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Chemical Evolution in the Order Peltigerales: Triterpenoids

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Abstract

The order Peltigerales in the Ascomycotina consists of five families and 16 genera. It is presumed to be of great antiquity as evidenced by a very wide diversity of lichenicolous fungi coevolved with the major lichen-forming genera, by the great diversity of secondary metabolites found in the order, and by global distribution patterns. Both primary (lipids and proteins) and secondary metabolites may have taxonomic utility in the Peltigerales in addition to their presumed biological functions. Triterpenoids which are widespread in certain general in the order are discussed as an example of a group of compounds that may have implications for the detection of evolutionary relationships in this group of lichen-forming fungi.

Keywords: Peltigerales, triterpenoids, evolution, biogeography, hopane, stictane, fernene, lupane

1. Introduction

The Ascomycete order Peltigerales as presently understood comprises 5 families, 16 genera and about 600 species (Henssen and Jahns, 1973; Eriksson, 1981; Hale, 1983; Hawksworth et al., 1983; Eriksson and Hawksworth, 1990). Eriksson (1981) and Eriksson and Hawksworth (1990) regard the family Placynthiaceae (syn. Leciotheciaceae) [*Hertella, Koerberia, Leptochidium,*

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Placynthiopsis, Placynthium, Polychidium, Vestergrenopsis] as a distinct clade, and the families Lobariaceae [*Lobaria, Pseudocyphellaria*], Stictaceae [*Dendriscocaulon, Sticta*], Nephromataceae [*Nephroma*], and Peltigeraceae [*Hydrothyria, Massalongia, Peltigera, Solorina*] as another distinct clade. I have separated Stictaceae from Lobariaceae on grounds suggested earlier (Galloway, 1988b, p. 26) and consider these four latter families as most likely constituting Peltigerales sens. str. Certainly there are strong morphological, anatomical, morphogenetic, and chemical arguments which would support this disposition [see also Hafellner (1988)].

A scheme correlating ascus morphology, photobionts, and habitat ecology in the Ascomycotina gives the Peltigerales a central position (Dick and Hawksworth, 1985) reflecting several lines of evidence that the order includes taxa retaining characters of particular antiquity (Hawksworth, 1982, 1988a, 1988b, 1988c). These include: (1) Studies on ascus structure (see Eriksson, 1981; Hawksworth, 1982), the semifissitunicate ascus of the Peltigerales theoretically being a possible progenitor of both ascohymenial unitunicate, and ascolocular bitunicate lines; (2) The exceptional number of genera and species of obligately lichenicolous fungi parasitizing members of the Peltigerales and in the Peltigeraceae in particular, have significant coevolutionary or coadaptationary implications (Hawksworth, 1982, 1988a,b). (3) Evidence from known geographical distributions of taxa in the order when present distributions are discussed in the light of current biogeographical theories (Hawksworth, 1982, 1988a; Galloway, 1987, 1988a,b). (4) Cyanobacterial symbionts are common in the order both as primary photobionts and as cephalodia (James and Henssen, 1976; Galloway, 1988b). Cyanobacteria are known to be of ancient origin (Schopf and Walter, 1982) and would have been among the earliest potential photobionts available for any emerging fungi (Hawksworth, 1988a). The Peltigerales may therefore preserve elements from palaeophytic or proterophytic times (see terminology in Traverse, 1988) in aspects of their symbiotic associations involving consideration of photobionts, coevolved or coadapted lichenicolous fungi and also their chemistry which is discussed further below.

2. Chemistry

Lichens produce a wide range of primary and secondary metabolites (Culberson, 1969; Elix et al., 1984; Huneck, 1984, 1991), the latter being widely used by lichenologists in systematic studies, with chemical characters commonly invoked to support or deny variation in morphological characters and in geographical distributions of taxa (Elix, 1982; Brodo, 1986; Egan, 1986; Rogers, 1989). W.L. Culberson (1986) has drawn attention to the importance

of chemical characters in sibling speciation in lichen-forming fungi where chemical and ecological differentiation is observed in taxa having highly conservative morphologies, underlining the view that chemistry is a major marker of evolutionary change in the lichens. His view is further vindicated by the demonstration for the first time of gene flow in lichen fungi by an analysis of secondary metabolites in the progeny of individuals from natural populations of mixed chemodemes of the *Cladonia chlorophaea* complex (Culberson et al., 1986).

The increasing sophistication of techniques for detection of secondary metabolites in lichens (Culberson et al., 1987; Tabacchi, 1991) allows the elaboration of biosynthetic hypotheses which can be used in cladistic analyses of evolutionary relationships among taxa (Culberson, 1986; Culberson et al., 1987), a field ripe for further investigation not least in the Peltigerales.

In genera of the Peltigerales sens. str., a wide chemical diversity is found, with substances represented from all three major pathways of secondary metabolism in lichens viz., the acetate-polymalonate pathway (producing orcinol depsides, β-orcinol depsides and depsidones and usnic acid), the shikimic acid pathway (producing terphenylquinones and pulvinic acid derivatives) and the mevalonic acid pathway (producing sterols and terpenoids). The family Stictaceae produces no acetone-soluble compounds and instead has quantities of simple carbohydrates and methylamine. In the families Peltigeraceae, Solorinaceae, and Nephromataceae, compounds from both the acetate-polymalonate and mevalonic acid pathways are produced. In the Lobariaceae the most complex chemistry in the order is encountered, with *Pseudocyphellaria* having the most richly diverse chemistry of any genus, with several compounds synthesized via the shikimic acid pathway in addition to contributions from the other biosynthetic pathways. This genus is also rich in triterpenoids from several different series, exhibiting an evolution in chemical complexity (Galloway, 1988b).

Triterpenoids

Terpenoids are compounds with varying numbers of carbon atoms derived from C-5 (isoprenoid) units. They are widespread in the plant world and it is probable that more terpenes and terpenoids exist than any other group of plant products. Photosynthesis, the essential process on which all plant life depends, has a mandatory requirement for certain terpenoids and their derivatives (e.g. carotenoids and chlorophylls) and many plant hormones are terpenoids. Studies of carotenoids from various areas of the world (Czeczuga, 1988) and from examples of the families Lobariaceae, Peltigeraceae and Stictaceae in the Peltigerales (Czeczuga, 1980, 1988; Czeczuga and Richardson, 1989) show that

a considerable diversity of these compounds occurs in lichens, with some being specific to particular taxa. Although lichen carotenoid content may be influenced by environmental factors, carotenoid profiles in lichens may be useful in taxonomic studies (Czeczuga and Richardson, 1989). The rich speciation of taxa in the Peltigerales in the Southern Hemisphere would seem to offer considerable scope for further studies in lichen carotenoids and their possible use in phylogenetic reconstructions.

Chemically all terpenes and terpenoids are derived from a basic 5-carbon isoprene building block, and they are classified according to the number of such units in the molecule. Triterpenoids usually have a skeleton of 30 carbon atoms derived from the acyclic hydrocarbon squalene, itself formed through the mevalonic acid pathway (Goodwin and Mercer, 1983, Nes, 1990). In a recent review of the biochemistry of the mevalonic pathway, Towers and Stafford (1990) record "Life, as we know it, would not be possible without the ability of living organisms to employ this metabolic sequence which proceeds from condensations of three molecules of acetyl-CoA and terminates with the elaboration of the terpenoid precursors, isopentenyl pyrophosphate and dimethylallyl pyrophosphate. In addition to producing obviously essential compounds that are partially or completely of isoprenoid origin such as hormones, photsynthetic pigments, compounds involved in electron transport in respiration and in photosynthesis, oxidative enzymes and membrane components, plants elaborate thousands of novel terpenoids, many of which do not as yet have identifiable physiological, biochemical or even ecological roles, e.g. the cardenolides, ecdysones or saponins... Studies of the chemical signalling between plants and organisms, ranging from bacteria to mammals have expanded tremendously in recent years, and, in many cases terpenoids have been shown to be involved in these interactions".

As a group, triterpenoids occur widely in plants, many being well-known toxins such as saponins and cardiac glycosides, with the structures of some 750 triterpenoid glycosides presently known (Hiller, 1987). The comparative biochemistry of triterpenoids has been extensively studied in higher plants, pteridophytes (Berti and Bottari, 1968), bryophytes (Markham and Porter, 1978), algae and fungi, with taxonomic implications being deduced from their distribution patterns (Harborne and Turner, 1984).

In the order Peltigerales, triterpenoids are especially richly developed in the genera *Nephroma* (Wetmore, 1960; Galloway, 1985; James and White, 1987; White and James, 1988), *Peltigera* (Tonsberg and Holtan-Hartwig, 1983; Vitikainen, 1985; Galloway, 1985; Holtan-Hartwig, 1988) and *Pseudocyphellaria* (Galloway, 1988b) and besides having an obvious and important utility in separating taxa at the species level they may also prove to be of

importance in phylogenetic studies in these genera. Brodo (1984) used triterpenoids (only zeorin identified) to help separate taxa in the *Lecanora subfusca* group in North America. In four new species of *Ramalina* from Port Santo, Madiera (Krog, 1990) triterpenoid patterns were known to be constant in the four taxa although the terpenoids were not identified.

Triterpenoids have rather complex structures with the main groups consisting of tetracyclic derivatives based on parent hydrocarbons such as lanostane, cycloartane and dammarane, and pentacyclic compounds derived from ursane, oleanane, lupane, hopane and related skeletons.

They are readily detected on thin layer chromatograms being visualised only on developed plates after acid spray and charring (see White and James, 1985, pp. 32–34), giving purple or mauve spots in daylight and appearing distinctively salmon pink under UV at 350 μm (Wilkins and James, 1979). Triterpenoid patterns from thin layer chromatograms run in several solvent systems (Culberson et al., 1981; White and James, 1985) are shown in Fig. 1 for species of *Pseudocyphellaria*; in Fig. 2 for species of *Peltigera*; and in Fig. 3 for species of *Nephroma*.

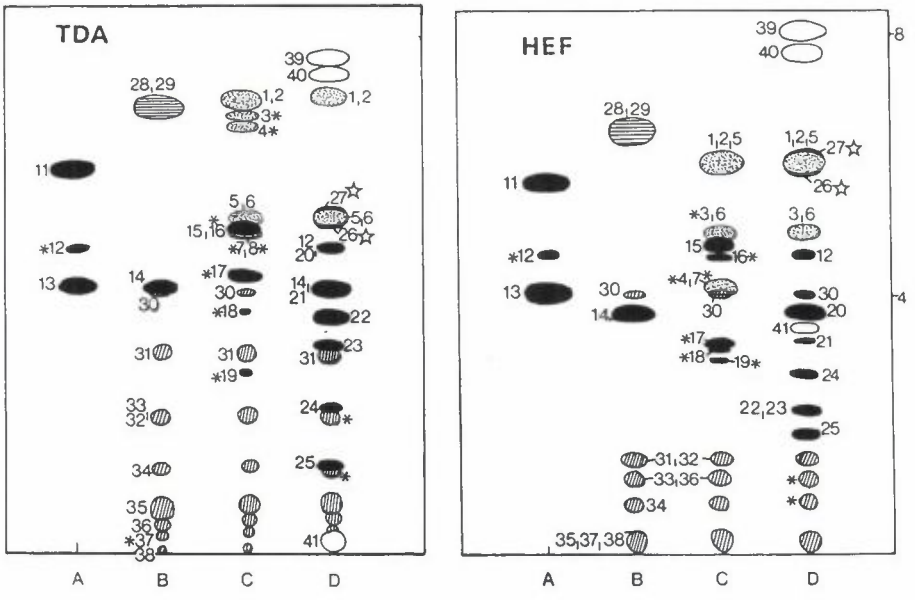


Figure 1. Triterpenoids (black spots) from four species of *Pseudocyphellaria* from New Zealand, run in solvents TDA and HEF (after Wilkins and James, 1979). (A) *P. rufovirescens*, (B) *P. faveolata*, (C) *P. billardierei*, (D) *P. carpoloma*.

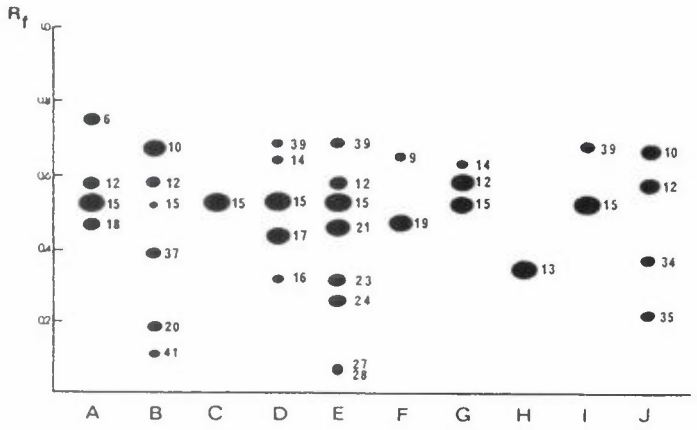


Figure 2. Chromatogram of the triterpenoid composition in Scandinavian species of *Peltigera* and their chemodemes, developed twice in solvent EHF (after Holtan-Hartwig, 1988), (A) *P. scabrosella*; (B–C) *P. scabrosa*; (D) *P. frippii*; (E) *P. neckeri*; (F–I) *P. malacea*; (J) *P. lyngei*.

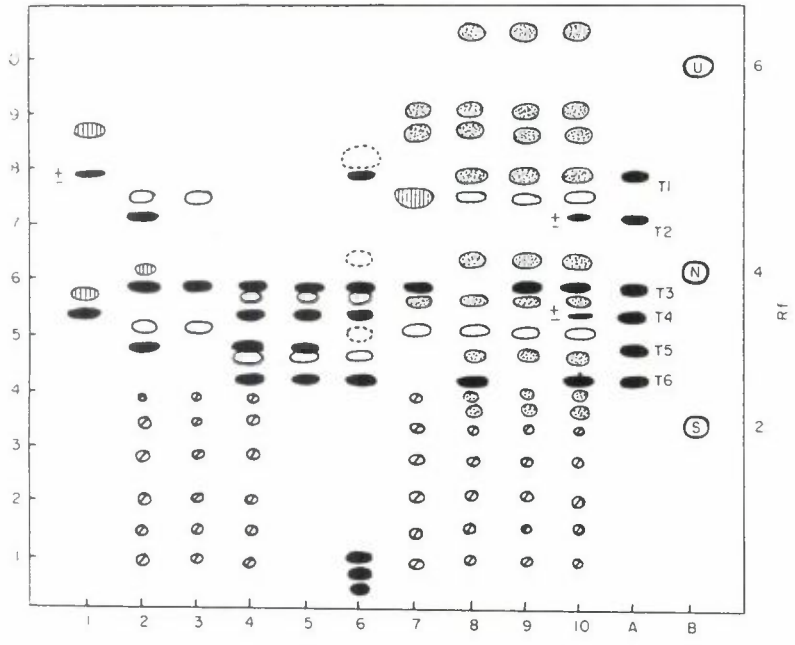


Figure 3. Characteristic TLC patterns of Macaronesian species of *Nephroma* in solvent G. Triterpenoids are blackspots (after James and White, 1987). (1) *N. helveticum*; (2) *N. parile*; (3) *N. foliolatum*; (4) *N. areolatum*; (5) *N. sulcatum*; (6) *N. hensseniae*; (7) *N. venosum*; (8) *N. laevigatum* (race 1); (9) *N. laevigatum* (race 2); (10) *N. tangierense*.

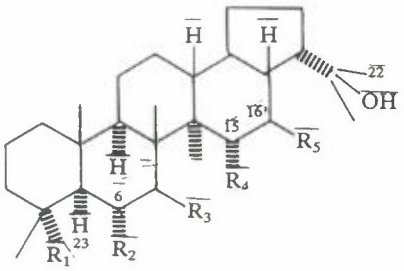
Hopanes

Triterpenoids of the hopane series are the most widely distributed of triterpenoids in lichens being known from species of *Heterodermia, Lobaria, Nephroma* (White and James, 1987, pp. 218–220), *Peltigera* and *Pseudocyphellaria* (Galloway, 1988b), and *Physcia* and *Rinodina* (Elix et al., 1982; Wilkins et al., 1989). Hopanoids are present in a number of prokaryotes including *Nostoc* (a widespread photobiont in the Peltigerales) and it is thought that they play the same role as sterols in the eukaryotic membrane, since many prokaryotes lack sterols (Ourisson et al., 1979). That hopanoids may be primitive phylogenetic precursors of sterols is strongly suggested by several facts. Both hopanoids and sterols share a common acyclic precursor the C-30 molecule squalene. The primitiveness of hopanoids may therefore be sought for in the biosynthetic steps after squalene. A first primitive trait is the fact that squalene is the *direct* precursor of 3-deoxyhopanoids by cyclization and hydration, whereas it is first oxidised to squalene 2, 3-epoxide before this is cyclized to sterol precursors. Indeed, none of the steps between acetyl Co A and the hopanoids requires molecular oxygen and these compounds are compatible with archaebiotic, prephotosynthetic conditions. Sterol synthesis in contrast has a mandatory requirement for molecular oxygen and could have occurred only after the rise of photosynthesis led to an increase in molecular oxygen in the environment. Secondly, in contrast to the formation of other common types of pentacyclic triterpenes from oleanane, ursane and lupane groups, only that of hopane derivatives does not require rearrangements after simple cyclization of squalene. Hopanoid synthesis is thus simpler than that of sterols (Ourisson et al., 1979).

Four main hopanes are widely distributed in the Peltigerales, though at least 15 different compounds from the series are known. These widely occurring hopanes are: 7β-acetoxyhopane-22-ol, and hopane-15α, 22-diol (Corbett and Young 1966a,b), hopane-6α, 7β, 22-triol and hopane-6α, 22-diol or zeorin (Mattson, 1987; White and James, 1988). These hopanes occur in a series of species-specific patterns (Wilkins and James, 1979; Galloway et al., 1983; James and White, 1987; Galloway, 1988b; Holtan-Hartwig, 1988, and Figs. 1–3). Known lichen hopanes are shown in Table 1.

In *Pseudocyphellaria*, a two-hopane chemistry (7β-acetoxyhopane-22-ol and hopane-15α, 22-diol) is the most widespread and probably also a primitive pattern, found in taxa with a white medulla and white pseudocyphellae. Taxa with a white medulla and yellow pigments in the pseudocyphellae (the *P. crocata* group) have hopane-6α, 7β, 22-triol as the major hopane and in addition

Table 1. Hopane skeleton and known lichen hopanes (after Galloway, 1988b)



	R_1	R_2	R_3	R_4	R_5	
1	CH_3	H	OAc	H	H	7β-acetoxyhopan-22-ol (peltidactylin)
2	CH_3	H	OH	H	H	hopane-7β, 22-diol
3	CH_3	H	H	OH	H	hopane-15α, 22-diol
4	CH_3	OH	OH	H	H	hopane-6α, 7β, 22-triol
5	CH_3	OH	H	H	H	hopane-6α, 22-diol (zeorin)
6	CH_3	OAc	H	H	OAc	6α, 16β-diacetoxyhopan-22-ol
7	CH_3	OAc	H	H	OH	6α-acetoxyhopane-16β-22-diol
8	COOH	OAc	H	H	H	6α-axetoxy-22-hydroxyhopan-23-oic acid
9	COOH	OH	H	H	H	6α-22-dihydroxyhopan-23-oic acid
10	CH_3	OH	OAc	H	H	7β-acetoxyhopane-6α, 22-diol
11	CH_3	OAc	OH	H	H	6α-acetoxyhopan-7β, 22-diol
12	COOH	H	H	OAc		15α-acetoxy-22-hydroxyhopan-24-oic acid

have both yellow pulvinic acid derivatives and a number of depsides and depsidones, a more diverse and advanced chemical pattern. The triol is more highly oxygenated than the two earlier hopane diols and represents both a chemical and an evolutionary advance. Green et al. (1980) claim that New Zealand species of *Pseudocyphellaria* contain some 15 distinct chemical strains and they show that species with an identical chemistry are also very similar in acetylene reduction activity and nitrogen content and that they group together when rates of acetylene reduction are plotted against thallus nitrogen content. Recently, Guzman et al. (1990) have shown that lichens with hopane triterpenoids are more resistant to decomposition in litter in cool temperate rainforests of southern Chile.

Pseudocyphellaria has the highest chemical diversity of any genus in the Peltigerales (Galloway, 1988b) and besides hopane triterpenoids, fernene, stictane, secostictane and lupane triterpenoids are known from species present in the Southern Hemisphere where the major areas of endemism and species diversity are New Zealand, South America, south east Australia and Tasmania and the palaeotropics. Compounds from each of these latter triterpenoid series are all more oxygen-rich than hopanes and from both a biosynthetic and

an evolutionary standpoint may be regarded as less primitive (apomorphic) compounds than hopanes.

Hopanes have also attracted considerable interest in the organic geochemistry of shales, coals, lignites, sediments and petroleum. Two triterpenes, 17α(H), 21β(H)-hopane and 17β(H), 21α(H)-hopane (moretane) have received wide attention as indicators of maturity, and to a lesser extent, sources of sedimentary organic matter (Czochanska et al., 1987). Their presence in sediments and oils is attributed to contributions from microorganisms, algae and vascular plant sources, and it is possible that they may even reflect a contribution from lichens. It is possible that lichen-derived triterpenoids may contribute to the pool of buried organic carbon derived from such resistant materials as sporopollenin, cutin, tannin, lignin etc. (Robinson, 1990). The similarity of lichen hopanes which are produced in quantity in species of *Nephroma, Peltigera* and *Pseudocyphellaria* in forests and grasslands of the Southern Hemisphere cool temperate belt, and the hopane biomarkers (VI and VII) suggests the possibility of relating chemical structures to geological formations of known age, which may have important implications in phylogenetic reconstructions in the Peltigerales.

Fernenes

Recorded in the Peltigerales only from yellow-medulla taxa in *Pseudocyphellaria*; *P. aurata* (Wilkins and Elix, 1990) and related species (viz: *P. arvidssonii, P. clathrata* (Galloway and Arvidsson, 1990) and *P. poculifera*). Wilkins and Elix (1990) have characterised four new fernene triterpenoids from *P. aurata* as: 3β-acetoxyfern-9(11)-en-12-one, 3β-acetoxyfern-9(11)-en-12β-ol, fern-9(11)-ene-3β, 12β-diol and 3β-acetoxyfern-9(11)-en-19β-ol. Fernenes are rearrangement products of hopanes and occur in ferns (Berti and Bottari, 1968) and in four orders of mosses, all in the Bryidae viz: the Eubryales, Isobryales, Hypnobryales and Dicranales (Markham and Porter, 1978). These authors claim that the distribution of fernene derivatives is suggestive of a possible phylogenetic link between lichens, mosses and vascular plants.

Stictanes

These are triterpenoids isolated from yellow-medulla species of *Pseudocyphellaria* in Australasia, viz: *P. colensoi, P. coronata, P. pickeringii* (Chin et al., 1973), and from vicariant yellow-medulla taxa in cool temperate South America: *P. compar, P. coerulescens, P. endochrysa, P. flavicans*, and *P. scabrosa* (Wilkins, 1977b). Stictanes are also present in the Northern

Hemisphere species, *Cetraria nivalis* (Wilkins, 1977a). Known lichen stictanes are shown in Table 2.

Secostictanes

Three of these compounds (Table 3) were isolated from the New Zealand yellow-medulla species *Pseudocyphellaria degelii* (Goh et al., 1978). They are not known in taxa from other Southern Hemisphere areas.

Lupanes

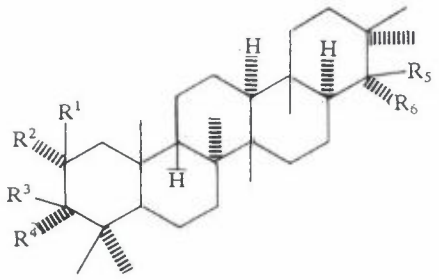
Twenty lupane triterpenoids (Table 4) were isolated from the yellow-medulla, non-glabrous species *Pseudocyphellaria rubella* which occurs in New Zealand and Tasmania (Corbett et al., 1987). They have not so far been detected in any other species.

It is noteworthy that these more highly oxidised triterpenoids all co-occur in species of *Pseudocyphellaria* having the yellow pigments calycin, pulvinic acid and pulvinic dilactone present either in the medulla or in pseudocyphellae (Galloway, 1988b). That hopanes may be considered pleisiomorphic to these other triterpenoids is supported by the fact that hopane-containing taxa in *Pseudocyphellaria* are both more numerous and also much more widely distributed geographically.

3. Discussion

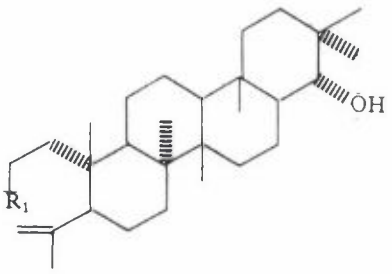
The possible biological role of triterpenoids as distinct from their taxonomic utility in lichens and especially in general of the Peltigerales where they are particularly diverse and produced in some quantity (James and White, 1987; Galloway, 1988b) invites considerable speculation. In plants generally, many triterpenoids are well-known toxins (Goodwin and Mercer, 1983; Harborne and Turner, 1984) and are implicated, as are many other secondary compounds, in a variety of ecological roles as defensive agents in plant-plant (allelopathic), plant-herbivore, and plant-pathogen interactions. In this regard it is significant that very few studies have been made of the relationships between insects on the one hand and terpenoids generally and triterpenoids in particular on the other, since these compounds are widely distributed in the plant and fungal kingdoms (Herout, 1970). The considerable adaptive advantage to lichens in the production of secondary metabolites with these presumed functions suggest a functional role and a co-evolutionary importance for such compounds (Harborne and Turner, 1984; Lawrey, 1984, 1986, 1989; Hawksworth, 1988a).

Table 2. Stictane skeleton and known lichen stictanes (after Galloway, 1988b)



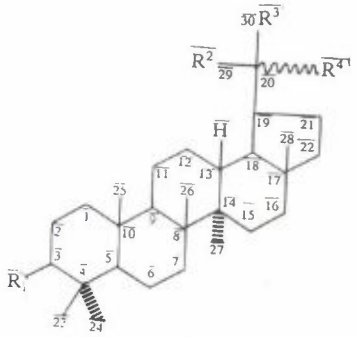
	R_1	R_2	R_3	R_4	R_5	R_6	
1	H	OH	OH	H	H	OH	stictane-2α, 3β, 22α-triol
2	H	OAc	OAc	H	H	OAc	2α, 3β, 22α-triacetoxystictane
3	H	OAc	OAc	H	H	OH	2α, 3β-diacetoxystictan-22α-ol
4	H	OH	OH	H	H	OH	2α-acetoxystictane-3β, 22α-diol
5	H	OAc	OAc	H	H	OH	3β-acetoxystictane-2α, 22α-diol
6	H	OAc	OAc	H	O		2α, 3β-diacetoxystictan-22-one
7	H	H	OH	H	H	OH	stictane-3β, 22α-diol
8	H	H	OAc	H	H	OAc	3β, 22α-diacetoxystictane
9	H	H	OAc	H	H	OH	3β-acetoxystictan-22α-ol
10	H	H	O		H	OH	22α-hydroxystictane-3-one
11	H	H	H	H	H	H	stictane

Table 3. Secostictane skeleton and known lichen secostictanes (after Galloway, 1988b).

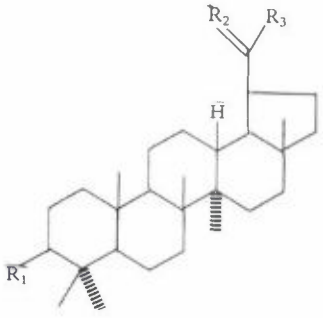


	R_1	
1	COOH	22α-hydroxy-3,4,-secostict-4(23)-ene-3-oic acid
2	CHO	22α-hydroxy-3,4,-secostict-4(23)-en-3-ol
3	CH_2OAc	3-acetoxy-3,4,-secostict-4(23)-en-22α-ol

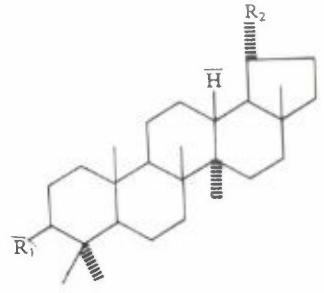
Table 4. Lupane skeletons and known lichen lupanes (after Galloway, 1988b)



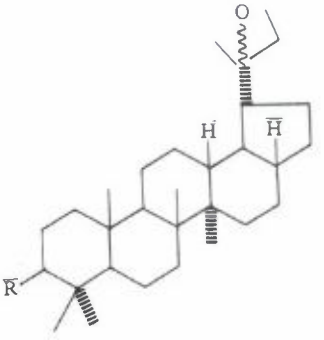
R_1	R_2	R_3	R_4	
OAc	CHO	CH_3	H	3β-acetoxylupan-29-al
OAc	CH_3	CH_3	OH	3β-acetoxylupan-20-ol
OH	CHO	CH_3	H	(20RS)-3β-hydroxylupan-29-al
OH	COOH	CH_3	H	(20RS)-3β-hydroxylupan-29-oic acid
OAc	COOH	CH_3	H	(20RS)-3β-acetoxylupan-20-oic acid
OH	CH_3	CH_3	OH	lupan-3β, 20-diol
OH	CH_2OH	CH_3	H	(20RS)-lupan-3β, 29-diol
OH	CH_3	H	OH	(20RS)-30-norlupane-3β, 20-diol
OH	CH_2OH	CH_3	OH	(20RS)-lupane-3β, 20, 29-triol
OAC	CH_2OH	CH_3	OH	(20RS)-3β-acetoxylupane-20, 29-diol



R_1	R_2	R_3	
OAc	CH_2	CH_3	3β-acetoxylup-20(29)-ene
OAc	O	CH_3	3β-acetoxy-30-norlupan-20-one
OAc	CH_2	CH_2OH	3β-acetoxylup-20(29)-en-30-ol
OH	O	CH_3	3β-hydroxy-30-norlupan-20-one
OH	CH_2	CH_3	3β-hydroxylup-20(29)-ene (lupeol)
OH	CH_2	CH_2OH	Lup-20(29)-en-3β, 30-diol



R_1	R_2	
OH	OH	20,29·30-trinorlupane-3β, 19α-diol
OAc	OAc	3β, 19α-diacetoxy-20,29,30-trinorlupane



R	
OAC	3β-acetoxylupan-20(29)-epoxide
OH	3β-hydroxylupan-20(29)-epoxide

In cool temperate rainforests of the Southern Hemisphere species of *Nephroma, Pseudocyphellaria* and *Sticta* are richly developed, many species reaching a great size and representing a considerable epiphytic and terricolous biomass. They have rapid growth and appear to be strongly competitive. Rogers (1990) discuses these ecological strategies further. According to Denison (1973) and Pike (1978) epiphytic lichens, when abundant, can contribute significantly to the nutrient budget (especially nitrogen and phosphorus) in some forest ecosystems. Green et al. (1980) suggested a possible nitrogen contribution of between 1–10 kg per hectare per annum from lichens in *Nothofagus* forest in New Zealand. In *Nothofagus* forests in southern Chile where the proportion of species of *Nephroma*, *Pseudocyphellaria* and *Sticta* with cyanobacterial photobionts is higher than it is in New Zealand, one could expect a similar or higher level of nitrogen enrichment. The input through lichen decomposition could potentially also be an important factor in the forest nitrogen budget (Guzman et al., 1990). Kershaw (1985, pp. 136–140) reviews fixation and release of nitrogen from lichens in field conditions and a more recent statement is given by Crittenden (1989).

The rich biomass of species of Peltigerales in these Southern Hemisphere forests constitutes a significant protein source for potential herbivores. The fact that they are not significantly grazed in these areas (Rundel, 1978; Galloway, 1988b, 1990) suggests that an anti-herbivore defence system may be present in these genera. While it is tempting to suggest that triterpenoids may function as anti-herbivore compounds in *Nephroma* and *Pseudocyphellaria* for example, the absence of any secondary metabolites in species of *Sticta* and in two species of *Pseudocyphellaria* (*P. gretae* in New Zealand and *P. nitida* in South America) makes it appear that other factors are involved in anti-herbivory, since species of *Sticta* reaching great size and being unattacked by herbivores, are frequently sympatric with taxa of *Nephroma* and *Pseudocyphellaria* which produce triterpenoids in quantity.

However, the investment in highly metabolised carbon that pools of triterpenoids represent in certain taxa of the Peltigerales is remarkable. The formation of complex molecules such as triterpenoids and their co-occurrence in closely related taxa in genera such as *Nephroma, Peltigera* and *Pseudocyphellaria* indicates conservation of biosynthetic pathways in phylogenetic lineages, with the products of biosyntheses, in this case triterpenoids, serving adaptive functions (Rodman, 1987). Since there is a diverse chemistry between genera and also within genera in the Peltigerales, it should be possible to use a variety of chemical characters to detect evolution of relationship between taxa at various levels of complexity and in such studies triterpenoids are likely to prove key compounds.

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