Symbiosis, 16 (1994) 117–165
Balaban, Philadelphia/Rehovot

LIBRARY
JAN 1 1 2010
SAINT MARY'S U
HALIFAX, CANADA
B3H 3C

Review article

Secondary Biochemistry of Lichens

DIANNE FAHSELT
Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada
Tel. 1-519-679-211 ext. 6480, Fax 1-519-661-3935

Received October 21, 1993; Accepted December 25, 1993

Abstract

A review is presented on secondary products of lichens, including distribution according to developmental stage of the thallus and synthesis of these compounds in relation to the two symbiotic partners. Techniques for analyzing secondary substances are considered, as well as biosynthetic pathways and regulatory controls. The adaptiveness of secondary metabolites in a natural thallus and applications to human uses are also discussed.

Keywords: secondary metabolism, acetate-polymalonate pathway, polyketides, UV-B protection, glycans, anti-tumor activity, antimutagens, human immunodeficiency virus (HIV)

Contents

1. Introduction 119
2. Which Symbionts Synthesize Lichen Products? 119
3. Intrathalline Location of Lichen Products 122
4. Development Effects 123
5. Adaptiveness 124
   Weathering 124
   Surface sealants 125

0334-5114/94 /$05.50 ©1994 Balaban

Metabolic regulation 125
Defence and aggression 125
Procuring and excluding radiation energy 126
6. Methods Used to Study Secondary Products 130
General tests 130
Thin-layer chromatography (TLC) 130
Gas-liquid chromatography (GC) 131
High performance liquid chromatography (HPLC) 131
Spectrometry 132
7. Biosynthesis 133
Raw materials for secondary pathways 133
Enzymes of secondary pathways 133
Controls on levels of lichen products 135
Light/pH 137
Temperature 139
Moisture content 140
Intrathalline differences in secondary pathways 140
Methods of studying pathways 141
8. Acetate-polymalonate Synthesis 141
Ester-linked polyphenolics: depsides/usnic acids 141
Ester- and ether-linked polyphenolics: depsidones 143
Phenolic diversity 144
Single chain polyketides 145
9. Mevalonic Acid Derivatives 145
Carotenoids 146
Triterpenoids 146
10. Shikimic Acid Derivatives 146
11. Amino Acid Derivatives 147
12. Pathways in Related Taxa 147
13. Application of Lichen Products to Human Uses 148
Dyes 148
Food 148
Cosmetics 149
Pest control 149
Antibiotics for use in medicine 150
Antimutagenicity 151
Anti-tumor effects 151
Inhibition of HIV 152
Utilization and conservation 152

1. Introduction

Secondary products are unusual, low-molecular weight compounds that are structurally related to primary metabolites, but have a more restricted distribution. In microbes they are generally formed under limiting conditions, and are thought of as overflow metabolites. Individual products occur in a limited number of families, genera, species or races, though some are more widely distributed than others. They are not essential for growth, and though they may appear unnecessary, many are highly adaptive (Domain, 1992).

Early in the century, Zopf prepared a monograph on lichen substances and, later, Ashahina and Shibata became leading authorities on lichen chemistry. Huneck (1968) authored a major review of lichen substances, while C.F. Culberson and co-workers (Culberson, 1969, 1970; Culberson et al., 1977a) produced a comprehensive three-volume series systematically covering both chemical products and individual lichen species with their constituents.

At the present time there are more than 600 known secondary products in lichens (Lawrey, 1993), and new ones are continually being described. Even common pigments, such as the dark ones frequently encountered in arctic species, have yet to be identified. Some, such as anthraquinone pigments, are typically found in both lichens and free-living fungi, but most secondary substances produced by lichens generally do not occur in other organisms. A few characteristic hydrophobic polyphenolic lichen substances (Fig. 1) are distributed sporadically in non-lichenized fungi, but fungi more typically produce water-soluble monocyclic phenolics. Secondary products in lichens may comprise a fair proportion of the thallus dry weight, commonly constituting 0.1 to 5% (w/w), though in some cases much more than this.

2. Which Symbionts Synthesize Lichen Products?

Since fungi are often rich in secondary products, it is not surprising that many typical lichen substances are synthesized by the mycobiont. This capability for independent synthesis is illustrated by the production of lichen compounds in mycobionts that are cultured without photosynthetic partners (Table 1). Most products are based on acetate, but some are produced from other starting materials. A few classes of lichen substances, such as naphthoquinones and xanthones, have not been detected in cultures of lichen fungi. However, failure to find them in isolated mycobionts does not rule out the possiblity that they are synthesized by the fungal component in a lichen, because secondary metabolism is extremely dependent on culture environment. Although depsides and depsidones, for example, have been found by a number of workers, they are produced only under specific conditions and are not

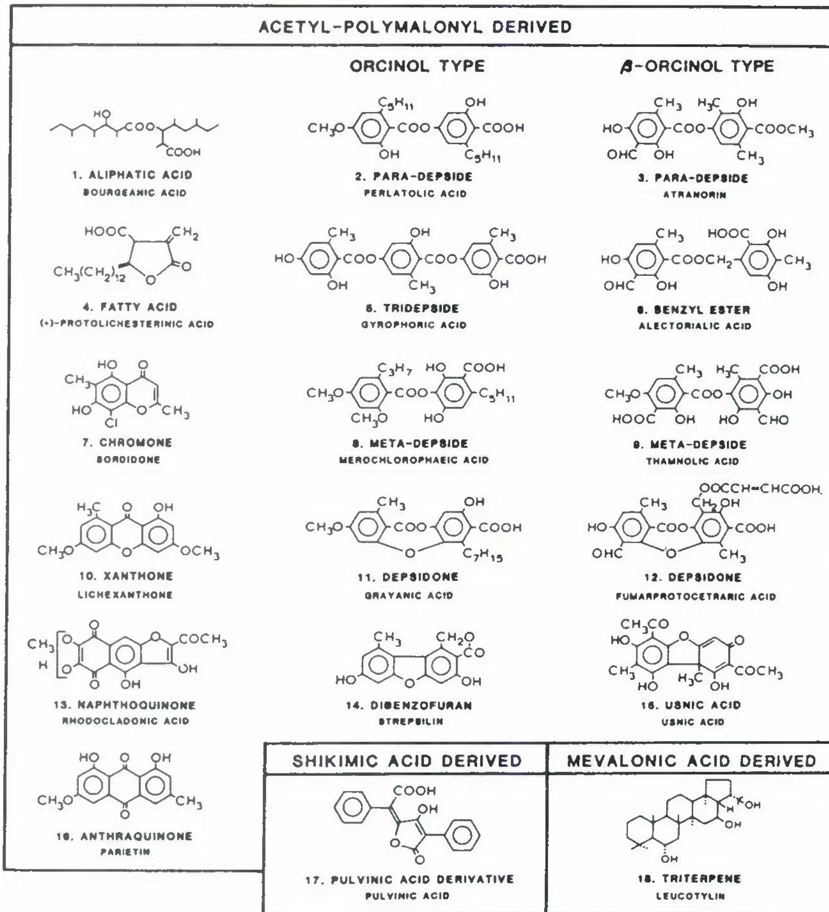

Figure 1. Representative structures of major classes of lichen secondary products (from Culberson, 1986)

detectable routinely (Ahmadjian and Jacobs, 1983; Culberson and Ahmadjian, 1980).

No typical lichen phenolics were found in algae isolated from six species of lichens (Harmala et al., 1992). Phenolics would be lacking if the photobiont were able to synthesize them, but their absence does not prove inability. Culberson, Culberson and Johnson (1985a) showed that experimentally-produced combinations of a mycobiont with foreign photobionts generate the same lichen products as the mycobiont in a natural thallus with its usual partner. These results indicate that the photobiont neither establishes the course of secondary metabolism nor deters a mycobiont from producing its normal products. It is of interest however, that products of

Table 1. Lichen products synthesized by cultured mycobionts without participation of the photobiont

	Secondary products	Reference
Depsides	Squamatic acid	Ejiri and Shibata (1975)
	4-O-demethylbarbatic acid	Hamada and Ueno (1987)
	4-O-demethylbarbatic acid	Hamada (1988)
	Lecanoric acid	Hamada and Ueno (1990)
	4-O-methylsphaerophorin	Culberson and Armaleo (1992)
Depsidones	Salazinic acid	Komiya and Shibata (1969)
	Salazinic acid	Yoshimura et al. (1989)
	Protocetraric acid	Yoshimura et al. (1989)
	Pannarin	Leuckert et al. (1990)
	Dechloropannarin	Leuckert et al. (1990)
	Grayanic acid	Culberson and Armaleo (1992)
	4-O-demethylgrayanic acid	Culberson and Armaleo (1992)
Dibenzofurans	Didymic acid	Castle and Kubsch (1949)
	Didymic acid	Ahmadjian and Jacobs (1983)
Usnic acids	D-usnic acid	Castle and Kubsch (1949)
	+-usnic acid	Komiya and Shibata (1969)
	D-usnic acid	Yamamoto, Mitsuguchi and Yamada (1985)
Anthraquinones	Rhodocladondic acid	Thomas (1939); Castle and Kubsch (1949)
	Emodin	Nakano, Komiya and Shibata (1972)
	Erythroglaucin	Nakano, Komiya and Shibata (1972)
	Fragilin	Nakano, Komiya and Shibata (1972)
	Fallacinal	Nakano, Komiya and Shibata (1972)
	Fallacinol	Nakano, Komiya and Shibata (1972)
	Parietin	Nakano, Komiya and Shibata (1972)
	Bellidiflorin	Ejiri, Sankawa and Shibata (1975)
	Graciliformin	Ejiri, Sankawa and Shibata (1975)
	Skyrin	Ejiri, Sankawa and Shibata (1975)
	Several	Renner and Gerstner (1980)
	Parietin, others	Honegger and Kutasi (1990)
Chromones	Eugenetin	Fox and Huneck (1969)
	Rupicolon	Fox and Huneck (1969)
	Eugentiol	Fox and Huneck (1969)
High aliphatic acids	Rocellic acid	Fox and Huneck (1969)
Pulvinic acid derivatives	Calycin	Mosbach (1967)
	Vulpinic acid	Mosbach (1967)
	Pulvic acid	Mosbach (1967)
	Pulvic dilactone	Mosbach (1967)

cultured mycobionts are often but not always, different from those produced by an intact thallus (Kinoshita et al., 1993; Kon et al., 1993; Yamamoto et al., 1993).

3. Intrathalline Location of Lichen Products

Pigmented secondary constituents, such as usnic acid and anthraquinones (Fig. 1), are considered to be located almost exclusively in the upper cortex, but distribution over the thallus surface is not uniform. In *Ramalina siliquosa*, usnic acid is preferentially associated with, but not restricted to, the apothecial disk and spermagonia (Culberson, Culberson and Johnson, 1993). Unpigmented compounds, such as atranorin and squamatic acid, are also found in the upper cortex. Although atranorin and usnic acid are the most common cortical substances, they usually do not occur together. Depsides and depsidones are typically found in the medulla.

Synthesis of secondary compounds in lichens as in free-living fungi, may be affected by the juxtaposition of hyphae within the thallus. In cultures of *Neurospora crassa*, mycelia are adherent in the upper stratum of a colony and loosely arranged below, and enzyme activities differ between the two regions (Toledo et al., 1986). The cortex and medulla of a stratified lichen may also be different enzymatically, a possibility that seems likely since particular secondary products are associated with each layer. Secondary products may also be restricted to specific lichen structures, with individual compounds localized, for example, in apothecia, podetia, soralia (White and James, 1985), hymenium (Culberson, 1969) or umbilicus (Posner et al., 1990).

Secondary metabolites are deposited in extracellular locations where they appear as surficial crystals (Fahselt et al., 1973; Ahmadjian and Jacobs, 1985; Honegger, 1986). They are easily extracted in acetone or ethanol and, depending on the method of preparation, may be clearly visible in scanning electron micrographs. Crystalline deposits on the surface give lichen hyphae a "rough" appearance (Fig. 2a), unlike that of hyphae in many mycobiont cultures (Fig. 2b). Crystals, or impressions where crystals have been, are evident not only on hyphae but also on photobiont cells, an indication that secondary products are transported within the thallus (Honegger, 1986). There are reports that some phenolics are taken into the photobiont cell wall and that others enter the cytoplasm (Avalos and Vicente, 1987a; Legaz and Vicente, 1989), but uptake by algal cells has not been demonstrated experimentally. However, usnic acid is apparently transported through the plasmalemma of mesophyll cell protoplasts (Vavasseur et al., 1991). An enzyme that reduces usnic acid, D-usnic acid dehydrogenase, has been reported in photobionts

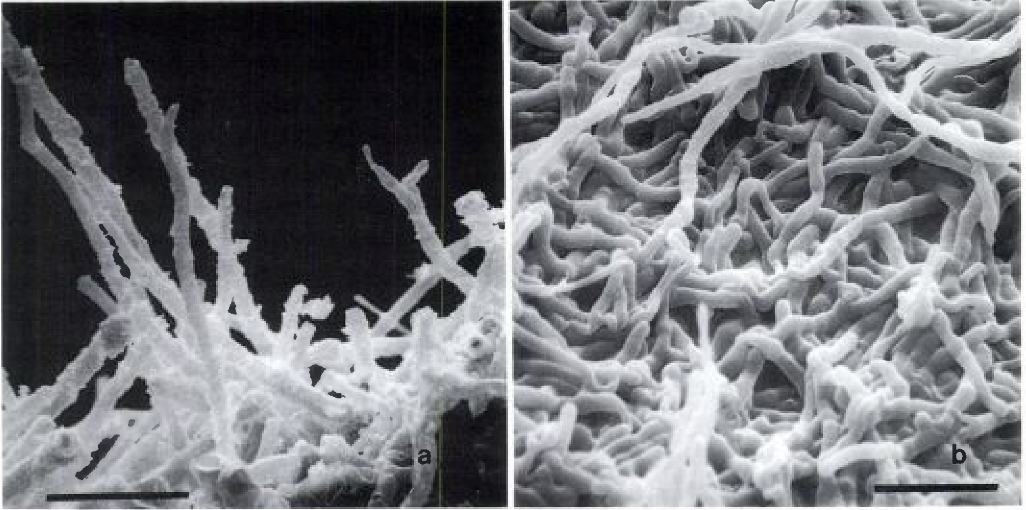

Figure 2. Mycobiont hyphae. (a) medullary hyphae of *Parmotrema hypotropum* with deposits of norstictic acid. Bar = 10 μm. (b) bare hyphae of *Cladonia verticillata* mycobiont grown in pure culture and generating no polyphenols. Bar = 10 μm.

as well as fungal hyphae (Avalos and Vicente, 1987b). Indeed, it has been suggested that usnic acid regulates enzyme activity within the photobiont wall (Legaz and Vicente, 1989).

4. Development Effects

In umbilicate lichens, Culberson and Culberson (1958) found no difference in secondary products between younger, or smaller thalli and older ones. Similarly, Mirando and Fahselt (1978) detected no consistent differences between a marginal band and the center of a thallus, while Seriina, Arroyo and Manrique (1991) found decreasing levels of the depside, lecanoric acid, toward the umbilicus, while a related tridepside, gyrophoric acid, was somewhat erratic, showing no progressive trend. However, in view of the physiological and enzymatic mosaicism which has become apparent within the umbilicate thallus (Larson, 1983; Larson and Carey, 1986) and the suspicion that growth is intercalary rather than marginal, umbilicates are probably not the best lichens in which to examine age-related trends.

With fruticose species, there is a much more straight-forward relationship between the stage of development and thallus form, with apical or terminal regions being the youngest. In most foliose lichens, the distal edge of each

advancing lobe comprises the most recently formed tissue. Many phenolics show gradients of intrathalline distribution with respect to presumed age.

In cortical tissues amounts of usnic acid were found to be highest in apical parts of the thallus of *Cladina* spp. and lowest in older tissues (Mirando and Fahselt, 1978; Fahselt, 1984). Vulpinic acid, another cortical substance, was also in greater concentrations in terminal tissues of *Letharia vulpina*, but atranorin concentrations were higher in the basal regions of the thallus (Stephenson and Rundel, 1979). Atranorin levels were greater in the youngest branches of *Cladina rangiferina* (Mirando and Fahselt, 1978) and *Pseudevernia furfuracea* (Manrique and Lopez, 1991). Overall, the highest concentrations of cortical secondary substances seem to be apical, and these could be adaptive if they provide protection against possible damage during a crucial formative period of the thallus.

The concentration of medullary substances may also show a tip-to-base, or young-to-old trend. For example, levels of the para-depsides, perlatolic acid (Mirando and Fahselt, 1978) and olivetoric acid (Manrique and Lopez, 1991), were higher in young tissues. In one chemical race of *P. furfuracea*, the decline of perlatolic acid with age was accompanied by a concomitant increase in the concentration of physodic acid. Depsidones of *Cladonia cristatella* did not change in concentration, but dibenzofurans increased significantly with developmental stage (Culberson et al., 1983). Mechanisms that might be responsible for age-related gradients are discussed in Section 7.3.

5. Adaptiveness

Many secondary metabolites in lichens are excreted from the cells that make them. Since phenols are toxic, this is probably adaptive, as it precludes autotoxicity. Excretion of secondary products could be a means of correcting metabolic imbalances created by excess photosynthates and insufficient nitrogen. Discharging energy-rich constituents from mainstream metabolism could also reduce resources available to the mycobiont and, thereby, restrict its aggressiveness. While removal of lichen products from the cytoplasm may be beneficial to the symbiosis, extracellular deposits themselves probably contribute significantly to thallus functioning, as discussed below.

Weathering

Phenolic acids have chelating abilities and are capable of solubilizing cations from mineral substrates and increasing their availability (review: Jones, 1988). Stictic acid and physodic acid, for example, are particularly active in

weathering rocks and minerals. However, it has been suggested that wall and membrane proteins (Brown and Beckett, 1985), or organic acids such as oxalic acid that are produced by the mycobiont (Wilson, 1992), are more important as mineral releasing agents.

Surface sealants

Due to the hydrophobicity of many lichen products, copious deposits on the upper cortex would prevent wetting from above, but might aid also in the retention of water acquired otherwise. This could be adaptive in species contending with either extremely wet or very dry conditions. Crystalline depositions of secondary products on individual hyphae may also help to seal the other wall surface and, thus, facilitate intrathalline movement of solutes within the apoplastic space in the cell wall (Honegger, 1991; Armaleo, 1993).

Metabolic regulation

It has been suggested that secondary products may suppress growth of the photobiont in older, less active parts of the thallus (Stephenson and Rundel, 1979; Honegger, 1987), a possibility since some phenolics appear to regulate aspects of primary metabolism (Kinraide and Ahmadjian, 1970). Urea can be derived from pools of free arginine (Vicente, 1985) and degraded by urease into CO_2 and NH_3, both critical during times of resource depletion (Blanco, Suarez and Vicente, 1984). Urease, thus, could provide a source of CO_2 for photosynthesis, as well as NH_3 that could be used in protein synthesis. Atranorin was found to enhance urease function (Perez-Urria and Vicente, 1988), but other phenolics, including usnic acid, block the thiol (-SH) groups, irreversibly aggregating and inactivating the enzyme (Vicente et al., 1978). Because photosynthesis is limited by low levels of CO_2, hydrolysis by the photobiont of phenolics that prevent release of CO_2 from urea has been interpreted as a form of self defense (Mosbach and Ehrensvard, 1966; Avalos and Vicente, 1987b; Herrero-Yudega et al., 1989). Arginase, which acts on arginine, is also affected by lichen phenols (Planelles and Legaz, 1987; Pedrosa and Legaz, 1991).

Defence and aggression

Secondary products may serve to defend the whole thallus against other organisms, and the antibiotic, allelopathic and antiherbivore potential of these substances was reviewed by Lawrey (1984, 1986). The most susceptible microbes are gram-positive bacteria and fungi, and more than half of all lichen

species have products capable of antibiosis. The active substances are usually usnic acids, pulvinic acid derivatives, aliphatic acids and orcinol-based depsides and depsidones (Fig. 1), although some substances are not yet identified.

Secondary metabolites of lichens may have inhibitory effects on spore and seed germination, as well as on the growth of sporelings, protonema and seedlings. Such allelopathic interactions have been documented between lichen species, as well as between lichens and bryophytes or higher plants. Effects on trees, both native and cultivated species, may be at least partly mediated through effects on ectotrophic mycorrhizal fungi (Goldner et al., 1986). Lichen secondary substances strongly inhibit photosynthesis in plant protoplasts and isolated chloroplasts and, applied to the roots of higher plants, limit transpiration also (Vavasseur et al., 1991). However, there is no experimental confirmation that lichen products disadvantage species that compete with lichens in nature, nor has it been demonstrated that chemically well-defended lichen species are more successful than poorly-defended ones (Lawrey, 1991).

Lichen products also seem to play an antiherbivore role. Avoidance of lichens by the gypsy moth (*Lymantria dispar*), which is presently defoliating forests in Eastern North America, may be related to the presence of undetermined water-soluble secondary lichen substances (Blewitt and Cooper-Driver, 1990). *Parmelia pulla* has an unidentified toxin, which can be precipitated with ammonium sulfate, that hemolyzes the erythrocytes of various vertebrate species (Hunaita et al., 1988). There are several known lichen products that are associated with grazer avoidance, including stictic acid and protocetraric acid (Froberg et al., 1993; Hale, 1972), parietin (Yom-Tov and Galun, 1971) and vulpinic acid (Stephenson and Rundel, 1979), and these discourage herbivory by snails, slugs, woodlice and earwigs. Lichen compounds seem to provide a generalized defence since, for example, the same lichen extracts that discourage feeding by a slug, *Pallifera varia*, are also effective against gram-positive bacteria (Lawrey, 1989).

Fruticose lichens, especially *Cladina* species, are consumed by larger animals, such as reindeer, and are utilized mainly in winter when other types of forage are unavailable. Nutritionally, lichens have less to offer than higher plants, in that they are particularly poor in proteins and minerals (e.g., Nieminen and Heiskari, 1989). The preferences of vertebrate grazers in relation to secondary products have only been established circumstantially.

Procuring and excluding radiation energy

Since a large proportion of lichen species in the High Arctic are darkly pigmented, absorbance in the visible region of the spectrum may be an

adaptation that raises thallus temperature (Thomson, 1984). Another role that has been suggested for lichen substances is that they increase light energy available for photosynthesis (Rao and LeBlanc, 1965). Atranorin absorbs in the ultraviolet and re-emits at 425 nm (in the blue range), which is an absorbance maximum of chlorophyll. However, since atranorin does not accumulate under increased illumination (Klee and Steubing, 1977) and shade-grown lichens have no more atranorin than those in the sun (Rundel, 1979), the light-gathering propensities of atranorin may be of limited importance.

In some species, there is a direct relationship in natural habitats between the thallus concentration of usnic acid and the intensity of available light (Rundel, 1979), or between parietin levels and light (Hill and Woolhouse, 1966). It has been considered that these substances are formed in response to high levels of visible light and protect the photobiont from damage. Under culture conditions, however, usnic acid is produced by synthetic lichens under even low light intensities (Ahmadjian and Jacobs, 1985). The amount of usnic acid in cultured mycobionts is not altered by increasing the intensity of light (Hamada, 1991). Experiments on nutrient media thus suggest that usnic acid is not formed in response to light, but results are consistent with the possibility that synthesis of this substance is dependent upon available carbohydrate. Under controlled conditions it can be shown that, up to a point, usnic acid levels in *Ramalina siliquosa* are increased in response to temperature (Hamada, 1991).

There is a possibility that usnic acid production is induced by ultraviolet light, rather than by visible light or perhaps is constitutive. Also, since considerable interthalline differences in levels of usnic acid occur within one environmentally homogeneous population (Fahselt, 1984), high-usnic thalli could simply be favored in more highly illuminated exposures.

Protection provided by usnic acid against high levels of visible light must be minimal, however, compared to that against UV. Although it is a pigment, the major absorbance peaks of usnic acid are in the ultraviolet (Fig. 3a), rather than the visible range (Huneck, 1968). Only slight absorbancy is evident in the blue region, and even with a highly concentrated solution, there are no absorbance maxima within the visible range. The spectrum of usnic acid, thus, bears little resemblance to that of pigments such as carotenoids, that actually protect from excess light in the visible.

Biological damage is more likely to occur as a result of radiation with wavelengths shorter than visible light, particularly those that interact with DNA and alter the bonding of pyrimidine bases. Most lichen products are colorless and, like usnic acid, absorb strongly in the ultraviolet and barely, if at all, in the visible (Hale, 1956; Rao et al., 1967; Huneck, 1968). Typical absorbance of a whole thallus ethanolic extract is shown in Fig. 3b. Absorbance

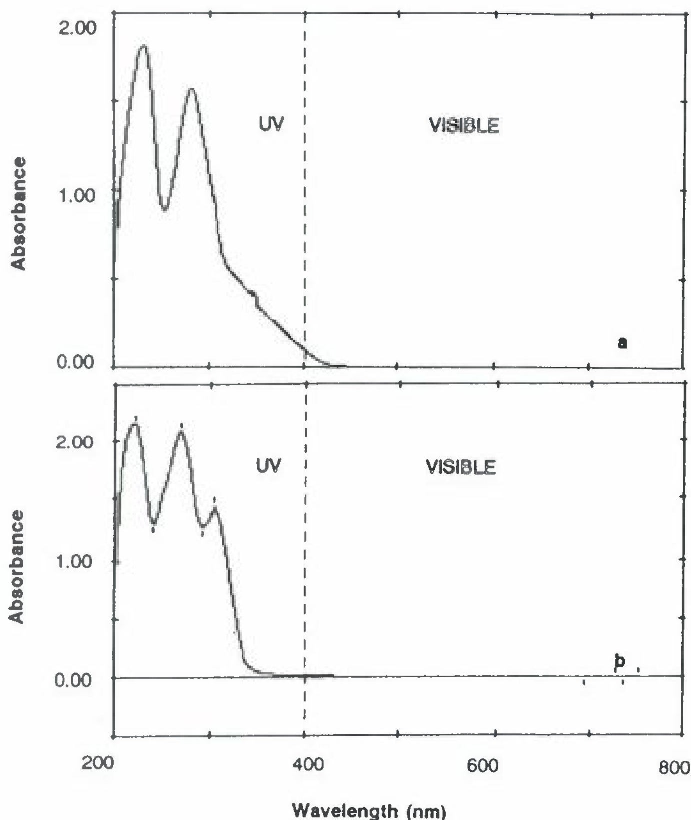

Figure 3. Absorbance spectra (200–800 nm) in 95% ethanol. (a) usnic acid, maxima 232 and 280 nm. (b) extract from whole thallus of *Umbilicaria deustra*, maxima 220, 269 and 305 nm, containing gyrophoric acid, lecanoric acid and umbilicaric acid.

properties of individual cortical substances and some representative medullary constitutents are given in Table 2; many absorb in both UV-C and UV-B, and the spectra of substances that are chemically related to one another are similar. The peak in the UV-B appears to be generally at a somewhat lower wavelength for cortical substances than for medullary compounds. Xanthone and anthraquinone pigments also absorb strongly in the ultraviolet.

Although the proportion of usnic acid to protocetraric acid was shown to be highest in reproductive structures of *Ramalina siliquosa*, the highest total amounts of secondary products (μg/mg tissue) were in the medulla (Culberson et al., 1993). Medullary polyphenolics may protect vegetative hyphae against irradiation as well as perform other functions. Free-living ascomycetes could have alternate means of protection, but many are ephemeral and may not require the same level of defence for the relatively short periods when they are exposed to UV-B.

Table 2. Absorbance maxima of lichen products in the ultraviolet region of the spectrum

Lichen substance	Wavelength (nm)			Solvent	Reference
	200–280 UV-C	280–320 UV-B	320–400 UV-A		
Cortical					
Usnic acid	232	282		methanol	Huneck, 1968
Atranorin	239	285		ethanol	Culberson, 1965
Parietin	220, 254, 264	286	374	methanol	Huneck, 1968
Medullary					
Lecanoric	270	307		95% ethanol	Hale, 1956
Evernic acid	267	300		95% ethanol	Rao et al., 1967*
Physodic acid	256	310–300 (sh)		95% ethanol	Hale, 1956
Barbatic acid	275	308		95% ethanol	Rao et al., 1967*
Salazinic acid	239	312		95% ethanol	Hale, 1956
Norstictic acid	239	320		95% ethanol	Rao et al., 1967*

* Rao, Sarma and Seshadri (1967)

While the ozone layer screens out UV-C (200–280 nm), some UV-B (280–320 nm) penetrates the atmosphere and is also capable of genetic damage. Though ultraviolet radiation was more intense hundreds of millions of years ago, it may still be a problem, especially for slow-growing, long-lived organisms such as lichens. Preliminary studies indicate that stands of lichens capable of less UV-absorbance by secondary products tend to have more highly polymorphic enzymes (Fahselt, 1993). This is what would be expected if lichen phenolics provide a degree of protection against UV-induced changes. That higher plants have photo-receptors, that respond to UV as well as visible light (Stafford, 1990) is an indication of the biological importance of shorter wavelengths.

6. Methods Used to Study Secondary Products

A practical guide to a variety of microchemical tests that aid in the identification of lichen substances has been prepared by White and James (1985). A recent review of separation and identification procedures that are currently used in lichen chemistry is found in Culberson and Elix (1989).

General tests

There are a number of tests that permit general characterization, but not identifications, of individual compounds. Crystal tests, developed by Asahina, involve the application of glycerin-containing solutions to solid residues obtained by extracting with acetone. A key which can be used to evaluate crystals is given in Hale (1979). Fluorescence is an attribute of some lichen products which permits the location of substances to be determined within the intact thallus. Inexpensive equipment is available for field use.

Color tests involve placing drops of chemical solution on cut thallus surfaces. Reagents react with lichen substances to produce colors that are useful when using some taxonomic keys or making identifications in the field. These tests cannot be used to identify specific compounds and have been largely surpassed by techniques that are more informative.

Thin-layer chromatography (TLC)

Lichen products are routinely analyzed by thin-layer chromatography (TLC), a method pioneered by C.F. Culberson and co-workers (Culberson and Kristinsson, 1970; Culberson, 1972a). Unknowns are characterized on the basis of their performance in three solvent systems in comparison to the performance of standards. Absorbance properties and reactions to acidic spray are other

criteria used to evaluate and identify unknown substances. This method has undergone a few modifications to improve safety of solvents or accommodate to changes in commercially available thin-layer plates and been translated into German (Culberson and Ammann, 1979). Compound characterizations are included in the computer software, Mactabolites (Elix, Johnston and Parker, 1988). Identification of secondary metabolites is often possible by TLC and the method is employed widely along with other technologies for studying lichen products.

High performance TLC (HPTLC) (Arup et al., 1993) has recently been described. This requires even less lichen material than standard TLC and permits detection of many minor compounds. It is somewhat faster and uses much smaller volumes of solvent. At present, however, published information on the performance of lichen products in HPTLC is available for about half as many compounds as for the Culberson TLC system. Quantification can be accomplished through densitometric scanning of thin-layer plates, using either TLC or HPTLC (Fahselt, 1981; Polonia et al., 1991; Arup et al., 1993). Sophisticated integrating scanners, e.g., LKB Ultroscan, are available.

Gas-liquid chromatography (GC)

Gas-liquid chromatography was explored by Culberson (1972b) as a rapid technique for the separation of typical lichen products from minimal amounts of lichen material. However, lichen depsides have low volatility and they are degraded by the high temperatures that are necessary to convert them to the gaseous form. Depsides and many other secondary compounds can only be detected, therefore, after they are made more volatile, and this is done by converting them to trimethylsilyl derivatives. Usnic acid is less labile and does not require derivatization (Fahselt, 1975).

High performance liquid chromatography (HPLC)

Although instrumentation is costly, HPLC permits the isolation of pure substances, separation of compounds that are different to resolve using TLC and simultaneous quantification (Schultz and Albroscheit, 1989). Derivatization is not necessary. Initially, investigations involving gas-liquid chromatography of lichen substances used non-polar solvents for the mobile phase (Culberson, 1972b), but polar mobile phases (reversed-phase HPLC) provide much improved resolution (Culberson and Culberson, 1978) and are safer. High performance liquid chromatography is being used extensively when quantification is important (e.g., Culberson et al., 1983, 1985b; Hamada, 1984, 1988b; Fahselt, 1984; Huovinen et al., 1985; Huovinen et al., 1989,

1990; Manrique and Lopez, 1991). Lichen substances are characterized on the basis of retention times, but since two or more compounds by chance may be retained on the column to the same degree, separations should be performed under a variety of conditions. This can be done through use of two or three different reversed-phase columns (Huovinen, 1987) or by supplementing HPLC, for example, with standard TLC. Impurities can be discovered by monitoring absorbance at two different wavelengths. When impurities are present, the ratio between absorbancies at the two wavelengths probably will vary from that of a pure compound (Huovinen, Hiltunen and von Schantz, 1985).

Spectrometry

Since related structures have very similar absorbances (Huneck, 1968), ultraviolet spectra are only capable of characterizing classes of lichen substances, rather than individual compounds. Therefore, UV spectrometry is not used for determination of unknown products, but only to assess the type of molecule. Infrared spectrometry and nuclear magnetic resonance spectrometry are important for the determination of unknown substances, and are employed to decipher the structure of many new lichen products. These techniques are used by some lichenologists (e.g., Blanco et al., 1984) but are not options for most.

Mass spectrometry, which determines the mass of ions derived from any molecule, as well as the mass of ions from its larger fragments, has more frequently been employed for confirming the identity of lichen substances (Santesson, 1969). It provides precise information about structure but it, too, is costly in terms of time and equipment. Mass spectrometry of substances sublimated from a tiny fragment of lichen thallus, using a direct inlet system, is referred to as lichen mass spectroscopy (LMS). If there are only a few major secondary products in a thallus, they can be analyzed successfully by directly heating the lichen fragment under greatly reduced pressure (Leuckert et al., 1990). For complex mixtures, tandem mass spectroscopy (MS-MS) permits the identification of compounds that can not be seen with ordinary mass spectroscopy (Tabacchi et al., 1991). In the case of anthraquinones, which are not degraded by high temperatures (Santesson, 1970), gas chromatography can be used in sequence with mass spectrometry (GS-MS) (Fahselt, 1993).

Unequivocal identification involves the use of complimentary techniques, such as TLC with HPLC or LMS, an approach used by Leuckert et al. (1990) for determining the substances generated by cultured mycobionts, by Culterson et al. (1985b) for the *Cladonia chlorophaea* group of lichens and by Posner,

Feige and Leuckert (1991) for *Lasallia*. Proton and ^{13}C NMR spectroscopy can also aid in identification (e.g., Wilkins et al., 1989).

7. Biosynthesis

Raw materials for secondary pathways

Primary metabolites serve as precursors to secondary products, but relatively few intermediates function in such a dual capacity. In lichens, the main point of departure to secondary metabolism is acetyl-CoA (Fig. 4), which in some cases, is processed through the mevalonate pathway and converted to terpenoids or sterols. However, acetyl-CoA is usually directed into polyketides, which include a great variety of products, such as the widely distributed usnic acid and numerous depsides and depsidones that are characteristic of lichens. The term, polyketides, is used to refer only to secondary compounds and not fatty acids, although all are made up of sequentially linked C-2 units derived from acetyl-CoA. Polyketides are found in higher plants, but occur mainly in lichens and fungi (Packter, 1980).

Secondary products may also be synthesized from intermediates of both the Embden-Meyerhof and pentose phosphate pathways, in this case generating end products such as pulvinic acid and related compounds. Some recently discovered products probably require amino acid presursors (details below).

Enzymes of secondary pathways

The major enzymes involved in secondary metabolism are probably derived from enzymes of primary pathways, possibly through duplication of genes that code for primary enzymes, followed by independent evolution of the copies (Luckner, 1984). The enzyme system required for polyketide synthesis in fungi functions very much like that which synthesizes fatty acids (Hardie and McCarthy, 1986). The metabolic sequence culminating in polyketides is a little simpler, in that it involves fewer reduction steps and thus generates a first product that retains oxygen functions on alternate carbons. However, in both syntheses, a series of parallel steps achieve step-wise chain extensions of two-carbons each.

An interesting possibility is that secondary enzymes are not just derived from those operating in primary pathways but, in some unknown way (viral transfer?), from enzymes of primary metabolism in other organisms (Vining, 1992). The evidence is molecular. For example, the free-living fungus, *Penicillium urticaea*, produces a polyketide mycotoxin called patulin, the synthesis of which requires 6-methylsalicylic acid as an intermediate. A specific

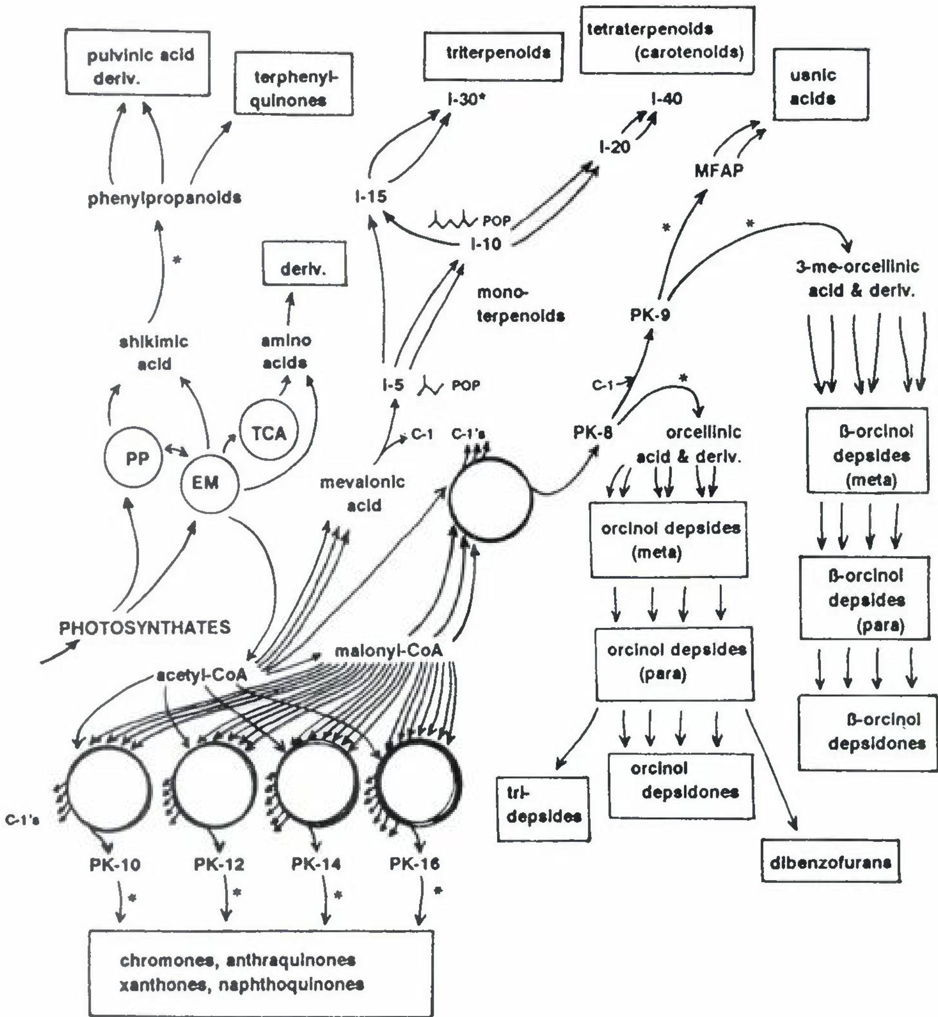

Figure 4. Major secondary pathways in a mycobiont. C-1 = one carbon unit; I = isoprenoid intermediate, with figure after hyphen indicating number of carbons in molecule; PK = straight chain polyketide, with figure after hyphen indicating number of carbons; deriv. = derivatives; PP = pentose phosphate pathway; EM = Embden-Meyerhoff pathway; MFAP = methylphloroacetophenone; * = cyclization.

polyketide synthetase (PKS) produces this intermediate, and a partial base sequence was determined in the DNA coding for it (Wang et al., 1991). Only about 13% similarity was found between the sequence coding for polyketide synthetase (PKS) in *P. urticaea* and the sequence for fatty acid synthetase (FAS) in the same species, and a low similarity was also found between the genes for *P. urticaea* PKS and the FAS of yeast. However, similarity between comparable coding regions for *Penicillium* PKS and vertebrate FAS was much higher (67%). Both fungus PKS and vertebrate FAS are multifunctional enzymes and the order of the coding regions is also the same. Such striking similarities probably could not occur by chance, and may be accounted for by past transfer of genes between species (Vining, 1992).

Further evidence cited to support the idea of intraspecific gene transfer is found in Luckner (1984), but the possibility that genes might be introduced from one lineage to another certainly represents a departure from most current thinking about biological evolution. Perhaps such transfers occur relatively infrequently. This is of particular interest because of the importance of polyketides in lichens. Culberson et al. (1990) previously suggested an evolutionary connection between the fatty acid synthesis and secondary metabolism in lichens.

While enzymes synthesizing polyketide chains probably arose from those of primary metabolism, enzymes needed for subsequent reactions in the formation of lichen products perform other types of conversions, such as oxidation, decarboxylation and methylation. Therefore, although the actual nature of these enzymes is not known (Garcia-Junceda and Vicente, 1986), they must be different from PKS and FAS. However, enzymes of these later synthetic steps may have been derived from other primary pathway enzymes.

Controls on levels of lichen products

Just as secondary metabolism in microbes may be stimulated by excess carbon, so a massive influx of assimilates from the photobiont could promote secondary syntheses in lichens. However, there is little experimental evidence, and not all is consistent with this possibility. For example, high nutrient media cause mycobionts of *Xanthoria* to grow more rapidly than in a nutrient-free medium (Honegger and Kutasi, 1990), but the effect on anthraquinone content varies depending on the species. In *X. elegans*, anthraquinone levels are enhanced when the growth rate increases, while in *X. parietina* accumulation of these substances appears to be severely impaired.

The fate of intermediates with the potential for entering either primary or secondary pathways could be determined by the supply of reductive

power. Photosynthesis generates NADPH and Mosbach (1973) suggested that, because there are fewer reductive steps in polyketide synthesis, cofactor availability may determine the balance between lichen polyketides and fatty acids.

There may be a link in lichens, as in microbes, between overflow metabolism and slow growth. For example, in the isolated mycobionts of *Cladonia grayii*, maximal generation of secondary products occurs near the end of a period of rapid hyphal growth (Culberson and Armaleo, 1992). The low growth rates (Hale, 1983) and abundant secondary products of intact lichens are well known, but a causal relationship between the two has not been established.

The levels of any metabolite are affected both by the rate of synthesis and degradation, and the breakdown of secondary metabolites can be enzymatic or otherwise. One of the first-studied lichen enzymes degrades lichen polyphenols. A depside hydrolyzing enzyme, orcellinate depside hydrolase is found in extracts of both whole thallus and *Trebouxia*. It hydrolyzes the ester bond between phenolic units in some closely related depsides (Mosbach and Ehrensvard, 1966; Schultz and Mosbach, 1971). However, if an ether bond is also present between the two phenolic units, as in a depsidone (Fig. 1), the same hydrolase cannot effect degradation. A different enzyme, a depsidone hydrolase, is required to break the ether linkage (Garcia-Junceda et al., 1991). The depside-hydrolyzing enzyme is activated by light in the red part of the visible spectrum (Vicente and Avalos, 1986), but little is known of controls on the depsidone hydrolase. Breakdown products of orcellinate depsides and depsidones may be decarboxylated by an enzyme of the mycobiont, orcellinic acid decarboxylase (Mosbach and Ehrensvard, 1966).

Both symbionts produce dehydrogenases that degrade usnic acid. One enzyme is stimulated under drought and nutrient-poor conditions (Vicente et al., 1980), and the other is inducible either by excess usnic acid (Vicente and Legaz, 1988) or light quality (Avalos and Vicente, 1987b). Usnic acid oxidoreductase, similarly induced by the quality of light, also degrades usnic acid (Avalos and Vicente, 1989).

In bacteria and fungi, individual secondary pathways may be subject to controls such as end product inhibition or end product regression (e.g., Martin and Demain, 1980), whereby an end product impairs the activity of an enzyme required to catalyze an early step in its own synthesis or prevents the synthesis of a necessary enzyme. However, since many secondary constituents of lichens are excreted from the site of production, and possibly even transported elsewhere in the thallus, synthesis may be regulated in a less direct way. A number of molecular species and both bionts could be involved. For example, Richardson (1985a) summarized the "urease hypothesis": lichen acids made

by the mycobiont are transported to the algal cell where they inhibit urease and reduce the amount of CO_2 available for photosynthesis. The amount of ammonia produced from urea is also lowered, reducing algal permeability. As a result, fewer photosynthates are released to the mycobiont and there are fewer precursors for the synthesis of lichen acids.

Phenolics themselves may influence the course of secondary synthesis, for example, evernic acid reportedly inactivates an enzyme responsible for synthesizing usnic acid (Vicente and Perez-Urria, 1988). These authors found that when evernic acid was high in a thallus, levels of the usnic acid were relatively low.

The synthesis of secondary products must also be extremely sensitive to growing conditions, since the same single spore isolate can generate different secondary products in different laboratories, even under similar culture conditions (Yoshimura et al., 1993). Anthraquinone accumulation and colony color in cultured mycobionts are affected by the nature of culture media (Renner and Gerstner, 1980; Honegger and Kutasi, 1990), and the extent to which some xanthones are chlorinated probably depends on the availability of chlorine (Culberson, 1969).

Light/pH

Greater amounts of photoassimilates may yield increased levels of secondary products. Obtusatic acid and 4-O-demethylbarbatic acid may be increased under more intense light (Culberson et al., 1983) when *Cladonia cristatella* is grown under certain temperature conditions. The amount of perlatolic acid in *Cladina stellaris* also is greater with high illumination (Fahselt, 1981). A distinction should be made, however, between phenolic concentrations at any point in time and those products synthesized during the course of an experimental period. This can be done through the use of radioactively-labeled precursors. Lichens fed with ^{14}C-labelled urea incorporate a greater amount of labelled carbon into phenolics in the light than the dark, suggesting that assimilated carbon actually is directed toward polyketide synthesis during the course of an experiment (Blanco et al., 1984).

Not all lichens respond to increased levels of photosynthates by generating more secondary products. Herrero-Yudego et al. (1989) showed that accumulation of usnic acid and evernic acid in *Evernia prunastri* is impeded by photosynthesis. Vicente (1991) suggested that high concentrations of glucose affect DNA and prevent the generation of enzymes required for production of secondary metabolites. (A dye, acridine orange, which destroys the helical configuration of DNA has the same effect on synthesis of phenolics as glucose

does). This form of control, called the glucose effect or catabolite repression, is partially reversed by cyclic AMP, a substance which interacts with DNA to favor transcription in spite of the presence of glucose. Perhaps phenolics in *E. prunastri* are decreased after addition of glucose due to effects on their synthesis. However, in *Pseudevernia furfuracea*, levels of atranorin are reduced by glucose and not restored by cyclic AMP. Therefore, a mechanism other than catabolite repression was suspected. In this case, glucose was interpreted as controlling enzymes that are required for the mobilization of storage products prior to the synthesis of atranorin (Garcia-Junceda et al., 1987). In some situations, the production of secondary products seems to be independent of light (e.g., Culberson et al., 1983; Hamada, 1991), but the wavelengths, as well as the total flux, may be important. In the Antarctic lichen, *Himantormia lugubris*, there is no catabolite repression of secondary products and they appear to be limited instead by the availability of ATP (Mateos et al., 1991).

The effect of carbon on secondary synthesis depends on the form, as well as the amount, of the carbon source. This is illustrated by the fact that high levels of glucose supplied exogenously to some lichens reduce the content of phenolic secondary products, but high mannitol does not have the same effect (Vicente, 1991). In cultured fragments of *Usnea hirta*, usnic acid was not altered by higher concentrations of sucrose and mannitol, but was increased with more glucose (Kinoshita et al., 1993; Kinoshita, pers. commun.).

Because the forward and backward reactions for interconversion of fructose and mannitol have different pH optima, and because photosynthesis produces alkalinization, Vicente (1991) has suggested that photosynthesis could change the relative amounts of these sugars. Secondary metabolism could depend on sugar levels. In *Ramalina siliquosa* cultures, depsidone production was maximal in pH 6.5 media (Hamada, 1989), but internal pH has not been assessed nor has it been related to phenolic synthesis.

Light quality may affect secondary synthesis in lichens. The proportion of red to far-red light is greater in sun than in shade, in summer than in winter and at mid-day than at dusk or dawn. When the ratio of red/far red light is high, the photoreceptor, phytochrome, is converted to its active form (Pfr) and changes are effected in transcription, usually increasing the rate. In lichens Pfr seems to enhance production of cyclic AMP, and following this, particular enzymes of secondary metabolism are synthesized. Enzymes that are produced in response to high-red illumination may be catabolic or anabolic; some of the induced enzymes degraded phenols (Vicente and Avalos, 1986; Avalos and Vicente, 1987b, 1989) but, in cases where phenolic levels were increased (Avalos and Vicente, 1987c), perhaps production of synthetic enzymes was enhanced.

Since phytochrome attaches to the membrane of photobiont cells and phenolics are produced in the mycobiont, a message must be transmitted between the two symbionts. In plants, and at least some non-lichenized algae, phytochrome induces changes in Ca^{2+}, either affecting levels inside cells or the flux out (Sage, 1992). Calcium ions bind to a regulatory protein, and the protein activates key enzymes or hormones. In plants and algae, a calcium-binding regulatory protein, calmodulin, has been identified. The mechanism for message transfer is not known in lichens, but Avalos and Vicente (1987b) suggested that a messenger molecule may travel from the photobiont to the fungal partner. While manipulation of visible light appears not to alter the level of usnic acid, perhaps both visible and ultraviolet signals are critical. Honegger and Kutasi (1990) noted that while there was less anthraquinone in naturally-shaded lichens, *Xanthoria parietina* mycobionts on ribitol medium accumulated more anthraquinone in the dark. Such an apparent inconsistency could relate to differences between natural radiation and the quality of light provided experimentally.

Temperature

Studies by Hamada in both natural environments and under culture conditions indicate a relationship between temperature and the level of secondary products. Of several environmental factors, only mean annual temperature of collection site was correlated with the amount of the depsidone, salazinic acid, and the depside, divaricatic acid, in *Ramalina* spp. (Hamada, 1982, 1983). More secondary substances were produced in warm collection sites and on dark colored, or warmer rocks than than in other situations. Isolated mycobionts of *R. siliquosa* (Hamada, 1989) produced phenolics maximally at 15° C, and whole thalli maintained in growth cabinets show increased salazinic acid with temperatures up to 19° C (Hamada, 1984). Geographic variation was found in the levels of perlatolic acid in *Cladina stellaris* and fumarprotocetraric acid in *C. rangiferina*; the quantity of both compounds decreased in more northerly, or colder locations in Finland and Norway (Huovinen, 1985).

Culturing of *Cladonia cristatella* clones in a phytotron indicates that the effect of temperature and other environmental factors varies with respect to different biosynthetic pathways (Culberson, Culberson and Johnson, 1983). Depsides related to barbatic acid were found in significantly lower quantities at 18° C than 8° C, while the levels of dibenzofurans differed little at these temperatures.

Wtih excessively high temperatures, the chemical structure of lichen products is destroyed. Drying at 80° C caused degradation of lichen products

in two species of *Cladina* and *Umbilicaria muhlenbergii* (Mirando and Fahselt, 1978). Heating thalli of *Hypotrachina partita* at 85° C and 105° C resulted in thermal degradation of anziaic and perlatolic acids; higher temperatures apparently generate phenolics that mimic those found in lichens growing near a recently active volcano (Culberson et al., 1977b). Non-enzymatic degradation of lichen products, thus seems to occur in nature. Thermal decomposition of nine lichen depsides has been demonstrated experimentally by Huneck et al. (1989).

Moisture content

Exposure of fungal cultures to air enhances the formation of aerial hyphae (Toledo et al., 1986) and such hyphae are associated with the formation of secondary products in both free-living fungi and lichen mycobionts. Production of secondary metabolites was promoted when mycobiont cultures were transferred from liquid media to the surface of solid media. On solid media, synthesis was enhanced under the driest conditions provided (Culberson and Armaleo, 1992) and initiated near the tips of aerial hyphae. However, the reverse seems to apply to intact lichens. In thalli of *Hypogymnia physodes*, more atranorin increased under high moisture conditions (Klee and Steubling, 1977), a response attributed to increased photosynthesis when more water was available.

Intrathalline differences in secondary pathways

The range of photobiont cell sizes within a thallus, that is, smaller cells in newly formed parts of the thallus cells and larger in older tissues (Hill, 1985), suggests that photobiont metabolism changes with age. The rate of carbon assimilation is age-dependent (Lechowicz, 1983), with the greatest amount of fixation in more recently formed tissues and gradually decreasing levels in older tissue. In the foliose species, *Parmelia caperata*, both photosynthesis and secondary metabolism are most active in the marginal, or youngest tissue (Taguchi et al., 1969a).

There are several possible ways to account for age-related differences in phenolics. First, levels of secondary products could be determined by the manner in which photosynthates affect their synthesis. Compounds occurring in high amounts in new tissue could be those produced by enzymes whose activity is enhanced in the presence of photosynthates, and substances that increase in importance during aging may be generated by enzymes that are repressed by assimilates. Changes in secondary products during thallus development could also result if one product is converted into another during

aging, (Mirando and Fahselt, 1978; Manrique and Lopez, 1991) or substances are simply degraded. Alternatively, intrathalline gradients could be explained on the basis of solubility in a polar solvent, that is, on differential loss of extracellular compounds in rainfall or capillary water (Manrique and Lopez, 1991).

Differences in chemistry between layers of the thallus or between thallus structures are perhaps not surprising, since factors such as moisture and temperature may affect secondary substances. The mycobiont of *Lecanora dispersa*, produces xanthones in apothecia of the intact lichen, but mainly depsidones in cultured vegetative hyphae (Leuckert et al., 1990). The explanation for these differences is not known, but environmental/developmental effects could be responsible; acetyl-CoA and malonyl-CoA must be processed by a different synthase in each situation.

Methods of studying pathways

Early steps in the synthesis of depsides and depsidones have been partly determined through experiments with lichens using radioactive tracers, and inferences have been made based on the pathways of free-living fungi. Less is known, however, about later steps leading to particular end products (Turner, 1976). Since many metabolites are produced exclusively by lichens, parallels can not be found in other groups of organisms.

One indication of biochemical relationships among compounds is the combinations that occur together in a particular taxon (e.g., Elix and Crook, 1992) or are produced in closely related taxa (e.g., Culberson and Culberson, 1978; Huovinen and Ahti, 1982). Inferences can be made on the basis of co-occurrence along with structural similarities among secondary metabolites. Laboratory interconversions of lichen products also indicate synthetic routes that might be utilized within the thallus (e.g., Elix and Gaul, 1987; Elix and Parker, 1987).

The stage of synthesis at which modifications, such as hydroxylation and methylation, take place is not always clear, but laboratory syntheses suggest, for example, that meta-depsides (Fig. 1), are generated by C-hydroxylation of para-depsides. The temporal patterns of accumulation in cultured mycobionts as they first begin to synthesize secondary products may also be informative.

8. Acetate-polymalonate Synthesis

Ester-linked polyphenolics: depsides/usnic acids

The largest groups of lichen products are phenolics, in particular, orcinol- and beta-orcinol-based substances (Table 3). After feeding with ^{14}C-labelled

Table 3. The number of compounds in categories of lichen secondary products, as reported by Culberson and Elix (1989). For structural formulae of individual compounds, see Culberson (1969) and supplements.

Category	Number of compounds
Acetate-polymalonate pathway	
Secondary aliphatic acids, esters and related compounds	42
Simple phenolics	17
Depsides, tridepsides and related compounds	133
Depsidones, depsones and related compounds	88
Usnic acids, dibenzofurans	17
Chromones	13
Xanthones	26
Anthraquinones	54
Naphthoquinones	4
Mevalonic acid pathway	
Terpenoids	62
Steroids	20
Skikimic acid pathway	
Pulvinic acid derivatives	12
Terphenylquinones	2

malonate precursor the tridepside, gyrophoric acid, is labelled in the same way as molecules known to be synthesized in micro-organisms along the acetate-polymalonate pathway (Mosbach, 1964), and the incorporation of $^{14}CO_2$ into depsides, tridepsides, depsidones and dibenzofurans is likewise consistent with their origin through the activity of a typical aromatic synthetase (Fox and Mosbach, 1967). It is thus assumed that acetyl-CoA and malonyl-CoA must be precursors, as they are in free-living fungi (Turner, 1976), the first acting as a primer and the second condensing with the growing chain one molecule at a time.

At each condensation, malonyl-CoA undergoes decarboxylation, or loses one of its three carbons, and extends the chain by two. After three condensation cycles, a C-8 polyketide chain (tetraketide) is produced that is cyclized to form a simple phenolic acid, e.g., orcellinic acid (Fig. 4). The polyketide synthetase, orcellinate synthase, has been isolated from *Penicillium madriti* (Gaucher and Shepherd, 1968). In lichens, orcellinic acid is a precursor for orcinol depsides (Fig. 1); pairs of simple phenolic units based on orcellinic acid are joined together through an ester linkage. No condensing enzymes have been isolated, but several esterases may be involved in depside synthesis (Mateos et al., 1991). Structural similarities and co-occurrences of meta- and para-depsides suggest that the first may be synthesized from the second (Elix et al., 1987) (Fig. 4). Hydroxylated para-depsides are thermodynamically less

stable than meta-derivatives, and this may account for their infrequency in nature (Elix and Gaul, 1986).

Labelling experiments indicate that an additional C-1 unit must be added to a polyketide prior to cyclization in order to produce beta-orcinol depsides (Yamazaki et al., 1965; Yamazaki and Shibata, 1966). Polyketides with extra C-1 units are cyclized into 3-methyl orcellinate and, after undergoing side-chain modifications (Culberson, 1969), they too are condensed in pairs, in this case, into beta-orcinol depsides.

Early stages in the synthesis of usnic acid are the same as those for depsides (Fig. 4). Methylation of the C-8 tetraketide takes place before ring closure (Taguchi et al., 1966), as is the case for phenolic units that give rise to beta-orcinol derivatives. However, cyclization occurs differently. In the formation of beta-orcellinic acid, the carboxyl group at the end of the tetraketide chain becomes a substituent on the 6-membered ring. In the formation of methylphloroacetophenone (MFAP), which is a precursor of usnic acid, the carboxyl carbon becomes part of the ring and the other end of the chain is left as a substituent. Each type of cyclization is probably facilitated by a different enzyme. Two molecules of MFAP are coupled to form hydrated usnic acid, and a molecule of water is then removed to yield usnic acid (Taguchi et al., 1969b).

Ester- and ether-linked polyphenolics: depsidones

Depsidones are probably derived from depsides (Fig. 4), since structurally related depsides and depsidones often occur in the same lichen (Culberson and Elix, 1989). The sequence in which secondary products are accumulated in *Cladonia cristatella* mycobionts supports such a biogenetic relationship. In hyphae on moist agar, depsidones were present initially in only low amounts, but over a longer time accumulated to much higher levels than depsides (Culberson and Armaleo, 1992). The actual mechanism whereby a depside is transformed into a depsidone has not been demonstrated, but it could occur through oxidative coupling. Another possible mode of synthesis involves a series of intra-molecular rearrangements in depside molecules, including Smiles rearrangements (Fig. 5). This route involves more steps, but is highly favored thermodynamically (Elix et al., 1987). Since these intramolecular conversions take place so readily *in vitro*, it would be energetically expedient for lichens to make depsidones the same way. On a similar basis, it is believed that rearrangements within para-depside molecules can give rise to dibenzofurans (Elix and Parker, 1987).

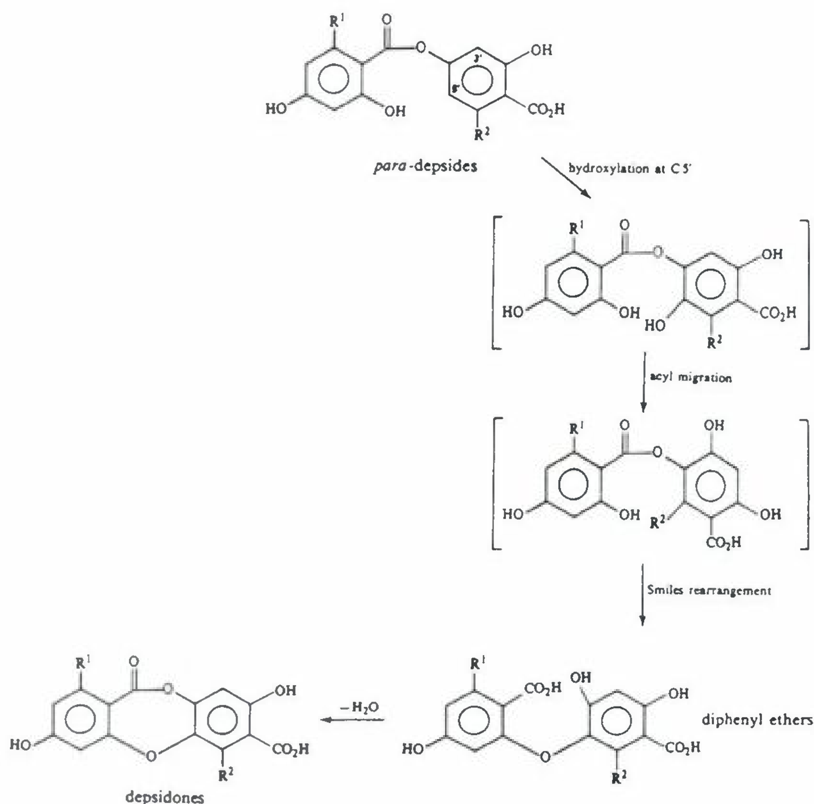

Figure 5. Proposed biosynthesis of depsidones from a para-depside (modified from Elix, Jenie and Parker, 1987)

Phenolic diversity

Enzymes with medium to low specificity occur in both primary and secondary metabolism, but are more common in secondary pathways (Bu'Lock, 1975), examples being flavonoid synthesis in higher plants and anthraquinone synthesis in fungi. A fungal transferase enzyme which glycosylates the anthraquinone, alizarin, is capable of glycosylating anthraquinones with closely similar structures in exactly the same way (Webb, 1984). Secondary pathways of lichens generate a great variety of different end products, and enzymes for the later steps in their syntheses may similarly have only moderate or low specificity (Culberson and Culberson, 1978; Mateos et al., 1991) and, thus, each could accommodate several similar substrates.

A series of closely related enzymes, each acting on a slightly different substrate, is an alternative explanation for the great variety of related end

products found in lichens. Mateos et al. (1991) suggested that similar esterases are used for the synthesis of atranorin and barbatolic acid.

In lichens with highly complex phenolic complements, much of the variety is due to the range of different simple phenolic precursor units that are available for condensation into pairs, as well as to the reactions that take place after condensation. Putative synthetic pathways can be devised to account for co-occurring secondary products, even for lichens with a highly diverse group of natural products (e.g., Culberson et al., 1990, 1992; Elix and Crook, 1992).

Single chain polyketides

Four classes of aromatic polyketide pigments are probably formed by cyclization of a single polyketide chain (Huneck, 1968; Culberson, 1969; Culberson and Elix, 1989): chromones, anthraquinones, xanthones and naphthoquinones (Fig. 1). Since distinct enzymes are seemingly required for the synthesis of different types of tetraketides (Vining, 1992), yet other synthases must be necessary to generate polyketide chains that are longer than this (Fig. 4). Secondary products based on polyketide chains with five to twelve two-carbon units are cyclized variously, either the same way as MFAP or as beta-orcellinic acid (Culberson, 1969). There are relatively small numbers of chromones and naphthoquinones, but many xanthones and anthraquinones. Rhodocladonic acid, a naphthoquinone, is the red pigment often seen in *Cladonia* species, and parietin is the most common anthraquinone.

9. Mevalonic Acid Derivatives

Many of the products of this pathway, such as the C-20 phytyl side chain of chlorophyll, are important in primary metabolism but others are secondary metabolites. The mevalonic acid pathway, like the acetate-polymalonate pathway, uses acetyl-CoA (Fig. 4), but malonate is not involved. Three acetate units are condensed into a six-carbon intermediate, mevalonic acid, which is decarboxylated and converted to a C-5 pyrophosphate form. The branched five-carbon modules, isoprenoid units, are joined in a head-to-tail fashion, to form a linear molecule that has a characteristic branching pattern (Fig. 4), and become the basis for all terpenoid molecules. Two five carbon units are combined into a C-10 unit (geranyl pyrophosphate), a ten and a five into a C-15 (farnesyl pyrophosphate) and two tens into a C-20 (geranylgeranyl pyrophosphate). Intermediates are in the form of pyrophosphates and with each condensation phosphate is lost.

Carotenoids

Carotenoids are essential both as accessory pigments for photosynthesis and molecular shields against destructive effects of excess irradiation. Thus, they are present in all lichens and total carotenoid content may be similar for many species (e.g., Czeczuga and Alstrup, 1987). However, the particular constituents vary, with certain carotenoids being restricted to some species and some compounds being quite rate.

Carotenoids are tetraterpenoids, that is, they are basically 40 carbons long and comprise eight isoprenoid units. They are formed by the condensation of two molecules of geranylgeranyl pyrophosphate. Though essentially linear molecules, with the same regular pattern of short branches that characterizes other products of the mevalonic acid pathway, the two ends are cyclized into six-membered rings.

Triterpenoids

Triterpenoids (Fig. 1) are formed by condensation of two molecules of farnesyl pyrophosphate, and thus are based on a 30 carbon skeleton (Fig. 4) which constitutes 6 isoprenoid units. Complex mixtures of terpenoids occurred in low concentrations (0.01–0.05%) in several lichen species examined by Tabacchi (1993). Zeorin is one example of a lichen triterpene that is cyclized into a series of five interconnected rings, and there are many structurally similar derivatives that are rarely found, except in lichens. New triperpenes are still being described (e.g., Wilkins et al., 1989; Wilkins and Elix, 1990; Tabacchi, 1993).

10. Shikimic Acid Derivatives

The most widely distributed end products of the shikimic acid pathway are yellow pigments, the pulvinic acid derivatives including vulpinic and rhizocarpic acids. Red and purple terphenylquinones made along this pathway are less common. Shikimic acid pathway intermediates originate from phosphoenolpyruvate, an intermediate in the Embden-Meyerhof pathway, and erythrose-4-phosphate, an intermediate of the pentose phosphate pathway, and generate phenylpropanoid compounds, such as phenylalanine. Labelling experiments by Mosbach (1967) indicate that phenylalanine in lichens is a substrate for vulpinic acid (Fig. 4), but non-nitrogenous phenylpropanoids can also be incorporated (Culberson, 1969). Two phenylpropanoids condense to form pulvinic acid derivatives. Terphenylquinones are also formed from shikimic acid.

11. Amino Acid Derivatives

A newly discovered class of lichen substances includes derivatives of amino acid and amino alcohol esters (Fig. 6). It is assumed that these compounds are

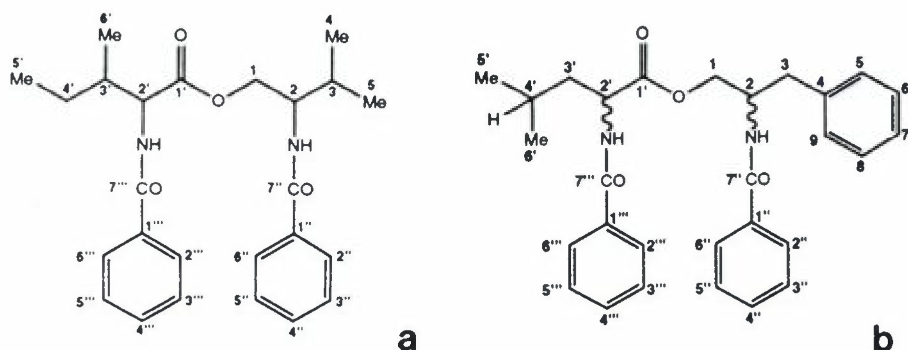

Figure 6. Compounds in a newly discovered class of lichen substances derived from amino acids, arthonic and hypothallin (from Huneck et al., 1992, 1993).

synthesized from L-amino acids. The structures of two substances have been described: arthonin and hypothallin, found in the lichens, *Arthonia endlicheri* and *Schismatomma hypothallinum*, respectively (Huneck et al., 1992; Huneck et al., 1993). A similar substance was reported previously in the *Penicillium*.

12. Pathways in Related Taxa

Although synthesis of secondary products is influenced to some extent by environmental factors, variation from one taxon to another is largely determined genetically. Variants of orcinol and beta-orcinol derivatives in the *Cladonia chlorophaea* group appear to have arisen by O-methylation, addition of shorter or longer aliphatic side chains, hydroxylation, oxidation, chlorination, as well as truncation of pathways leading to loss of some substances and accumulation of others (Culberson, 1986). Such variations in phenolic synthesis form the basis for extensive use of these compounds in systematic investigations.

Pathway divergence may be evident at the sectional level. For example, in *Cladonia*, the ability to synthesize beta-orcinol depsides is found in most sections. In section Unciales, however, usnic acid is always present, sometimes as the only secondary product (Huovinen, 1986); in section Perviae, the route to usnic acid appears to be almost never operative (Huovinen and Ahti, 1988).

Differences in secondary products, may also occur at the species level, e.g. difficult *Lecidella* specimens can be precisely determined using mainly chloroxanthones (Leuckert et al., 1990). Chemical variants, or chemotypes, have frequently been recognized within species such as *Cladonia chlorophaea* (Culberson et al., 1985b). Depsides are common in *Roccella capensis*, but a few chemotypes generate dibenzofurans as well (Huneck et al., 1991). In *Cetraria pinastri*, all specimens have products of the shikimic acid and acetate-polymalonate pathways, but some synthesize triperpenoids also (Mattsson, 1987).

13. Application of Lichen Products to Human Uses

Comprehensive and interesting papers on the relationship between lichens and man include those of Richardson (1985b, 1988), Hale (1983) and Lawrey (1986). The present account will briefly review and supplement these earlier papers with respect to secondary biochemistry of lichens as it relates to human useage.

Dyes

Secondary phenolic constitutents of lichens make them an excellent source of natural dyes, most of which color effectively without the need for mordants. Red and purple dyes derived from orcinol, which is produced by degradation of depsides and tridepsides (Richardson, 1985b), were utilized traditionally in several cultures. *Letharia vulpina* was an important source of bright yellow dyes for the Thompson Indians of British Columbia, Canada, who used it to color animal hides, horse hair bridles, wooden objects, faces and bodies (Turner et al., 1990). The Thompsons still dye woolen fibres with this lichen. Navajo Indians, also, depend on natural dye sources; they use a wide range of lichens, but predominantly *Xanthoparmelia* spp., and extract dyes by a boiling water method (Brough, 1988).

Food

There is continued interest in lichens as a food source, in spite of the unpleasant taste produced by secondary products. Lichens may be boiled repeatedly in water in an attempt to remove polyphenolics and then highly flavored to improve palatability. Thalli of the Japanese *Umbilicaria esculenta* (i-wa-ta-ke), are scraped, soaked, and seasoned with generous applications of soy sauce, sugar and perhaps sa-ke (strong Japanese wine). Three species of lichens, *Lasallia pustulata, Bryoria* sp. and *Cetraria islandica*, were included

in a survival diet during a recent Swedish attempt to subsist exclusively on wild plants (Kallman, 1992).

Cosmetics

Two species of lichens that grow on oak trees, *Pseudevernia furfuracea* and *Evernia prunastri*, figure prominently in the production of modern face and body preparations! These "oak moss" lichens are used in a variety of quality soaps and perfumes (Richardson, 1985b) because of their essential oils and odoriferous principles, which may include volatile isoprenoid compounds (monoterpenoids, Fig. 4) such as camphor, borneol, cineol, geraniol and others.

A small proportion of cosmetics users develop dermatitis, and among the most frequent sensitizers are perfumes, including those derived from lichens (Remaut and Thune, 1992). Sensitivity results from contact with lichen substances, either in commercial products or natural lichens. Allergens appear not to be the aromatic monoterpenes, but phenolics such as atranorin, usnic acid, evernic acid and fumarprotocetraric acid (Goncalo et al, 1988), which are essentially the same compounds that cause dermatitis in forest workers (Richardson, 1985b). A longer list of lichen substances producing contact allergies has been prepared recently by Brasch and Jacobsen (1991). Some phenolic products inhibit the enzyme, tyrosinase (Matubara et al., 1993; Takahashi et al., 1993), which is necessary for the formation of melanin. However, it seems unlikely, given current fashion in many countries, that blockage of melanin production would generate much interest in the cosmetics industry.

Pest control

Since many lichen substances naturally deter foraging, it is possible that some could be used against pests that are of concern to humans. Clothes moths, for example, are repelled by lichen substances remaining in lichen-dyed woolens. Secondary products appear to provide a defence for lichens against damage by the gypsy moth, but acetone extracts of five epiphytic lichens applied to *Quercus rubra* (red oak) did not reduce larval damage on leaves. Larval feeding was reduced, however, by treating with aqueous lichen extracts (Blewitt and Cooper-Driver, 1990). There were no detectable depsides or depsidones in these extracts. The active ingredients may be polypeptides or glycosides, although actual structures have not yet been determined. In any case, water-soluble pesticides would be impractical as a deterrent against gypsy damage. In regard to *Cladonia subtenuis*, however, acetone-extractable products reduced both larval growth and the apparent digestibility of

leaves. Of the lichens tested, it was the only species known to produce fumarprotocetraric acid. This constituent, or perhaps some others, may be useful in reducing the impact of insect damage on plants.

There were earlier indications that lichen products deter plant pathogens (Hale, 1983), but they have not been applied to any extent in agriculture or horticulture. Some lichen substances inhibit wood-rotting fungi (Lundstrum and Henningsson, 1993).

Antibiotics for use in medicine

Many lichens were screened for antibiotic properties by Burkholder et al. (1944), and preparations including usnic acid extracted from natural lichens have been available commercially in Europe for some time (Hawksworth and Hill, 1984). Thallus extracts have generally been most effective against gram-positive bacteria, probably because the cell membrane in Gram positive bacteria constitutes less of a barrier than that of gram negative bacteria. However, Pereira et al. (1991) have shown that crude extracts of several lichen species are active against both Gram positive and Gram negative bacteria, as well as yeasts.

The use of natural antibiotics extracted from lichens has declined due to the availability of synthetically produced substances. However, in many countries interest has been maintained, and surveys for active principles continue (e.g., Cosar et al., 1988; Xavier Filho et al., 1990; Pereira et al., 1991; Rowe et al., 1991; Harmala et al., 1992). Many of the organisms tested for susceptibility to lichen products are of medical interest, that is, bacteria such as *Staphlococcus aureus, Bacillus subtilis* and *B. cereus*, or yeasts. Extracts of *Stereocaulon ramulosum* are active against dermatophytic fungi, producing changes in their gross morphology as well as sterol metabolism (Hickey et al., 1990). *Tuberculosis bacillus* is also inhibited by lichen extracts (Pereira et al., 1991).

Usnic acid is often the active principle in commercial medicines from lichens. The (D)+ optical form of usnic acid is a more effective antibacteriocidal compound than the (L)+ form and, used as a mouthwash, selectively acts against *Streptococcus mutans* without disrupting the normal bacterial flora of the mouth (Ghione et al., 1988). Though pulvinic acid derivatives have not usually been considered antibiotics (Hale, 1983), vulpinic acid is reported to be effective against a wide range of bacteria and fungi (Xavier Filho et al., 1992). Another active component is methyl-haematommate, a simple phenolic based on benzoic acid, that is produced in *Stereocaulon ramulosum* (Hickey et al., 1990).

Antimutagenicity

The orcinol depsidone, physodalic acid, is reported to be mutagenic against a strain of the bacterium, *Salmonella typhimurium*. However, physodic acid, which is structurally similar, does not act as a mutagen and appears, on the other hand, to be antimutagenic (Osawa et al., 1991). It particularly inhibits the mutagenic effects of heterocyclic amines, such as are commonly found in heated foods, seemingly by preventing benign heterocyclic amines from being converted to more mutagenic forms that bond with DNA. Against certain strains of *Salmonella*, physodalic acid appears to be antimutagenic also. Thus, both physodic acid and physodalic acid are of potential medical importance.

Anti-tumor effects

To treat cancerous growths, it is essential to control cell proliferation, and one of the numerous interesting properties of usnic acid (Hawksworth and Hill, 1984) is its ability to inhibit tumors (Takai et al., 1979). The mode of action is not clear. Usnic acid has been reported to uncouple ATP production (Richardson, 1985b), but it may also interfere with functioning of the mitotic spindle (Al-Bekairi et al., 1991) and, thus prevent cell division. In the tissues of mice that are fed high doses of usnic acid, DNA replication appears to take place but cells do not always divide. The significance of this for medical treatment is uncertain, however, since the effect is only evident for 24 hr, after which the proportion of normal cells returns to control values.

A number of lichen polysaccharides also have anti-tumor activity. Effects have been demonstrated by injecting water-soluble glycans extracted from lichens into mice that were implanted with tumors. The polysaccharides tested were from *Umbilicaria, Lasallia, Cladina, Usnea* and a few other lichens. Within a genus, extracts of some species may be effective, while those of others are not, for example, *Cladonia* (Lima et al., 1990). An acetylated derivative of pustulan, which is a linear (1-6) linked β-D-glucan, is extremely effective (Nishikawa et al., 1970), as is an α-glucan, isolichenan, with alternating (1-3) and (1-4) linkages (Nishikawa et al., 1974). Other active forms are (1-3) and (1-4) linked β-D-glucans, or lichenans (Demleitner et al., 1992a), linear (1-3) β-D-glucans and a complex heteroglycan comprising mainly of mannose, galactose and glucose (Nishikawa et al., 1974). In contrast, β-(1-4) linked glucans have only moderate activity, or none (Demleitner et al., 1992b). The relationship between structure and antitumor activity is not yet fully understood, but several polysaccharides not only prevent the expansion of tumors, but actually reduce their size.

In some cases, tumors do not begin to regress until several days after a polysaccharide injection, while in other instances the antitumor effect is more rapid. Lichenan or an acetylated pustulan-type polysaccharide produces the earlier type of regression, but the most immediate effect is not tumor regression itself, but an increase in the level of a particular acidic glycoprotein (α1-AP). This glycoprotein is normally present in cells that are actively dividing, such as breast epithelial cells and tissues of cancer patients, and is thought to serve as a regulator or suppressor of mitosis. Injections of some lichen polysaccharides apparently stimulate the host organism to increase production of the α1-AP cell division suppressor for controlling tumors and bringing about regression (Watanabe et al., 1986). In other words, they may function by activating the organism's own natural defence system. There have apparently never been clinical tests of lichen anti-tumor polysaccharides in humans.

Inhibition of HIV

The same partly acetylated pustulan that has antitumor properties also has an inhibitory effect against human immunodeficiency virus (HIV), the agent which is considered to cause acquired immune deficiency syndrome (AIDS) (Hirabayashi et al., 1989). In lymphocyte cultures infected with HIV, cells that are necessary for a normal immune response first fuse into multinucleated giant cells, then lyse and die. The sulfate form of acetylated pustulan from *Umbilicaria esculenta* was found to suppress the formation of giant cells and increase the number of normal viable cells up to control levels (Hirabayashi et al., 1989). This pustulan derivative was the only lichen polysaccharide of seven tested that had anti-HIV properties.

Utilization and conservation

Lichens offer a variety of unique chemical attributes, but applications requiring large scale collection from natural populations are not recommended. On a world-wide scale, natural habitats are being heavily modified or eliminated altogether, and slow-growing, pollution-sensitive lichens are extremely vulnerable. Over-collection, added to other pressures, could jeopardize future scientific study as well as the possiblity of further applications.

Large quantities of lichen material are necessary for many commercial or semi-commercial purposes. For dyeing with lichens, the recommended dry weight lichen material is the same as the weight of wool to be colored (Bolton, 1960). *Evernia prunastri* and *Pseudevernia furfuracea* are collected in enormous quantities for the perfume industry and transported by the bale

(Richardson, 1985b). Some effects of over-use are already evident. *Umbilicaria esculenta*, in many heavily populated parts of Japan, has been removed from all but the most treacherous cliff faces. Only by careful management of sites where *Cladina stellaris* is collected for architects' models and wreaths, is it possible to ensure that supplies are perpetuated (Hale, 1983).

Culturing of lichens or mycobionts for pharmaceuticals and other products (e.g., Komamine et al., 1991) is therefore, highly desirable. University laboratories in several countries are growing mycobionts (Crittenden and Porter, 1991), and companies are in some cases also establishing large scale cultures for the extraction of commercially useful products (Yamamoto et al., 1993). Such interest will add impetus to the study of lichen secondary compounds and may stimulate further research on their roles in the intact lichen. It should also help to conserve the remaining natural lichen populations.

REFERENCES

Ahmadjian, V. and Jacobs, J.B. 1983. Algal-fungal relationships in lichens: recognition, synthesis and development. In: *Algal Symbiosis*. L.J. Goff, ed. Cambridge University Press, London, pp. 147–172.

Ahmadjian, V. and Jacobs, J.B. 1985. Artificial re-establishment of lichens. IV. Comparisons between natural and synthetic thalli of *Usnea strigosa*. *Lichenologist* **17:** 149–165.

Al-Bekairi, A.M., Qureshi, S., Chaudhry, M.A., Krishna, D.R., and Shah, A.H. 1991. Mitodepressive, clastogenic and biochemical effects of (+)-usnic acid in mice. *J. Ethanopharmacol.* **33:** 217–220.

Armaleo, D. 1993. Why do lichens make secondary products? In: *XV International Botanical Congress Abstracts*. International Union of Biological Sciences, Yokohama, Japan, p. 11.

Arup, U., Ekman, S., Lindblom, L., and Mattsson, J.-E. 1993. High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist*. **25:** 61–71.

Avalos, A. and Vicente, C. 1987a. The occurrence of lichen phenolics in the photobionts cells of *Evernia prunastri*. *Plant Cell Rep.* **6:** 74–76.

Avalos, A. and Vicente, C. 1987b. Equivalence between Pfr and cyclic AMP in the induction of D-usnic acid dehydrogenase in the lichen *Evernia prunastri*. *Plant Physiol.* **84:** 803–807.

Avalos, A. and Vicente, C. 1987c. Light mediates accumulation of lichen phenolics in the *Evernia prunastri* thalli. *Symbiosis* **5:** 251–258.

Avalos, A. and Vicente, C. 1989. Action of filipin on phytochrome-modulated D-usnic acid: NAD+ (H) oxido-reductase activity in the lichen *Evernia prunastri*. *J. Plant Physiol.* **135:** 478–482.

Blanco, M.J., Suarez, C., and Vicente, C. 1984. The use of urea by the lichen *Evernia prunastri*. *Ann. Bot.* **60:** 553–562.

Blewitt, M.R. and Cooper-Driver, G.A. 1990. The effects of lichen extracts on feeding by gypsy moths (*Lymantria dispar*). *Bryologist* **93:** 220–221.

Bolton, E.M. 1960. *Lichens for Vegetable Dyeing.* Studio Books, Longacre Press Ltd., London.

Brasch, J. and Jacobsen, P. 1991. Flechten und ihre Allergene. *Allergologie* **14:** 93–103.

Brough, S.G. 1988. Navajo lichen dyes. *Lichenologist* **20:** 279–290.

Brown, D.H. and Beckett, R.P. 1985. Minerals and lichens: acquisition, localisation and effect. In: *Surface Physiology of Lichens.* C. Vicente, D.H. Brown and M.E. Legaz, eds. Universidad Complutense de Madrid, Madrid, pp. 127–149.

Bu'lock, J.D. 1975. Secondary metabolism in fungi and its relationships to growth and development. In: *The Filamentous Fungi,* Vol. 1, Industrial Mycology. J.E. Smith and D.R. Berry, eds. Wiley, New York, pp. 33–58.

Burkholder, P.R., Evans, A.W., McVeigh, I., and Thornton, H.R. 1944. Antibiotic activity of lichens. *Proc. Nat. Acad. Sci. (USA)* **30:** 250–255.

Castle, H. and Kubsch, F. 1949. The production of usnic, didymic and rhodocladonic acids by the fungal component of the lichen *Cladonia cristatella. Arch. Biochem.* **12:** 158–160.

Cosar, G., Rumbay, E., Zeybek, N., and Ozer, A. 1988. The anti-bacterial and antifungal effect of some lichens growing in Turkey: Part I. *Evernia prunastri, Pseudevernia furfuracea* and *Alectoria capillaris. Fitoterapia* **0(6):** 505–507.

Crittenden, P.D. and Porter, N. 1991. Lichen-forming fungi: potential sources of novel metabolites. *Trends in Biotechnology* **9:** 409–414.

Culberson, C.F. 1969. *Chemical and Botanical Guide to Lichen Products.* University of North Carolina Press, Chapel Hill.

Culberson, C.F. 1970. Supplement to "Chemical and Botanical Guide to Lichen Products". *Bryologist* **73:** 177–377.

Culberson, C.F. 1972a. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *J. Chromatogr.* **72:** 113–125.

Culberson, C.F. 1972b. High-speed liquid chromatography of lichen extracts. *Bryologist* **75:** 54–62.

Culberson, C.F. 1986. Biogenetic relationships of lichen substances in the framework of systematics. *Bryologist* **89:** 91–98.

Culberson, C.F. and Ahmadjian, V. 1980. Artificial establishment of lichens. II. Secondary products of resynthesized *Cladonia cristatella* and *Lecanora chrysoleuca. Mycologia* **72:** 90–109.

Culberson, C.F. and Ammann, K. 1979. Standardmethode zur Dunnschichtchromatographie von Flechtensubstanzen. *Herzogia* **5:** 1–24.

Culberson, C.F. and Armaleo, D. 1992. Induction of a complete secondary-product pathway in a cultured lichen fungus. *Exp. Mycol.* **16:** 52–63.

Culberson, C.F. and Culberson, W.L. 1958. Age and chemical constituents of individuals of the lichen *Lasallia papulosa. Lloydia* **21:** 189–192.

Culberson, C.F. and Culberson, W.L. 1978. β-orcinol derivatives in lichens: biogenetic evidence form *Oropogon loxensis. Exp. Mycol.* **2:** 245–257.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1977a. *Second Supplement to "Chemical and Botanical Guide to Lichen Products"*. Missouri Botanical Garden, American Bryological and Lichenological Society, St. Louis.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1977b. Thermally induced chemical artifacts in lichens. *Phytochem.* **16:** 127–130.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1983. Genetic and environmental effects on growth and production of secondary compounds in *Cladonia cristatella*. *Biochem. Syst. Ecol.* **11:** 77–84.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1985a. Does the symbiont alga determine chemotype in lichens? *Mycologia* **77:** 657–660.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1985b. Orcinol-type depsides and depsidones in the lichens of the *Cladonia chlorophaea* group (Ascomycotina, Cladoniaceae). *Bryologist* **88:** 380–387.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1990. The *Ramalina americana* complex (Ascomytina, Ramalinaceae): chemical and geographic correlations. *Bryologist* **93:** 167–186.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1992. Characteristic lichen products in cultures of chemotypes of the *Ramalina siliquosa* complex. *Mycologia* **84:** 705–714.

Culberson, C.F., Culberson, W.L., and Johnson, A. 1993. Occurrence and histological distribution of usnic acid in the *Ramalina siliquosa* species complex. *Bryologist* **96:** 181–184.

Culberson, C.F. and Elix, J.D. 1989. Lichen substances. In: *Methods in Plant Biochemistry*, Vol. 1. J.B. Harborne, ed. Academic Press, New York, pp. 509–535.

Culberson, C.F. and Kristinsson, H. 1970. A standardized method for the identification of lichen products. *J. Chromatogr.* **46:** 85–93.

Culberson, W.L. 1969. Norstictic acid as a hymenial constituent of *Letharia*. *Mycologia* **61:** 731–736.

Czeczuga, B. and Alstrup, V. 1987. The carotenoid content of lichens from Greenland. *Biochem. Syst. Ecol.* **15:** 297–301.

Demain, A.L. 1992. Microbial secondary metabolism: a new theoretical frontier for academia, a new opportunity for industry. In: *Secondary Metabolites: Their Function and Evolution*, Ciba Foundation Symposium 171. Wiley, New York, pp. 3–16.

Demleitner, S., Kraus, J., and Franz, G. 1992a. Synthesis and antitumour activity of derivatives of curdlan and lichenan branched at C-6. *Carbohydr. Res.* **226:** 239–246.

Demleitner, S., Kraus, J., and Franz, G. 1992b. Synthesis and antitumour activity of sulfoalkyl derivatives of curdlan and lichenan. *Carbohydr. Res.* **226:** 247–252.

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

Ejiri, H. and Shibata, S. 1975. Squamatic acid from the mycobiont of *Cladonia crispata*. *Phytochemistry* **14:** 2505.

Elix, J.A. and Crook, C.E. 1992. The joint occurrence of chloroxanthones in lichens and a further thirteen new lichen xanthones. *Bryologist* **95:** 52–64.

Elix, J.A. and Gaul, K.L. 1986. The interconversion of the lichen depsides para- and meta-scrobiculin and the biosynthetic implications. *Austr. J. Chem.* **39:** 613–624.

Elix, J.A. and Gaul, K.L. 1987. A novel synthesis of the naturally occurring dibenzofurans pannaric acid and schizopeltic acid. *Austr. J. Chem.* **40:** 187–192.

Elix, J.A., Jenie, U.A., and Parker, J.L. 1987. A novel synthesis of the lichen depsidones divaricatic acid and stenosporic acid, and the biosynthetic implications. *Austr. J. Chem.* **40:** 1451–1464.

Elix, J.A., Johnston, J., and Parker, J.L. 1988. A computer program for the rapid identification of lichen substances. *Mycotaxon* **31:** 89–99.

Elix, J.A. and Parker, J.L. 1987. A novel synthesis of the naturally occurring dibenzofurans pannaric acid and schizopeltic acid. *Austr. J. Chem.* **40:** 187–192.

Fahselt, D. 1975. Gas-liquid chromatography of the lichen substance usnic acid. *Bryologist* **78:** 452–455.

Fahselt, D. 1981. Lichen products of *Cladonia stellaris* and *C. rangiferina* maintained under artificial conditions. *Lichenologist* **13:** 87–91.

Fahselt, D. 1984. Interthalline variability in levels of lichen products within stands of *Cladina stellaris*. *Bryologist* **87:** 50–56.

Fahselt, D. 1993. UV-absorbance in thallus extracts of umbilicate lichens. *Lichenologist* **25:** 415–422.

Fahselt, D., Hayden, D.B., and Mirando, M. 1973. Structure of the lichen *Parmelia aurulenta*. *Canad. J. Bot.* **51:** 2197–2200.

Fox, C.H. and Huneck, S. 1969. The formation of rocellic acid, eugenitol, eugenitin and rupicolin by the mycobiont of *Lecanora rupicola*. *Phytochemistry* **8:** 1301–1304.

Fox, C.H. and Mosbach, K. 1967. On the biosynthesis of lichen substances. Part 3. Lichen acids as products of a symbiosis. *Acta Chem. Scand. Ser. B* **21:** 2327–2330.

Froberg, L., Baur, A., and Baur, B. 1993. Differential herbivore damage to calcicolous lichens by snails. *Lichenologist* **25:** 83–95.

Garcia-Junceda, E., Cambon, M.C., and Vicente, C. 1991. Enzymatic hydrolysis of physodic acid in *Psrudevernia furfuracea*. Characterization of the product of depsidone hydrolysis. *Plant Sci.* **77:** 197–206.

Garcia-Junceda, E., Gonzalez, A., and Vicente, C. 1987. Photosynthetical and nutritional implications in the accumulation of phenols in the lichen *Psuedevernia furfuracea*. *Biochem. Syst. Ecol.* **15:** 289–296.

Garcia-Junceda, E. and Vicente, C. 1986. The use of immobilized cells to stabilize orcellinate depside hydrolase of *Pseudevernia furfuracea*. *Plant Cell Rep.* **5:** 156–157.

Gaucher, G.M. and Shepherd, M.G. 1968. Isolation of orsellinic acid synthase. *Biochem. Biophys. Res. Commun.* **32:** 664–671.

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

Goldner, W.R., Hoffman, F.M., and Medve, R.J. 1986. Allelopathic effects of *Cladonia cristatella* on ectomycorrhizal fungi common to bituminous strip-mine spoils. *Can. J. Botany* **64:** 1586–1590.

Goncalo, S., Cabral, F., and Goncalo, M. 1988. Contact sensitivity to oak moss. *Contact Dermatitis* **19:** 355–357.

Hale, M.E. 1956. Ultraviolet absorption spectra of lichen depsides and depsidones. *Science* **123:** 671.

Hale, M.E. 1972. Natural history of Plummers Island, Maryland. XXI. Infestation of the lichen, *Parmelia baltimorensis* Gyel. & For. by *Hypogastrura packardi* Folsom (Collembola) *Proc. Biol. Soc. Wash.* **85:** 287–296.

Hale, M.E. 1979. *How to Know the Lichens.* Wm. C. Brown Co., Dubuque.

Hale, M.E. 1983. *The Biology of Lichens.* Edward Arnold, Baltimore.

Hamada, N. 1982. The effect of temperature on the content of the medullary depsidone salazinic acid in *Ramalina siliquosa* (lichens). *Canad. J. Bot.* **60:** 383–385.

Hamada, N. 1983. The effect of temperature on lichen substances in *Ramalina subbreviuscula* (Lichens). *Bot. Mag. Tokyo* **96:** 121–126.

Hamada, N. 1984. The content of lichen substances in *Ramalina siliquosa* cultured at various temperatures in growth cabinets. *Lichenologist* **16:** 96–98.

Hamada, N. 1988a. Depside from isolated lichen mycobiont. II. *Lichenologist* **20:** 294–295.

Hamada, N. 1988b. Individual variation in the contents of lichen substances in *Ramalina nervulosa* and *R. subcomplanata. Bryologist* **91:** 95–97.

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

Hamada, N. 1991. Environmental factors affecting the content of usnic acid in the lichen mycobiont of *Ramalina siliquosa. Bryologist* **94:** 57–59.

Hamada, N. and Ueno, T. 1987. Depside from an isolated lichen mycobiont. *Agric. and Biol. Chem.* **51:** 1705–1706.

Hamada, N. and Ueno, T. 1990. Lecanoric acid from the mycobiont of the lichen *Stereocaulon curtatum. Phytochemistry* **29:** 678–679.

Hardie, D.G. and McCarthy, A.D. 1986. Evolution of fatty acid synthase systems by gene fusion. In: *Multidomain Proteins – Structure and Evolution.* D.G. Hardie and J.R. Coggins, eds. Elsevier, Amsterdam, pp. 229–258.

Harmala, P., Hiltunen, R., Oksman-Caldentey, K.M., Laasko, T., and Kauppinen, V. 1992. Isolation and *in vitro* cultivation of lichen algae and their antimicrobial properties. *Fitoterapia* **63:** 217–225.

Hawksworth, D.L. and Hill, D.J. 1984. *The Lichen-forming Fungi.* Blackie, London.

Herrero-Yudego, P., Martin-Pedrosa, M., Morato, J., and Vicente, C. 1989. Some features about usnic acid accumulation and its movement between the symbionts of *Evernia prunastri. J. Plant Physiol.* **135:** 170–174.

Hickey, B.J., Lumsden, A.J., Cole, A.L.J., and Walker, J.R.L. 1990. Antibiotic compounds from New Zealand plants: methyl hematommate, an anti-fungal agent from *Stereocaulon ramulosum*. *N. Z. Nat. Sci.* **17:** 49–54.

Hill, D.J. 1985. Changes in photobiont dimensions and numbers during development of lichen symbionts. In: *Lichen Physiology* and *Cell Biology*. D.H. Brown, ed. Plenum Press, New York, pp. 303–317.

Hill, D.J. and Woolhouse, H.W. 1966. Aspects of the autecology of *Xanthoria parietina* agg. *Lichenologist* **3:** 207–214.

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*. *Chem. Pharm. Bull.* **37:** 2410–2412.

Honegger, R. 1986. Ultrastructural studies in lichens II. Mycobiont and photobiont cell wall surface layers and adhering crystalline lichen products in four Parmeliaceae. *New Phytol.* **103:** 797–808.

Honegger, R. 1987. Questions about pattern formation in the algal layer of lichens with stratified (heteromerous) thalli. *Bibl. Lichenol.* **25:** 59–71.

Honegger, R. 1991. Functional aspects of the lichen symbiosis. *Ann. Rev. Plant Physiol. and Plant Mol. Biol.* **42:** 533–578.

Honegger, R. and Kutasi. 1990. Anthraquinone production in three aposymbiotically cultured teloschistalean lichen mycobionts: the role of the carbon source. In: *Endocytobiology IV*. P. Nardon, V. Gianinazzi-Pearson, A.M. Grenier, L. Margulis and D.C. Smith, eds. Institut National de la Recherche Agronomique, Paris, pp. 175–178.

Hunaiti, A.A., El-Oqlah, A.A., and Abu-Gharibeh, K. 1988. Hemolytic toxin of the lichen *Parmelia pulla*. *J. Biol. Sci. Res.* **19 (Supp.):** 843–852.

Huneck, S. 1968. Lichen substances. In: *Progress in Phytochemistry*, Vol. 1. L. Reinhold and Y. Liwschitz, eds. Interscience Publishers, New York, pp. 223–346.

Huneck, S., Jakupovic, J., and Follman, G. 1991. 3-O-Methylpannaric acid from the lichen *Rocella capensis*. *Z. Naturforsch.* **46b:** 969–970.

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

Huneck, S., Porzel, A., Schmidt, J., and Follman, G. 1992. Hypothallin, ein weiterer Vertreter eines Aminosäure-aminoalkahol-esters aus der Krustenflecthe *Schismatomma hypothallinum*. *Z. Naturforsch.* **47c:** 785–790.

Huneck, S., Schmidt, J., and Tabacchi, R. 1989. Thermal decomposition of lichen depsides. *Z. Naturforsch.* **44b:** 1283–1289.

Huovinen, K. 1985. Variation of lichen acids in *Cladina stellaris* and *Cladina rangiferina* in Finland and North Norway. *Acta Pharm. Fenn.* **94:** 113–123.

Huovinen, K. 1986. *Chromatographic Studies on the Aromatic Lichen Substances in Cladina and Cladonia, Section Unciales.* Ph.D. thesis. University of Helsinki, Helsinki.

Huovinen, K. 1987. A standard HPLC method for the analyses of aromatic lichen compounds. *Bibl. Lichenol.* **25:** 457–466.

Huovinen, K. and Ahti, T. 1982. Biosequential patterns for formation of depsides, depsidones and dibenzofurans in the genus *Cladonia* (lichen-forming ascomycetes). *Ann. Bot. Fenn.* **19:** 225–234.

Huovinen, K. and Ahti, T. 1988. The composition and contents of aromtic lichen substances in *Cladonia* section Perviae. *Ann. Bot. Fenn.* **25:** 371–383.

Huovinen, K., Ahti, T., and Stenroos, S. 1989. The composition and contents of aromatic lichen substances in *Cladonia*, section *Cladonia* and group Furcatae. *Bibl. Lichenol.* **38:** 209–241.

Huovinen, K., Hiltunen, R., and Schantz, M. von. A high performance liquid chromatographic method for the analysis of lichen compounds from the genera *Cladina* and *Cladonia*. *Acta Pharm. Fenn.* **94:** 99–112.

Jones, D. 1988. Lichens and pedogenesis. In: *Handbook of Lichenology*, Vol. III. M. Galun, ed. CRC Press, Boca Raton, FL, pp. 109–124.

Kallman, S. 1992. Wild plants as food during survival situations. *Sven. Bot. Tidskr.* **86:** 49–59.

Kinoshita, Y., Yamamoto, Y., Yoshimura, I., and Yamada, Y. 1993. Effects of culture condition on lichen metabolite production by *Usnea hirta*. In: *XV International Botanical Congress Abstracts*, International Union of Biological Sciences. Yokohama, Japan, p. 372.

Kinraide, W.T.B. and Ahmadjian, V. 1970. The effects of usnic acid on the physiology of two cultured species of the lichen alga *Trebouxia* Puym. *Lichenologist* **4:** 234–247.

Klee, R. and Steubing, L. 1977. Der Veränderung der Atranorinkorzentration in Laubflechten durch Licht, Wasserversorgung und SO_2-Begasung. *Bryologist* **80:** 171–174.

Komamine, A., Misawa, M., and DiCosmo, F. 1991. *Plant Cell Culture in Japan.* CMC Co., Ltd., Tokyo.

Komiya, T. and Shibata, S. 1969. Formation of lichen substances by mycobionts of lichens. Isolation of (+)-usnic acid and salazinic acid from mycobionts of *Ramalina* species. *Chem. Pharm. Bull.* **17:** 1305–1306.

Kon, Y., Yoshida, Y., Kashiwadani, H., Masada, M., and Tamura, G. 1993. Compound produced by the *Usnea confusa* ssp. *kitamiensis* mycobiont cultured with sugar alcohols. In: *XV International Botanical Congress Abstracts*, International Union of Biological Sciences, Yokohama, Japan, p. 372.

Larson, D.W. 1983. The pattern of production within individual *Umbilicaria* lichen thalli. *New Phytol.* **94:** 409–419.

Larson, D.W. and Carey, C.K. 1986. Phenotypic variation within "individual" lichen thalli. *Am. J. Bot.* **73:** 214–223.

Lawrey, J.D. 1984. *Biology of Lichenized Fungi.* Praeger Publishers, New York.

Lawrey, J.D. 1986. Biological role of lichen substances. *Bryologist* **89:** 111–122.

Lawrey, J.D. 1989. Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. *Bryologist* **92:** 326–328.

Lawrey, J.D. 1991. Biotic interactions in lichen community development: a review. *Lichenologist* **23:** 205–214.

Lawrey, J.D. 1993. Lichen allelopathy. *Am. J. Bot.* **80 (suppl.):** 103.

Lechowicz, M.J. 1983. Age dependence of photosynthesis in the caribou lichen *Cladina stellaris. Plant Physiol.* **71:** 893–895.

Legaz, M.E. and Vicente, C. 1989. Regulation of urease activity of *Cladina dendroides* and its photobiont by lichen phenols. *Plant Sci. (Shannon)* **63:** 15–24.

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

Leuckert, C., Knopf, J.-G., and Hertel, H. 1990. Saxicolous European *Lecidella* species (Lichenes) containing chloroxanothones. In: *International Mycological Congress Abstracts*, Regensburg, Germany, p. 33.

Lima, R.C., Nascimento, S.C., Pereira, E.C.G., and Campos-Takaki, G.M. 1990. Cytotoxic and antitumor activity of lichen extracts. *Biol. Soc. Brot.* **63:** 339–348.

Luckner, M. 1984. *Secondary Metabolism in Microorganisms, Plants and Animals.* Springer-Verlag, Berlin.

Lundstrum, H. and Henningsson, B. 1973. The effect of ten lichens on the growth of wood-destroying fungi. *Mater. Org. Beih.* **8:** 233–246.

Manrique, E. and Lopez, F. 1991. Intrathalline variation in concentration of lichen substances in *Pseudevernia furfuracea. Bryologist* **94:** 207–212.

Martin, J.F. and Demain, A.L. 1980. Control of antibiotic biosynthesis. *Microbiol. Rev.* **44:** 230–251.

Mateos, J.L., Conde, E., Miranda, T., and Vicente, C. 1991. Regulation mechanisms of phenolic production in the lichen *Himantormia lugubris*, as deduced from the analysis of metabolite accumulation. *Plant Science* **77:** 1–9.

Mattsson, J.-E. 1987. Zeorin in *Cetraria pinastri* from an unusual habitat. *Bibl. Lichenol.* **25:** 207–208.

Matubara, H., Takatori, K., Koyama, K., and Takahashi, K. 1993. Antityrosinase active compounds from lichens, *Protousnea magellanica, P. malacea* and *P. dusenii.* In: *XV International Botanical Congress Abstracts*, International Union of Biological Sciences, Yokohama, Japan, p. 380.

Mirando, M. and Fahselt, D. 1978. The effect of thallus age and drying procedure on extractable lichen substances. *Canad. J. Bot.* **56:** 1499–1504.

Mosbach, K. 1964. On the biosynthesis of lichen substances. Part 1. The depside gyrophoric acid. *Acta Chem. Scand.* **18:** 329–334.

Mosbach, K. 1967. On the biosynthesis of lichen substances. Part 4. The formation of pulvic acid derivatives by isolated lichen fungi. *Acta Chem. Scand.* **21:** 2331–2334.

Mosbach, K. 1973. Biosynthesis of lichen substances. In: *The Lichens.* V. Ahmadjian and M.E. Hale, eds. Academic Press, New York, pp. 523–546.

Mosbach, K. and Ehrensvard, U. 1966. Studies on lichen enzymes I. Preparation and properties of a depside hydrolysing esterase and of orsellinic acid decarboxylase. *Biochem. Biophys. Res. Commun.* **22:** 145–150.

Nakano, H., Komiya, T., and Shibata, S. 1972. Anthraquinones of the lichens of *Xanthoria* and *Caloplaca* and their cultivated mycobionts. *Phytochemistry* **11:** 3505–3508.

Nieminen, M. and Heiskari, U. 1989. Diets of freely grazing and active reindeer during summer and winter. *Rangifer* **9:** 17–34.

Nishikawa, Y., Ohki, K., Takahashi, K., Kurono, G., Fukuoka, F., and Emori, M. 1974. Studies on the water soluble constituents of lichens. II. Antitumor polysaccharides of *Lasallia, Usnea* and *Cladonia* species. *Chem. Pharm. Bull.* **22:** 2692–2702.

Nishikawa, Y., Tanaka, M., Shibata, S., and Fukuoka, F. 1970. Polysaccharides of lichens and fungi. IV. Antitumour active O-acetylated pustulan-type glucans from the lichens of *Umbilicaria* species. *Chem. and Pharm. Bull.* **18:** 1431–1434.

Osawa, T., Kumon, H., Reece, C.A., and Shibamoto, T. 1991. Inhibitory effect of lichen constituents on mutagenicity induced by heterocyclic amines. *Environ. Mol. Mutagen.* **18:** 35–40.

Packter, N.M. 1980. Biosynthesis of acetate-derived phenols (polyketides). In: *The Biochemistry of Plants*, Vol. 4. P.K. Stumf and E.E. Conn, eds. Academic Press, New York, pp. 535–570.

Pedrosa, M.M. and Legaz, M.E. 1991. Binding studies of evernic acid to purified constitutive arginase of *Evernia prunastri* by SE-HPLC. *Symbiosis* **11:** 345–357.

Pereira, E.C., de Campos-Takaki, da Silva, N.H., Vicente, C., Legaz, M.E., and Xavier-Filho, L. 1991. Fractionation of *Cladonia substellata* crude extracts and detection of antimicrobial activity. *Bol. Soc. Brot. Ser. 2* **64:** 173–186.

Perez-Urria, E. and Vicente, C. 1988. Purification and some properties of a secreted urease from *Evernia prunastri* thallus. *J. Plant Physiol.* **133:** 692–695.

Planelles, V. and Legaz, M.C. 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.

Polonia, M.A.J., Carneiro, A.F., and Pinto, M.M.M. 1991. Quantification of usnic acid in *Usnea florida* by densitometric high performance thin layer chromatography. *J. Liq. Chromatogr.* **14:** 1447–1452.

Posner, B., Feiger, G.B., and Huneck, S. 1990. Phytochemische Untersuchungen an westeuropäischen *Lasallia-* Artin. *Z. Naturforsch.* **45C:** 161–165.

Posner, B., Feige, G.B., and Leuckert, C. 1991. Beitrage zur Chemie der Flechtengattung *Lasallia* Merat. *Z. Naturforsch.* **46C:** 19–27.

Rao, D.N. and LeBlanc, F. 1965. A possible role of atranorin in the lichen thallus. *Bryologist* **68:** 284–289.

Rao, P.S., Sarma, K.G., and Seshadri, T.R. 1967. The ultraviolet and infrared spectra of some lichen depsides and depsidones. *Proc. Indian Acad. Sci.* **66A:** 1–14.

Remaut, K. and Thune, P. 1992. Allergic contact dermatitis caused by cosmetic products: frequency. *Tidsskr. Nor. Laegeforen.* **112:** 1275–1277.

Renner, B. and Gerstner, E. 1980. Correlation between media and anthraquinoids produced by the isolated mycobiont of *Caloplaca ferruginea. Naturwiss.* **67:** 352–353.

Richardson, D.H.S. 1985a. The surface physiology of lichens with particular reference to carbohydrate transfer between the symbionts. In: *Surface Physiology of lichens.* C. Vicente, D.H. Brown and M.E. Legaz, eds. Universidad Complutense de Madrid, Madrid, pp. 25–55.

Richardson, D.H.S. 1985b. *The Vanishing Lichens.* Douglas David and Charles Limited, Vancouver.

Richardson, D.H.S. 1988. Medicinal and other economic aspects of lichens. In: *Handbook of Lichenology,* Vol. III. M. Galun, ed. CRC Press, Boca Raton, pp. 93–108.

Rowe, J.G., Saenz, M.T., Garcia, M.D., and Gil, A.M. 1991. New study of antimicrobial activity and identification of lichenical substances of some lichens from south Spain. *Ann. Pharm. Fr.* **49:** 278–285.

Rundel, P.W. 1979. Clinal variation in the production of usnic acid in *Cladonia subtenuis. Bryologist* **72:** 40–44.

Sage, L.C. 1992. *Pigments of the Imagination. A History of Phytochrome Research.* Academic Press, New York.

Santesson, J. 1969. Chemical studies on lichens. 10. Mass spectrometry of lichens. *Ark. Kemi* **30:** 363–377.

Santesson, J. 1970. Identification and isolation of lichen substances. In: *The Lichens.* V. Ahmadjian and M.E. Hale, eds. Academic Press, New York, pp. 633–652.

Schultz, H. and Albroscheit, G. 1989. Characterization of oak moss products used in perfumery by high-performance liquid chromatography. *J. Chromatogr.* **466:** 301–306.

Schultz, J. and Mosbach, K. 1971. Studies on lichen enzymes. Purification and properties of an orsellinate depside hydrolase obtained from *Lasallia pustulata. Eur. J. Biochem.* **22:** 153–157.

Serina, E., Arroya, R., and Manrique, E. 1991. Variaciones intratalinas de las sustancias liquenicas con la edad en *Lasallia pustulata* (L.) Merat. *Acta Bot. Malacitana* **16:** 157–163.

Stafford, H.A. 1990. *Flavonoid Metabolism.* CRC Press, Boca Raton, FL.

Stephenson, N.L. and Rundel, P.W. 1979. Quantitative variation and the ecological role of vulpinic acid and atranorin in the thallus of *Letharia vulpina. Biochem. Syst. Ecol.* **7:** 263–267.

Tabacchi, R. 1993. Terpenoids and steroids of lichens. In: *XV International Botanical Congress Abstracts,* International Union of Biological Sciences, Yokohama, Japan, p. 99.

Tabacchi, R., Allemand, P., and Tsoupras, G. 1991. Direct analysis of lichens by tandem mass spectrometry. *Symbiosis* **11:** 193–206.

Taguchi, H., Sankawa, U., and Shibata, S. 1966. Biosynthesis of usnic acid in lichens. *Tetrahed. Let.* **42:** 5211–5214.

Taguchi, H., Sankawa, U., and Shibata, S. 1969a. Biosynthesis of natural products. VII. Biosynthesis of usnic acid in lichens, seasonal variation observed in usnic acid biosynthesis. *Chem. Pharm. Bull.* **17:** 2061–2064.

Taguchi, H., Sankawa, U., and Shibata, S. 1969b. Biosynthesis of natural products. VI. Biosynthesis of usnic acid in lichens (1). A general scheme of biosynthesis of usnic acid. *Chem. Pharm. Bull.* **17:** 2054–2060.

Takahashi, K., Koyama, K., Takatori, K., and Matubara, H. 1993. Topics in the chemistry of lichen compounds. In: *XV International Botanical Congress Abstracts*, International Union of Biological Sciences, Yokohama, Japan, p. 99.

Takai, M., Uehara, Y., and Beisler, J.A. 1979. Usnic acid derivatives as potential antineoplastic agents. *J. Med. Chem.* **22:** 1380–1384.

Thomas, E. 1939. Über die Biologie von Flechtenbildnern. *Beitr. Kryptogamenflora Schweiz* **9:** 1–108.

Thomson, J.W. 1984. *American Arctic Lichens 1. The Macrolichens.* Columbia University Press, New York.

Toledo, I., Aguirre, J., and Hansberg, W. 1986. Aerial growth in *Neurospora crassa:* characterization of an experimental model system. *Exp. Mycol.* **10:** 114–125.

Turner, N.J., Thompson, L.C., Thompson, M.T., and York, A.Z. 1990. *Thompson Ethnobotany.* Royal British Columbia Museum, Victoria.

Turner, W.B. 1976. Polyketides and related metabolites. In: *The Filamentous Fungi, Vol. 2, Biosynthesis and Metabolism.* J.E. Smith and D.R. Berry, eds. Edward Arnold, Baltimore, pp. 445–459.

Vavasseur, A., Gautier, H., Thibaud, M.-C., and Lasceve, G. 1991. Effects of usnic acid on the oxygen exchange properties of mesophyll cell protoplasts from *Commelina communis* L. *Plant Physiol.* **139:** 90–94.

Vicente, C. 1985. Surface physiology of lichens: facts and concepts. In: *Surface Physiology of Lichens.* C. Vicente, D.H. Brown and M.E. Legaz, eds. Universidad Complutense de Madrid, Madrid, pp. 11–24.

Vicente, C. 1991. Biochemical and environmental influence on the synthesis and accumulation of lichen phenolics. *Symbiosis* **11:** 279–297.

Vicente, C. and Avalos, A. 1986. Phytochrome activates orcellinate depside hydrolase. *Lichen Physiol. Biochem.* **1:** 77–84.

Vicente, C., Azpiroz, A., Estevez, M.P., and Gonzalez, M.L. 1978. Quaternary structure changes and kinetics of urease inactivation by L-usnic acid in relation to the regulation of nutrient transfer between lichen symbionts. *Plant Cell Environ.* **1:** 29–33.

Vicente, C. and Legaz, M.E. 1988. Lichen enzymology. In: *Handbook of Lichenology*, Vol. I. M. Galun, ed. CRC Press, Boca Raton, FL, pp. 239–281.

Vicente, C. and Perez-Urria, E. 1988. Tolbutamide, a urea-derivative, impedes phenolic accumulation in the lichen *Evernia prunastri. J. Plant Physiol.* **132:** 580–583.

Vicente, C., Ruiz, J.L., and Estevez, M.P. 1980. Mobilization of usnic acid in *Evernia prunastri* under critical conditions of nutrient availability. *Phyton Rev. Int. Bot. Exp.* **39:** 15–20.

Vining, L.C. 1992. Roles of secondary metabolites from microbes. In: *Secondary Metabolites: Their Function and Evolution.* Ciba Foundation Symposium 171, Wiley, New York, pp. 184–194.

Wang, I.K., Reeves, C., and Gaucher, M. 1991. Isolation and sequencing of a genomic DNA clone containing the 3′ terminus of the 6-methysalicylic acid polyketide synthetase gene of *Penicillium urticae. Canad. J. Microbiol.* **37:** 86–95.

Watanabe, M., Iwai, K., Shibata, S., Takahashi, K., Narui, T., and Tashiro, T. 1986. Purification and characterization of mouse, alphal-acid glycoprotein and its possible role in the antitumor activity of some lichen polysaccharides. *Chem. Pharm. Bull.* **34:** 2532–2541.

Webb, E.C. 1984. *Enzyme Nomenclature.* Academic Press, New York.

White, F.J. and James, P.W. 1985. A new guide to microchemical techniques for the identification of lichen substances. *British Lichen Soc. Bull.* **57 (Suppl.):** 1–41.

Wilkins, A.L. and Elix, J.A. 1990. New fernene triterpenes from the lichen *Pseudocyphellaria aurata. Austr. J. Chem.* **43:** 623–628.

Wilkins, A.L., Elix, J.A., Gaul, K.L., and Moberg, R. 1989. New hopane triperpenoids from lichens in the family Physiaceae. *Austr. J. Chem.* **42:** 1415–1422.

Wilson, M.J. 1992. Interactions between saxicolous lichens and rocks; a review of developments in the past decade. In: *Second International Lichenological Symposium Abstracts.* I. Karnefelt, ed. Department of Systematic Botany, Lund, p. 101.

Xavier-Filho, L., Leite, J.B.M., and de O. Lima, E. 1990. Atividade antimicrobiana de liquens antarticos. *Bol. Soc. Brot. (Ser. 2)* **63:** 93–98.

Xavier-Filho, L., de O. Lima, E., and de Queiroz Paulo, M. 1992. Atividade antimicrobiana de substancias liquenicas I. *Rev. Bras. Farm.* **73:** 15–18.

Yamamoto, Y., Mitsuguchi, R., and Yamada, Y. 1985. Tissue culture of *Usnea rubsecens* and *Ramalina yasudae* and production of usnic acid in their cultures. *Agric. and Biol. Chem.* **49:** 3347–3348.

Yamamoto, Y., Miura, Y., Higuchi, M., Kinoshita, Y., and Yoshimura, I. 1993. Using lichen tissue cultures in modern biology. *Bryologist* **96:** 384–393.

Yamazaki, M., Matsuo, M., and Shibata, S. 1965. Biosynthesis of lichen depsides, lecanoric acid and atranorin. *Chem. Pharm. Bull.* **13:** 1015–1017.

Yamazaki, M. and Shibata, S. 1966. Biosynthesis of lichen substances. II. Participation of C1-unit to the formation of beta-orcinol type lichen depside. *Chem. Pharm. Bull.* **14:** 96–97.

Yom-Tov, Y. and Galun, M. 1971. Note on feeding habits of the desert snails *Sphincterochila boissieri* Charpentier and *Trochidea (Xerocrassa) seetzeni* Charpentier. *Veliger* **14:** 86–88.

Yoshimura, I., Kinoshita, Y., Kurokawa, T., and Yamamoto, Y. 1993. Lichen substances of some cultured lichens. In: *XV International Botanical Congress Abstracts*. International Union of Biological Sciences, Yokohama, Japan, p. 99.

Yoshimura, I., Kurokawa, T., Takano, T., and Yamamoto, Y. 1989. Production of secondary metabolic substances by cultured tissues of *Usnea flexilis*. *Bull. Kochi Gakuen* **20:** 535.