(2), whose structures have been elucidated via a combination of single crystal X-ray diffraction and spectroscopic analyses[20,21].

Ambewela has an elevation of 6000 ft. above sea level and is located 14 miles from Nuwara Eliya on Pattipola road. The lichen *Usnea* sp. is a fruticose lichen growing on the surface of rotting *Acacia decurrens* trees. *A. decurrens* is an introduced plant to that region. Recently, we have located the same lichen growing on dead *Pinus spathula* trunks, also from Ambewela. The thallus of the lichen is grey to pale green and erect or pendulous.

The dried lichen was exhaustively extracted with CH_2Cl_2 to give a crude gum that was fractionated via silica gel MPLC, silica gel preparative TLC, and Sephadex LH-20 chromatography to give a crystalline fraction that contained a mixture of ambewalamides A (1) and B (2). Final purification was accomplished using normal phase HPLC (Waters Radial PAK cartridge; eluent: CH_2Cl_2/EtOAc (20:1)) to give 13.4 mg of ambewalamide A (1) and 1.4 mg of ambewalamide B (2), both as optically active opaque crystalline plates.

Ambewalamides A(1) and B(2) are new members of a family of highly modified diketopiperazines that include fungal metabolites such as aranotin[22], apoaranotin[23], emethallicins[24], exserohilone[25], and SCH64847[26]. There are reports of antiallergy[27,28], antiviral[20], EGF receptor binding[25], phytotoxic[23] for members of the family of diketopiperazines, but the ambewelamides are the first to show potent cytotoxicity. Ambewelamides are the only members of the family have ring A/E epoxides and a diketopiperazine epidisulphide bridge, suggesting that these funtionalities might be necessary for cytotoxicity. The amebewelamides are the first examples of this family isolated from a lichen.



Ambewelamide **A 1** R = -$(CH_2)_2CH_3$

Ambewelamide **B 2** R = -$(CH_2)_4CH_3$

Allergenic acivity:

Contact dermatitis among forestry and horiticultural workers has been attributed to the exposure of lichen substances. Among the many lichen substances responsible for such allergenic action are usnic acid, evernic acid, fumaroprotocetraric acid, stictic acid and atranorin. Atranorin and stictic acid are capable of photosensiting human skin as well as being contact allergens[27]. Atranorin and other β-orcinol type depsides absorb UV radiation and this may account for their phototoxicity.

Dyes:

*Lichen dyes were mainly used for commercial production of brown- and purple-*coloured woolen yarn. However, lichens as a source of dyes was abandoned gradually in favour of synthetic dyes. Using lichens for commercial production of dyes, causes further decline of rare lichens which have been threatened by rising air pollution in rural areas and by destruction of their natural habitat by felling of deciduous woodland. The dye is due to the reaction between free amino acid groups in wool or silk with the aldehyde group of the lichen forming a stable azomethine link. Thus, there is no correlation between the colour of a lichen and the colour which it yields on boiling with wool. Such dyes are colourfast, unfading and give the fabric a pleasant aroma and are mothproof due to the lichens acids present in the dye[28].

Perfumes:

Evernia prunastri (oakmoss) and *Pseudoevernia furcuracea* (treemoss) are two common lichens used in perfumery. Lichens have the ability to act as both fixatives and perfumes[7,27]. They provide the "bass note" of a perfume and the "top notes" are the main flower ingredients and other ingredients which combine to make up the whole fragrance. The use of lichens in perfumery is the largest commercial exploitation of these cryptogams. Petroleum ether is commonly used in the extraction of lichens in pefumery. The extract thus obtained is further purified to remove the waxes and other impurities by heating with warm alcohol and cooling to about 20° C. Between 1-5 % of the lichen extract is added to perfumes and other products[29].

Food:

Although lichens are not used as a major food item in Man's diet, they are added to enhance the flavour of food. Shops and street sellers of Poona and Aurangabad often carry "dagaful" (stone flowers). This is an admixture of *P. tinctorum*, *P. nilgherrense*, *P. reticulata*, and *P. sancti-algelia*. *Ramalina* and *Usnea* may sometimes be included. Kabul Garam Masala is a prepacked version, in which one of the major ingredients is dagaful. The lichens in this Garam Masala may add flavour and antibiotic activity, thereby enhancing the keeping qualities of cooked food[30]. The interior people of Western North America formerly used *Bryoria fremntii* as an important component of their diet[31]. In the semiarid lands of the Iranian-Turanian steppes , highlands of North Africa and deserts of West Central Asia, *Lecanora esculenta* and related species, when old, gets detached and washed into depressions during rains. This sudden appearance of lichens is called "manna lichen". This large collections lichens have been used as a source of food by man or for his animals, usually sheep. *Umbilicaria esculenta* is eaten as a delicacy in Japan [28,32].

Anti-herbivore compounds:

Both vertebrate and invertebrate herbivores consume considerable quantities of lichens. The reasons for the low observed levels of herbivory may be due to low nutrient content of lichens, the difficulties in metabolising lichens, and the patchiness of lichens as a resource. Lichens comprise a major source of winter food supply of caribou and reindeer in boreal areas in the Northern Hemisphere. Although lichens are a poor source of proteins (2-3%), they are an easily accessible carbohydrate source when higher plant foliage is not available[33]. In addition, herbovory on lichens by insects, mites, molluscs are important [34,35,36]. Despite the lichens being a potential food source for herbivores, predation on lichens is uneven. In temperate evergreen forests, there is little evidence of vertebrate or invertebrate herbivory. The resistance of individual lichens to herbivory may be due to a variety of factors. Lichens with gelatinous sheaths covering their surface are relatively immune to predation[37] as seen in some species of Collemataceae. Thick cortical structure with heavily agglutinated hyphae may also provide some protection from small invertebrate herbivores. Inorganic chelate agents present in many saxicolous crustose lichens are toxic to numerous insects[38]. Four groups of lichen substances have possible defensive functions in lichens: pulvinic acid derivatives, terpenes, depsides and depsidones and anthraquinones.

Several studies support the primary defensive role of pulvinic acid derivatives. Lichens containing these compounds have relatively high nitrogen content thus making them potentially valuable for predation. Yet, predation in these taxa are virtually unknown. Vulpinic acid, the most widespread of these compounds is toxic to both vertebrates and invertebrates[39]. The cortical presence of most of the pulvinic acid derivatives is unusual since they show no variation along light gradients. But this histological position is consistent with their defensive role against predators. Since these compounds are bright yellow in colour, their cortical position indicates an example of warning coloration. The medullary position pulvinic acid derivatives in the Stictaceae and two species of *Parmelia* suggest a defensive role against small, nonvisually-oriented invertebrate herbivores[4].

In vascular plants, several studies have shown that sesqui- and diterpenoids may act as defense substances restricting predation[40]. The nutrient rich species of *Pseudocyphellaria*, *Sticta* and *Nephroma* show a surprising lack of predation indicating that terpenes may play an important role in protecting these species with a high nitrogen content[4].

Fumaroprotocetraric acid is a bitter depside occuring in many lichens which appear to deter predation. For example, neither *Caldonia arbuscula* nor *C. rangiferina* (both contain fumaroprotocetraric acid) are not eaten by reindeer, while they are attracted to *C. alpestris* which has no fumaroprotocetraric acid[41]. Concentrations of fumaroprotocetraric acid vary greatly between younger and older thallus parts in *Cladonia*, with the greatest concentration occuring in metabolically active growing tips[42]. Species containing depside and depsidone bitter principles such as diffractic acid, physodic and physodalic acids and salazinic acids are avoided by Caribou in Canada[43].

Parietin, an anthroquinone lichen substance appears to have a defensive role against herbivore[44]. Lichen species of Teloschistaceae contain relative high contents of parietin[45].

Chemotaxonomy of lichens

The application of chemical discriminators to lichen taxonomy is widely accepted. The secondary metabolites in about 5000 species (33 % of all known species) have been studied and the data are used in the routine identification of lichens than any other group of organisms.

Cortical chemistry:

It has now been recognised by many lichenologists that some cortical substances are correlated with higher taxonomic ranks. For example, vulpinic acid in *Letharia* (at the genus level) or anthraquinones such as parietin in Theloschistaceae (at the family level). There is clear evidence indicating their biological role (see above).

Medullary chemistry:

Variations in medullary constituents are used mainly as discriminators at the species level. Most morphologically defined species have a constant chemistry, regardless of their geographical location, substrate or ecology. Within a complex of morphologically similar species, three common patterns of chemical variation can be discerned:

(a) Replacement compounds
(b) Chemosyndromic variation
(c) Accessory type compounds

With replacement compounds, cogeneric chemotypes show simple replacement of one substance by another. Morphologically these lichen populations are sometimes indistinguishable, but possess well-defined and constant variations in chemical compositions. For example, *Psuedovernia furfaceae* has three chemical races. One race contains olivetoric acid and is found in Northern Europe, a second is found in Southern Europe and Northern Africa and contains physodic acid and third containing lecanoric acid is found in North America. Biogenetically the first two compounds are closely related because one can be derived from the other by one single step. But lecanoric acid is not biosynthetically related to these two compounds. It is therefore now recognised that when biogenetic demarcations complement biogeographic variations, such taxa should be recognised as separate species. Thus, the North American taxon is named as *P. consocians*[2]. However, it has been suggested that the best way to tell whether chemical variation is under genetic control or not is that chemical races, where sympatric, maintain their chemical integrity even when growing side by side[46].

Chemosyndrome refers to a group of biosynthetically related lichen substances and the major metabolite is accompanied by several minor constituents which are biosynthetically related[47]. The compounds which are major constituents in one species may occur as minor constituents in related taxa and vice versa. The ecological and biological features of major chemotypes can be better understood as sibling species rather than as components of traditional morphological species[48], where reproductive isolation of a species often leads to ecological differentiation. This is a common result of evolution in animals and vascular plants.

The occurrence of accessory compounds may tend to complicate the above arguments. These compounds are found at certain times in species, usually in addition to normal constituents, and have relation to the morphological or distributional variation. Thus these compounds are not accorded a chemotaxonomic significance. Such compounds often occur as accessory compounds in more than one species and often vary in quantity.

The following spot tests and microchemical methods will be used to garner taxonomical details of lichen specimens.

(a) Chemical (colour) tests conducted on lichen thalli

The common spot test reagents not only indicate where particular compounds are located in sectioned thalii, but may also indicate the chemical nature of the substance.

Reagents for thalline spot tests:

K = 10% Aqueous KOH solution

a. Turns yellow then red with most *o*-hydroxy aromatic aldehydes
b. Turns bright red to deep purple with anthraquinone pigments

C = Saturated aqueous $Ca(OCl)_2$ or common bleach NaOCl solution

a. Turns red with *m*-dihydroxy phenols, except for those substituted between the hydroxy groups with a –CHO or $-CO_2H$.
b. Turns green with dihydroxy dibenzofurans.

KC = 10% Aqueous KOH solution followed by saturated aqueous $Ca(OCl)_2$ or common bleach (NaOcl) solution

a. Turns yellow with usnic acid.
b. Turns blue with dihydroxy dibenzofurans.
c. Turns red with C-depsides and depsidones which, undergo rapid hydrolysis to yield a *m*-dihydroxy phenolic moiety.

PD = 5% alcoholic *p*-phenylenediamine solution

a. Turns yellow, orange or red with aromatic aldehydes.

(b) Microchemical methods

Standadised TLC methodology

The using commercial silica gel plates and three solvents systems (designated A, B & C) and two internal controls (atranorin and norstictic acid) are be used[49,50,51]. All R_f values are compared. An acetone extract of the lichen is spotted on the plate and subsequently eluted in each solvent system. For each solvent system, a spot is assigned to a R_f class determined by the position relative to the controls. Data are then sorted to find all compounds with the same R_f classes. Of these possibilities, those with similar spot characteristics (colour, fluorescence etc.,) are compared chromatographically with the unknown. Additional solvent systems will be used for compounds that do not separate well in the initial analysis. Two-dimensional TLC methods will be used to evaluate structurally similar compounds. Before spraying the dried plates will be examined in day light for pigments and for fluorescence or quenching under short and long wavelength ultraviolet (UV) light. Subsequently the plates will be sprayed with 10% sulfuric acid and heated at 110° C on a hot plate for 10 min to develop the spots.

The solvent systems:

Solvent A = Toluene-dioxane-acetic acid (180:45:5)
Solvent B = Hexane-methyl-*tert*-Butyl ether-formic acid (140:72:18)
Solvent C = Toluene-acetic acid (170:30)
Additional solvents:
Solvent E = Cyclohexane-ethyl acetate (75:25)
Solvent G = Toluene-ethyl acetate-formic acid (139:83:8)

High performance liquid chromatography (HPLC):

Aromatic lichen substances are well suited for analysis by HPLC. Samples are dissolved in methanol and dissolved into the appropriate partition column, through which an appropriate solvent or solvent system is passed under high pressure. The substances that separate are detected in a UV detector. The retention time Rt and peak intensity are recorded by a chart recorder. HPLC will also be used to measure either absolute or relative concentrations of lichen compounds. Gradient elution methods are used.

The RI value of an unknown peak is calculated as follows:

$$RI = \frac{R_t \text{ of Peak} - R_t \text{ of bezoic acid}}{R_t \text{ of Solorinic acid}} \times 100$$

RI values are very stable during the lifetime of a column. Substances which are eluted at the same time, often belong to different substance classes and can be distinguished by their UV spectra.

Gas Chromatography and Mass Spectrometry:

Xanthones, anthraquinones, dibenzofurans, terpenes and pulvinic acid that lack thermolabile ester groups can be studied by gas chromatography coupled with mass spectrometry (GCMS). Xanthones in lichens were studied by injecting a lichen extract directly into a mass spectrometer[52]. More recently, the main terpenoid components of the lichens of the family Picxinaceae were studied by GCMS)[53].

3. CONCLUSIONS

Lichen substances possess a variety of structural motifs and most of them do not have counterparts among fungi and higher plants. They exhibit a broad spectrum of biological activities and some of them have become commercially useful. They are extremely useful in lichen taxonomy unlike any other group of organisms.

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REFERENCES

1. Honegger, R., Functional aspects of the lichen symbiosis. - *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 553-578 (1991).
2. Huneck, S. and Yoshimura, I., Identification of Lichen Substances, Springer-Verlag Berlin Heidelberg (1996).

3. Elix, J.A. in *Lichen Biology* (Ed. Nash, T. III), Cambridge University Press, p.154-180 (1996).
4. Rudel, P.W., *Biochemical Systematics and Ecology*, **6**, 157-170 (1978).
5. Culberson, C.F., *Chemical and Botanical Giude to Lichen Products*, Univ. North Carolina Press, Chapel Hill, (1969).
6. Williams, M.E. and Rudolph, E.D., *Mycologia*, **66**, 648 (1974).
7. Brightman, F.H., *Biol. Hum. Affairs*, **26**, 1-5 (1960).
8. Smith A.L., *Lichens*, Cambridge University Press, London (1920).
9. Heinrich, G., Gries, A., Muller, H. and Oswald, K., *Mit Naturwiss Ver Steiermark*, **119**, 5-13 (1989).
10. Ingolfsdottir, K., Bloomfield, S.F. and Hylands P.J., *Antimicrobial Agents Chemother*, **28**, 550-553 (1985).
11. Savicz, V.P., Litvinov, M.A. and Moissejeva, E.M. *Planta Med.* **8**, 192-202 (1960).
12. Bombuwela, K., Subramaniam, V., De Silva, K.S., Karunaratne, V., Adikaram, N.K.B. and Herath, H.M.T.B., Ceylon Journal of Science, (in press).
13. Takai, M., Uehara, Y. and Beisler, J.A., *J. Med. Chem.*, **22**, 1380-1384 (1979).
14. Hirayama, T.,Fujikawa, F., Kasahara, T., Otsuka, M., Nishida, N. and Mizuno, D., *Yakugaku Zasshi*, **100**, 755-759 (1980).
15. Shibamoto, T and Wei, C-I., *Environmental Mutagenesis*, **6**, 757-762 (1984).
16. Hirabayashi, K., Iwata, S., Ito, M., Shigeta, S., Narui, T., Mori, T. and Shibata, S., *Chem. Pharm. Bull.*,**37**, 2410-2344 (1989).
17. Demleitner, S., Krams, J. and Franz, G., *Pharmazie Unserer Zeit*, **20**, 120 (1991).
18. Tokuzen, R., *Cancer Res.*, **31**, 1590 (1971).
19. Williams, D.E., Bombuwela, K., Lobkovsky, E., De Silva, E.D., Karunaratne, V., Allen, T., Clardy, J. and Andersen, R., *Tetrahedron Lett.*, **39**, 9579-9582 (1998).
20. Karunaratne, V., Bombuwela, K. De Silva, E.D. *Proceedings of Award Winning Papers*, University Research Sessions, University of Peradeniya (1998).
21. Neuss, N., Nagarajan, R., Molloy, B.B. and Huckstep, L.L., *Tetrahedron Lett.*, 4467 (1968).
22. Cutler, H.G., Hoogsteen, K., Litrell, R.H. and Arison, B.H., *Agri. Biol. Chem.*, **50**, 5631 (1985).
23. Kawahara, N., Nozawa, K., Nakajima, S., Kawai, K. and Yamazaki, M., *J. Chem. Soc., Chem. Commun.*, 951 (1989).
24. Sugawara, K., Sugawara, F., Stroebel. G.A., Fu, Y. and Clardy, J., *J. Org. Chem.*, **50**, 5631.
25. Hegde, V.R., Dai, P., Patel, M., Das, P.R. and Puar, M.S., *Tetrahedron Lett.*, **38**, 911 (1997).
26. Nagarajan, R., Huckstep, L. L., Lively, D. H., Delong, D.C., Marsh, M. M. and Neus, N., *J. Am. Chem. Soc.*, **90**, 2980 (1986).
27. Benzera, C., Ducombs, G., Sell., Y. and Foussereau, J., *Plant Contact Dermatitis*, Brian C. Decker, Toronto, 50-51 (1985).
28. Richardson, D.H.S., *The Vanishining Lichens*, David & Charles, North Pomfret, Vt., 95-97 (1975).
29. Bergwein, I.K., *Dragoco rep.* **3**,43-48 (1972).
30. Ingolfsdotter, K., Bloomfield, S.F. and Hylands, P.J., *Antimicrob. Agents Chemother.*, **28**, 289-292 (1985).
31. Turner, N.J., *Econ. Bot.*, **31**, 461-470 (1977).

32. Richardson, D.H.S., *Lichen Ecology*, Ed. Seaward, M.R.D., p.121-144, Academic Press, London (1977).
33. Hustich, I., *Acta. Geogr.*, **12**(1), 1 (1951).
34. Peake, J.F. and James, P.W., *Lichenologist*, **3**, 425 (1967).
35. Gerson, U., *Lichenologist*, **5**, 434 (1973).
36. Sowter, F.A., *Lichenologist*, **5**, 176 (1971).
37. Grandjean, F., *Bull. Mus. Nat. Hist. Natur.*, **22**, 224 (1950).
38. Sell, D.K. and Schmidt, C.H., *J. Econ. Entamol.*, **61**, 946 (1968).
39. Broderson, R. and Kjaer, A., *Acta Pharm. Toxic.*, **2**, 109 (1964).
40. Brown, K. and Sanchez, W., *Biochem. System. Ecol.*, **2**, 11 (1974).
41. Des Abbayes, H., *Bull. Soc. Scient. Bretagne*, **16**(2), 1 (1939).
42. Launden, J.R., *Lichenologist*, **5**, 175 (1971).
43. Ahti, T., *Ann. Bot.Soc. "Vanamo"* **30**(4), 1 (1959).
44. Yom-Tov, Y. and Galun, M., *The Veliger*, **14**, 86 (1971).
45. Masse, L., C.R. *Acad. Sci: Ser.* D., **262**, 1721 (1966).
46. Culberson, C.F. and Culberson, W.L., *Systematic Botany*, **1**, 325-39 (1976).
47. Culberson, W.L., *Brittonia*, 19, 333-52 (1967).
48. Culberson, C.F., *Bryologist*, **89**, 123-31 (1986).
49. Culberson, C.F., *Journal of Chromatography*, **72**, 113-25 (1972).
50. Culberson , C.F. and Anman, K., *Herzogia*, **5**, 1-24 (1979).
51. Culberson, C.F., Culberson, W.L. and Johnson, A., *Bryologist*, **84**, 16-29 (1981).
52. Santesson, J., *Arkiv för Chemie*, **30**, 363-77 (1969).
53. Aproot, A., *XIV International Botanical Congress, Abstracts*: 5-04-7, Berlin (1987).