

## New pigments of *Cortinarius* Fr. and *Dermocybe* (Fr.) Wünsche (Agaricales) from Australia and New Zealand\*

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The chemical structures and interrelationships of pigments from fruit-bodies of several *Dermocybe* and one *Cortinarius*, subgenus *Myxacium*, indigenous to Australasia are described. Most of the pigments are new and some chemotaxonomic inferences are drawn from biogenetic relationships.

Keywords: Agaricales, chemotaxonomy.

The present taxonomic structure of *Dermocybe* (Fr.) Wünsche and various sect. of *Cortinarius* Fr. is strongly influenced by chemotaxonomic data (Moser, 1985; Moser in Singer, 1986), particularly important being pigments present in the fruit-bodies (Gill & Steglich, 1987). Most of the pigments isolated hitherto from *Dermocybe* and *Cortinarius* in Europe are either anthraquinones or molecules that bear a close structural and biogenetic relationship to them (Gill & Steglich, 1987). In view of the relevance of these substances to taxonomy and the relative ease with which they can be extracted and analysed by thin-layer chromatography (Kidd & al., 1985), chemotaxonomic studies on European species of *Dermocybe* and *Cortinarius* by Moser (1972), Høiland (1980; 1983), Keller (1982), Moser & Keller-Dilitz (1983), and by Steglich & Oertel (1984) have proved most valuable. Similarly, the pigments of South American species of *Dermocybe* and *Cortinarius* have been studied using paper chromatography by Gruber (1975) and more recently thin-layer chromatography by Keller & al. (1987). Keller & Ammirati (1983) have reported on the pigmentation of North American *Dermocybe* taxa. In most previous studies, specific pigment combinations can be recognized that can be conveniently applied to the differentiation of infrageneric taxa and to the reinforcement of groupings made on the basis of other micro- and macroscopic criteria.

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\* Pigments of Fungi, Part 33; for Part 32 see Cotterill & Gill (1993). This paper is dedicated to Professor M. Moser on the occasion of his seventieth birthday.

To date, very little has been known about the chemical structure and properties of the pigments in any of the Australasian taxa. However, some progress has been made on the chemistry (Gill, 1994) and mycology of Australasian taxa (Horak, 1983; 1987; Keller & al., 1987; Grgurinovic, 1989; Horak & Wood, 1990; Høiland & Watling, 1990) and this has made it apparent that new features and phenomena are operating and that these facts require diagnosis and analysis from a standpoint completely different from that applied in the Northern Hemisphere.

In recent works by Horak (1987) and Keller & al. (1987) pigment profiles are presented for all known *Dermocybe* taxa in New Zealand and Papua New Guinea and mention is made of some of those that occur in Australia. It is apparent, even from this comparatively small survey, that *Dermocybe* species from the Southern Hemisphere are exceptionally diverse as indicated by a multitude of pigment types. Most significant from the taxonomic viewpoint is (a) the realisation that many of these pigments are different from those associated with the Northern Hemisphere taxa, (b) that they are often limited in their distribution to a small number of species or even to a single species, and (c), that most of the chemical structures are rare or hitherto unknown.

In this paper the structures of several unique pigments that have been isolated from Australian and New Zealand *Dermocybe* and *Cortinari* taxa are reported and some taxonomic inferences are drawn.

## Material and methods

Fungal material included in this study is listed in Tab. 1 along with geographic origin and accession number under which voucher specimens are held in the Herbarium of the Royal Botanic Garden, Edinburgh.

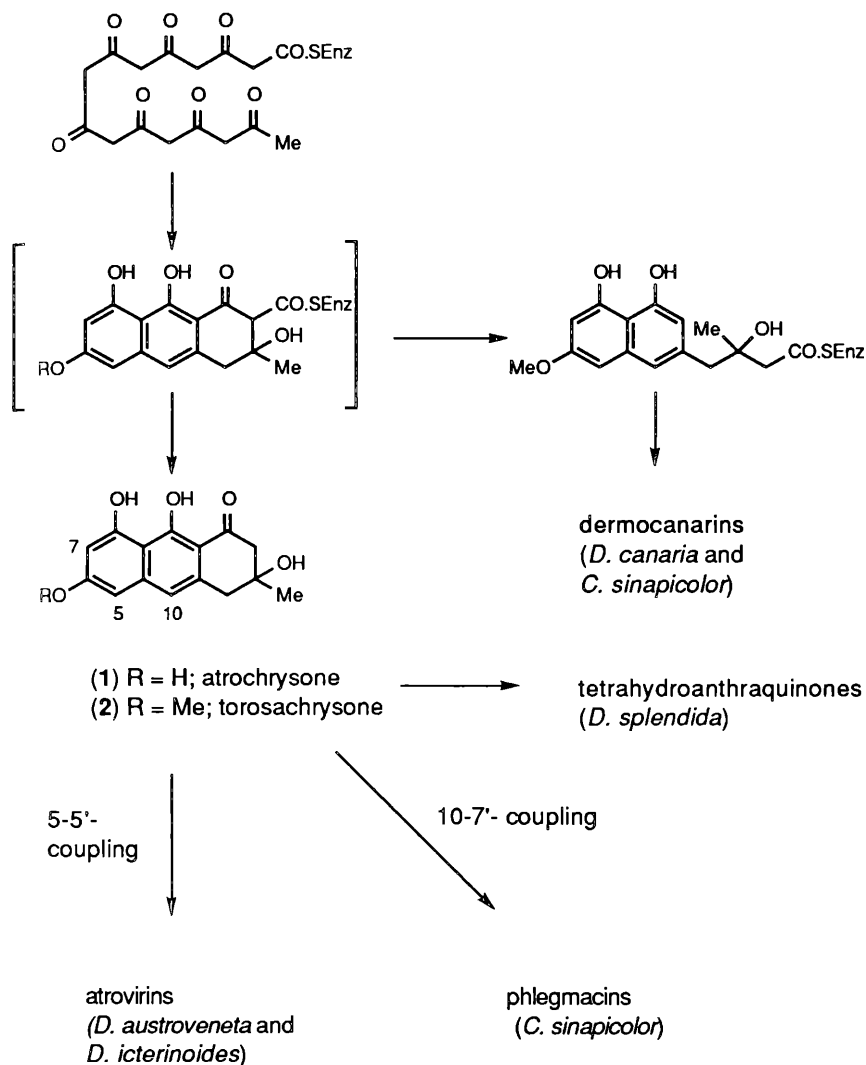
Tab. 1. – New pigments of *Dermocybe* and *Cortinari*: list of species examined. Abbreviations: AUS = Australia; NZ = New Zealand; E Herbarium of Royal Botanic Garden, Edinburgh, Scotland.

Species	Herbarium, Nr.	Origin
<i>Dermocybe splendida</i> Horak	E, WAT. 18086	AUS
<i>D. austroveneta</i> (Cleland) Moser & Horak	E, WAT. 19344	AUS
<i>D. icterinoides</i> Horak	E, WAT. 24300	NZ
<i>D. canaria</i> Horak	†	AUS
<i>D. canaria</i>	E, WAT. 22915	NZ
<i>Cortinari</i> <i>sinapicolor</i> Cleland	E, WAT. 24272	AUS
<i>Dermocybe cardinalis</i> Horak	E, WAT. 22916	NZ

† Identity confirmed by comparison with type material (E. Horak, personal communication to W. Steglich).

## Extraction and purification of pigments

Pigments were isolated from fresh or deep-frozen carpophores by maceration in ethanol. An exception applies to the extraction of *Dermocybe austroveneta*, which is discussed separately in the text. Extracts were routinely purified by preparative thin-layer chromatography on glass plates coated with a layer of silica gel (Merck Kieselgel GF<sub>254</sub>, 20 × 20 × 0.1 cm) using toluene:ethyl formate:formic acid 50:49:1 as eluant.



Scheme 1. – Biogenetic role of dihydroanthracenones.

## Identification of pigments

Pigments were identified by detailed analysis of the mass, infrared, ultraviolet,  $^{13}\text{C}$  and  $^1\text{H}$ -NMR spectra, including two-dimensional  $^1\text{H}$ - $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  correlation shift experiments.

## Results and discussion

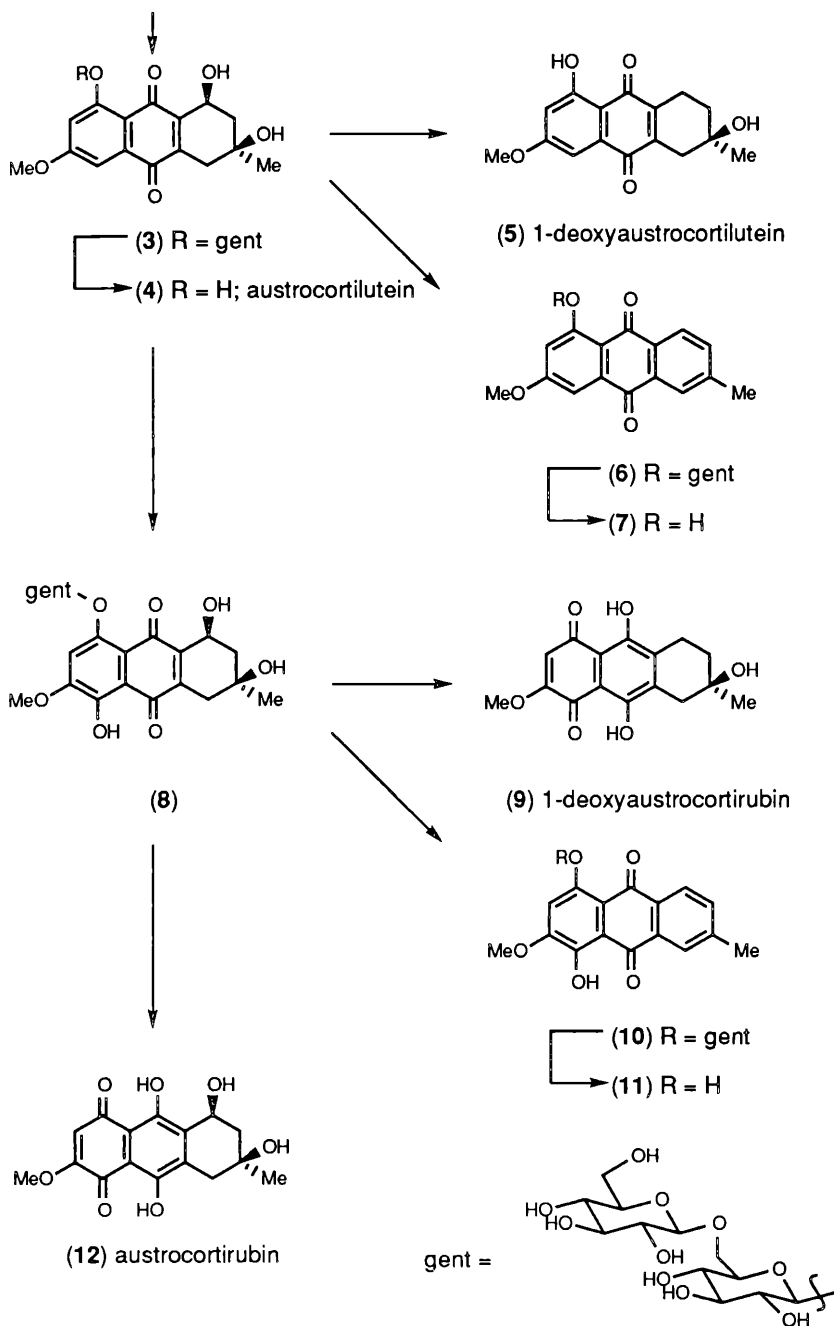
The majority of pigments isolated to date from *Dermocybe* and *Cortinarius* are derived biogenetically from so-called octaketide progenitors via dihydroanthracenones of the atrochrysone (**1**) and torosachrysone (**2**) types (Scheme 1) (Gill & Steglich, 1987; Gill & al., 1989; 1992). This pathway also operates in *Dermocybe splendida* (Gill & al., 1990), *D. austroveneta* (Gill & al., 1988; Gill & Gimenez, 1991), *D. icterinoides*, *D. canaria* (Keller & Steglich, 1987; Gill & Gimenez, 1990a), and in *Cortinarius sinapicolor*, but pigment biosynthesis in *Dermocybe cardinalis* takes a different route.

### *Dermocybe splendida*

*Dermocybe splendida* from New Zealand (Horak, 1983; 1987) was first examined chromatographically by Keller (1979) who was unable to identify any of the red and yellow pigments with anthraquinones known at that time. Later, Keller & al. (1987) were able to name six of the pigments by direct comparison with purified materials isolated from Australian specimens and supplied by our laboratories (Gill & Strauch, 1985; Archard & al., 1985; Gill & Smrdel, 1987; Gill & al., 1990). The principal yellow and red pigments isolated from the ethanol extracts of *D. splendida* are the tetrahydroanthraquinones austrocortilutein (**4**) and austrocortirubin (**12**), the former occurring in two stereoisomeric forms (Gill & al., 1990). Minor pigments are the 1-deoxy derivatives (**5**) and (**9**) of the major pigments (Gill & Smrdel, 1987), the anthraquinones (**7**) and (**11**) that are derived from the major pigments by loss of water (Archard & al., 1985), and finally torosachrysone (**2**) (Gill & al., 1989), which, as its 8-*O*- $\beta$ -**D**-gentiobioside is the natural progenitor of all other pigments in this fungus. Interrelationships between the pigments of *D. splendida* are shown in Scheme 2. Tetrahydroanthraquinones of the types (**4**) and (**12**) were known previously only from parasitic conidial fungi such as *Alternaria solani* and *Bostrychomonema alpestre*, the causal agents of blight disease of potato and water hyacinth, respectively (Thomson, 1987).

In the intact fruit-bodies of *D. splendida* the quinones (**4**), (**7**), (**11**), and (**12**) are present largely as their 8-*O*- $\beta$ -**D**-gentiobiosides (**3**), (**6**), (**10**), and (**8**), respectively, from which they arise naturally by

torosachryson-8-O- $\beta$ -D-gentiobioside



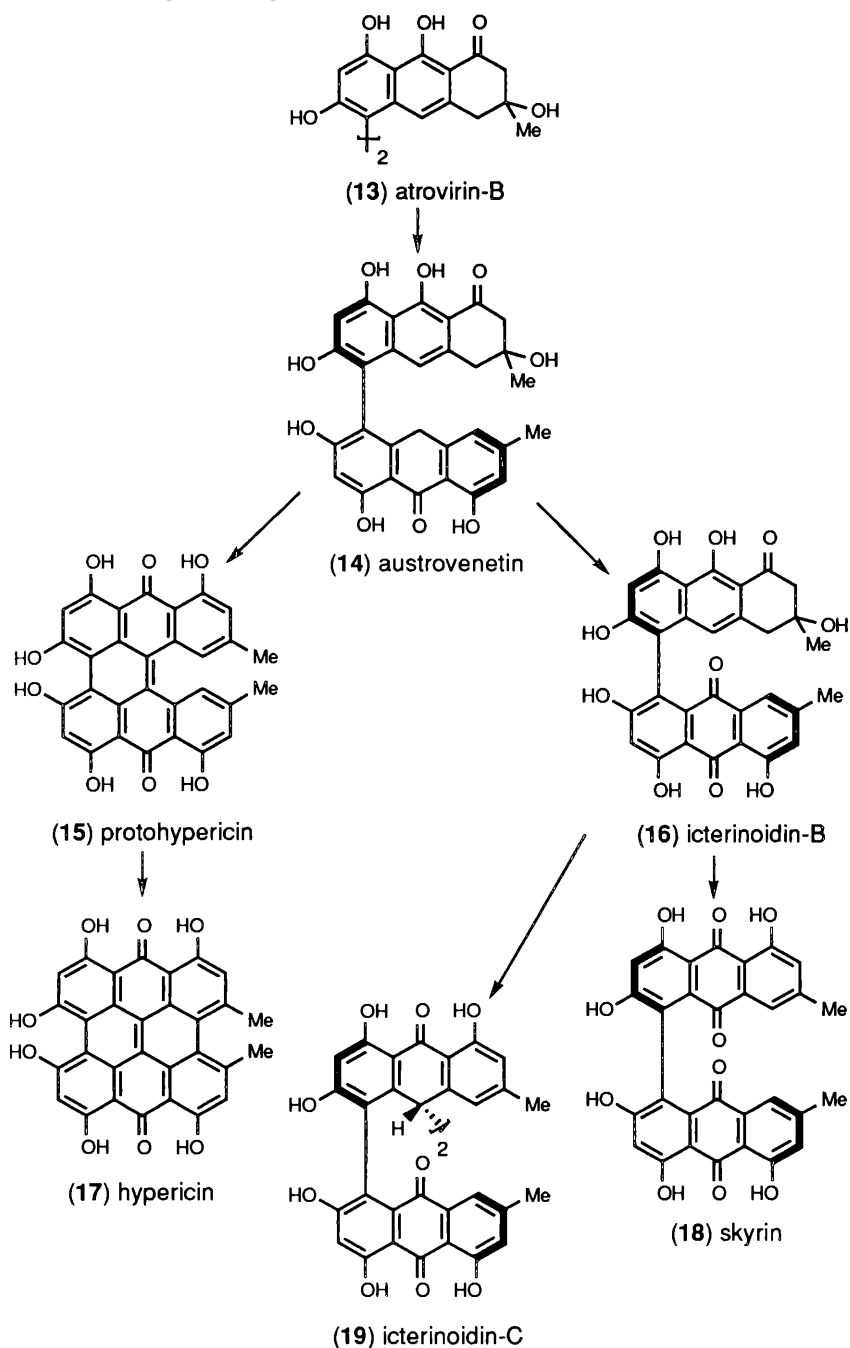
Scheme 2. – Biogenetic relationships between pigments of *D. splendida*.

glycolysis or during the course of the extraction and purification procedure. The gentiobiose residue in the pigments (3) and (8) is particularly labile being completely cleaved during twelve hours in ethanolic solution. Furthermore, the tetrahydroanthraquinones (4) and (12) are themselves prone to dehydration to afford the anthraquinones (7) and (11) if care is not taken to minimize their exposure to acid. Consequently, deliberate exposure of *D. splendida* extracts to acid (Keller & al., 1987) significantly alters the pigment profile compared to that which exists in the intact organism.

Examples from *Cortinarius* and *Dermocybe* in which pigments are present as sugar derivatives are extremely rare (Gill & Steglich, 1987). The gentiobiose residue has never been reported before in this context.

*D. splendida* has been related to the taxonomically yet doubtful (Moser & Horak, 1975) Australian taxon *Cortinarius umbonatus* (Cleland & Harris, 1948) by the presence of the quinones (4), (5), (7), (9), (11), and (12) (Keller & al., 1987). We have found red pigments of the austrocortirubin type in only two other Australian species. The first, *Dermocybe erythrocephala* (Dennis) Moser, is a blood-red toadstool collected by J. E. C. Aberdeen in Queensland and first placed in *Cortinarius* by Dennis (1955). It was later suggested by Moser (1972) that *D. erythrocephala* is synonymous with *D. sanguinea* (*sensu* Cleland, 1934) but our chemical work on the latter taxon (Gill & Gimenez, 1990b) coupled with the observations reported herein clearly establish that this can not be the case. Thus, extraction of a dried specimen of the type collection of *D. erythrocephala* provided on loan from the Plant Pathology Branch of the Department of Primary Industries, Queensland (BRIP), gave the red quinones (9), (11), and (12), but no trace of the yellow quinones (4) and (5) (Gimenez, 1990). The second is an incompletely described taxon in which the red pigments (9), (11), and (12) are restricted to a flame-red tip at the base of the stipe (*Cortinarius basirubescens?*; Keller, 1979). Other parts of the carpophore are yellow-brown and owe their pigmentation to the quinones (4), (5), and (7) (Gill & Yu, unpublished).

In contrast, austrocortilutein (4) and the related yellow pigments (5) and (7) have been isolated by us in various stereochemical forms from five as yet undescribed *Dermocybe* taxa (Gill & al., 1992) in which the red quinones, e.g. (12), are absent. It is suggested that these unnamed species, together with *D. splendida*, *D. erythrocephala* and *Cortinarius umbonatus* form a new group in which tetrahydroanthraquinones are the major pigments that are otherwise without affinities to other known taxa.



Scheme 3. – Biogenetic relationships between pigments of *D. austroveneta* and *D. icterinoides*.

*Dermocybe austroveneta* and *D. icterinoides*

*Dermocybe austroveneta* (Cleland) Moser is placed in the sect. *Pauperae* of the subgen. *Icterinula* by Moser (1986). Chromatographic analysis by Keller & al. (1987) of several South American taxa belonging to sect. *Pauperae*, and including two New Zealand taxa, namely *D. alienata* Horak and *D. icterinoides* Horak, revealed that the two major pigments present in the ethanolic extracts of fungi of this group are the purple extended quinone hypericin (17) and the orange anthraquinone skyrin (18).

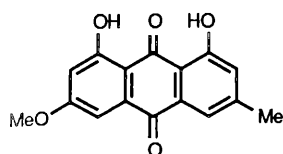
We have carefully examined the acetone extractives from fresh fruit-bodies of *D. austroveneta* and found that when these are obtained and processed in the absence of air and light the principal pigments are skyrin (18) and the green-yellow pre-anthraquinone, austrovenetin (14) (Gill & al., 1988; Gill & Gimenez, 1991). Austrovenetin (14) is extremely unstable to oxygen in the presence of which it is rapidly transformed to the violet pigment protohypericin (15). In turn, protohypericin (15) on exposure to sunlight is immediately converted to the final product, hypericin (17). As far as *D. austroveneta* is concerned, we are confident that the violet and purple pigments (15) and (17) are not present in fresh, undamaged carpophores but are instead artefacts of the extraction and purification procedure. The same could well apply to those taxa described by Keller & al. (1987) in which hypericin (17) is conspicuous in chromatograms by its colour and cherry red fluorescence under ultraviolet light.

Interestingly, when fruit-bodies of *D. austroveneta* become decayed and/or damaged by larval attack they develop violet-purple bruises that are no doubt due to the accumulation of hypericin (17) that arises by chemical modification of austrovenetin (14).

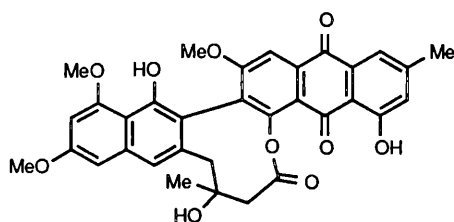
We have also studied the pigments of *D. icterinoides*, one of the New Zealand taxa examined by Keller & al. (1987). From ethanolic extracts we have isolated the known pigments atrovirin-B (13) and skyrin (18), and the new quinones icterinoidin-B (16), its atropisomer icterinoidin-A (not shown) and the curious tetramer, icterinoidin-C (19) (Gill & Morgan, unpublished). The presence of hypericin (17) is clear from the thin-layer chromatograms, it probably arises during extraction and purification from austrovenetin (14) present in the intact carpophores.

The pigments of *D. austroveneta* and *D. icterinoides* fall logically into the biogenetic pattern shown in Scheme 3. Chemically they have affinities, either actual or artefactual, not only to other members of the sect. *Pauperae* of subgen. *Icterinula* but also to subsect. *Atrovirentes* of sect. *Scauri* Fr. of subgen. *Phlegmacium* which is characterized by the presence of atrovirin-B (13), skyrin (18), and

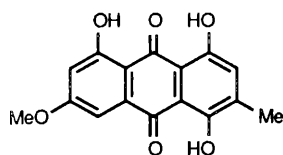




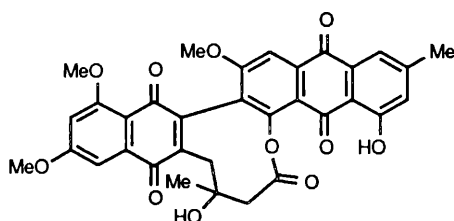
(20) physcion



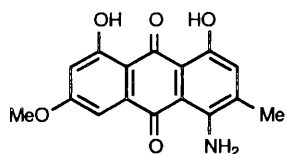
(23) dermocararin-1



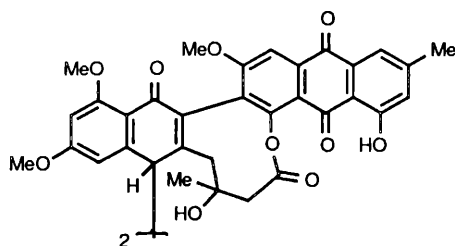
(21) erythroglauцин



(24) dermocararin-2



(22) 4-aminophyscion



(25) dermocararin-3

Scheme 4. – Pigments of *D. canaria*.

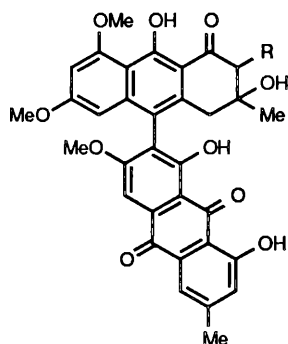
probably hypericin (17) (Steglich & Oertel, 1984). Our results provide further support to the suggestion by Keller & al. (1987) that sect. *Pauperae* of subgen. *Icterinula* should be grouped with subsect. *Atravirentes* of sect. *Scauri*.

*Dermocybe canaria* and *Cortinarius sinapicolor*

*Dermocybe canaria* Horak, a common species in New Zealand *Nothofagus* forests, occupies an isolated taxonomic position within the infragenic frame of *Dermocybe*. Keller & Steglich (1987) have shown that the brilliant yellow colour of the fruit-bodies is due to large quantities of physcion (20) together with lesser amounts of the related anthraquinones (21) and (22). Our own work with specimens collected both in New Zealand and Tasmania has confirmed the presence of the quinones (20) and (21) and further established that these pigments are present to a significant extent as their 1-O- $\beta$ -D-glucopyranosides (Gimenez, 1990). Studies on the intensely yellow mycelial mat (Gill & Gimenez, 1990a) led to the discovery of the first three members of a completely new class of coupled anthraquinonoid pigments, the so-called dermocanarins (Scheme 4). The two halves of the dermocanarin molecule, that is the anthracene moiety on one hand and the naphthalene ring together with the lactone bridge on the other, are formed independently from two molecules of a precursor of the torosachrysone (2) type. At some stage in the biogenesis these units are joined by an ester linkage and a biaryl bond to ultimately afford pigments such as the dermocanarins-1 (23) and -2 (24). Dermocanarin-3 (25), a complex tetramer, is formed by coupling together of two molecules of dermocanarin-1 (Gimenez, 1990). The dermocanarins-1, 2, and 3 are present in the carpophores of *D. canaria* only in trace proportions compared to the quinones (20) and (21) and their glucosides. The presence of physcion (20) as the most abundant pigment and the occurrence of the dermocanarins, hitherto found in no other *Dermocybe* taxon, supports the isolated taxonomic position proposed for *D. canaria*.

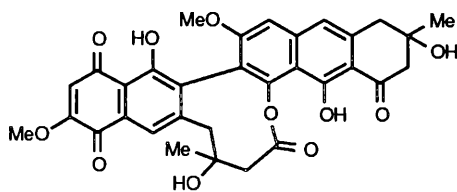
To date we have found pigments of the dermocanarin class in five other Australian taxa. Of these only one, *Cortinarius sinapicolor* Cleland, has been fully documented. The bright mustard yellow coloured basidiomes of *C. sinapicolor* have been beautifully illustrated (but erroneously identified as '*C. ochraceus*' ) by Cole & al. (1984) and Fuhrer (1985). It is placed in sect. *Pyromyxae* of subgen. *Myxacium* by Moser (1986), a placement supported by Grgurinovic (1989). Its taxonomic position and relationship with the confused '*C. ochraceus*' have recently been discussed by Horak & Wood (1990).

The pigments of *C. sinapicolor* are shown in Scheme 5. They fall into two different chemical groups, each one providing chemotaxonomic links with apparently diverse subgenera within the *Cortinarius* -*Dermocybe* framework. The most abundant yellow pigment is the new hydroxyphlegmacinquinone (26), which is accompanied by a minor quantity of the known phlegmacin derivative (27) (Gimenez, 1990; Gill & Milanovic, unpublished). A phlegma-



(26) R = OH

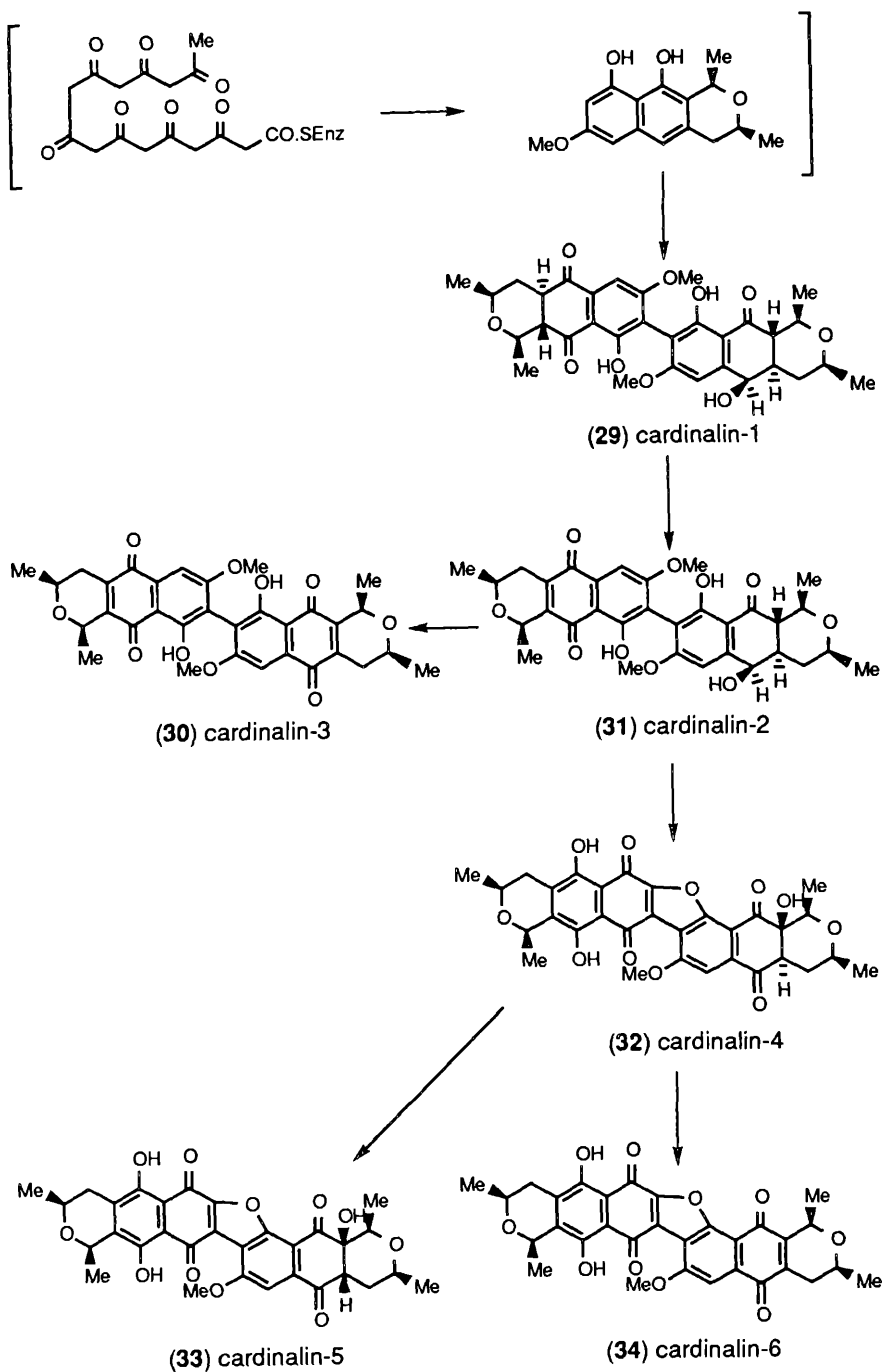
(27) R = H



(28) dermocanarin-4

Scheme 5. – Pigments of *Cortinarius sinapicolor*.

cinquinone with the same structure and chiroptical properties as the minor pigment (27) from *C. sinapicolor* has been described previously from *C. percomis*, *C. nanceiensis*, and *C. russeoides*, members of the subsect. *Percomes* of sect. *Scauri* (*Phlegmacium*) (Steglich & Oertel, 1984), where it and similar pigments are regarded as important taxonomic markers (Gill & Steglich, 1987). The occurrence of the phlegmacinquinones (26) and (27) in *C. sinapicolor* may reveal affinities to sect. *Scauri*, which has already been linked with sect. *Pauperae* of subgen. *Icterinula* by Keller & al. (1987). A link from *C. sinapicolor* to subgen. *Icterinula* of *Dermocybe* is further supported by our characterisation of dermocanarin-4 (28) as the second most abundant constituent of the mustard yellow *C. sinapicolor*. In the anthracene portion of dermocanarin-4 the ring system is at a torosachryson level of development, less advanced than in the dermocanarins-1, 2 and 3 from *Dermocybe canaria*, while the naphthalene ring is oxidized to a naphthoquinone. If the dermocanarins are to be taken as a guide, then



Scheme 6. – Biogenetic relationships between pigments of *D. cardinalis*.

*C. sinapicolor* has systematic affinities rather to *Dermocybe* subgen. *Icterinula* than *Cortinari* subgen. *Myxadium*, as is proposed by Horak & Wood (1990).

### *Dermocybe cardinalis*

In New Zealand forests *D. cardinalis* represents one of the visually most striking species of *Dermocybe*. Ethanolic extracts of the type collection have been examined chromatographically by Keller & al. (1987) who concluded that the pigments, which range from yellow to purple, are unique to this species and at that time mainly unidentified. Nevertheless, it was speculated, wrongly as it turned out, that the major pigments of *D. cardinalis* are anthraquinones.

In fact, as the structures collected and interrelated in Scheme 6 reveal, the principal yellow, red, and purple pigments of *D. cardinalis* constitute a series of unique naphthoquinone dimers that we have elected to call, collectively, the cardinalins. Cardinalins 2-6 belong to the group of so-called benzoisochromanquinones that have hitherto been restricted in their distribution to various molds, plants, and soil microorganisms (Thomson, 1987). Cardinalin-1 (**29**) is colourless since the quinone chromophore is incompletely developed, nevertheless, it may be readily identified as a putative precursor to the remaining five pigments (**30**)–(**34**). The benzoisochroman ring system in its simplest (and as yet hypothetical) form prior to biaryl coupling is depicted at the top of Scheme 6. It may be seen to arise by way of an octaketide precursor (shown alongside) that is folded according to a pattern that is fundamentally different to that which operates in the vast majority of other *Dermocybe* taxa (cf. Scheme 1). The fact that benzoisochromanquinones have never before been isolated from Basidiomycotina attests to the unique, isolated position that is currently accorded to *D. cardinalis*.

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