Pigments of Fungi. Part 17.[1] (*S*)-(+)-Dermochrysone, (+)-Dermolactone, Dermoquinone, and Related Pigments; New Nonaketides from the Fungus *Dermocybe sanguinea* (sensu Cleland)

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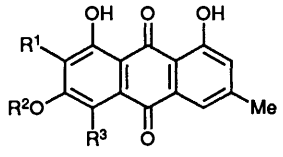
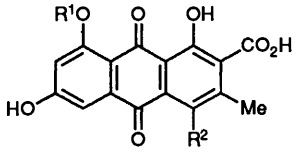
The novel dihydroanthracenones (**8**), (**11**), and (**12**), and the biogenetically related anthraquinones (**13**), (**14**), and (**15**) have been isolated from fruit bodies of the Australian toadstool *Dermocybe sanguinea* (sensu Cleland). The absolute stereochemistry of dermochrysone (**8**) is inferred by chiroptical comparison with fungal dihydroanthracenones of known chirality. Feeding experiments show that sodium [1-^{13}C]acetate is efficiently incorporated into dermochrysone (**8**) and dermolactone (**13**) by intact fruit bodies.

Although both Bachmann in 1886 and Zopf in 1890 were undoubtedly dealing with anthraquinones during their studies on the colouring matters of *Cortinarius bulliardi*[2] and *C. armillatus*,[3] respectively, the first anthraquinones to be chemically characterised from fungi emerged from an investigation of the pigments of the blood-red fruit bodies of *Dermocybe sanguinea* (Wulf. ex Fr.) Wünche by Kogl in 1925.[4] Kogl isolated emodin (**1**) and dermocybin (**2**). Later work by



(**1**) $R^1 = R^2 = R^3 = H$
(**2**) $R^1 = OH$, $R^2 = Me$, $R^3 = H$
(**3**) $R^1 = R^3 = H$, $R^2 = Me$
(**4**) $R^1 = R^3 = OH$, $R^2 = Me$



(**5**) $R^1 = R^2 = H$
(**6**) $R^1 = Me$, $R^2 = H$
(**7**) $R^1 = Me$, $R^2 = OH$

Steglich[5] on the same toadstool revealed that these pigments are present principally as glycosides and also discovered the presence of physcion (**3**), dermoglaucin (**4**), and the anthraquinone carboxylic acids endocrocin (**5**), dermolutein (**6**), and dermorubin (**7**). The quinones (**6**) and (**7**) are accompanied in *D. sanguinea* by their 5-chloro derivatives.[5] In recent years, the number of anthraquinonoid pigments isolated from the higher fungi has risen considerably and now more than 30 such pigments, many found only within the genera *Cortinarius* and *Dermocybe*, are known.[6] Significantly, the vast majority of these fungal anthraquinones are derivatives either of emodin (**1**) or endocrocin (**5**) and may therefore be confidently assumed to be derived from octaketide progenitors.[7] The only recorded exception to this pattern is the occurrence in some toadstools belonging to the subgenus *Leprocybe* of *Cortinarius* of small quantities of three anthraquinones (*vide infra*) for which a nonaketide biosynthesis has been suggested.[6,8]

We describe here the isolation and structural elucidation of six pigments, three new dihydroanthracenones and three new anthraquinones, from the blood-red fruit bodies of an Australian toadstool which was previously identified with the European *Dermocybe sanguinea*.[9] Feeding experiments using sodium [1-^{13}C]acetate support the fact that these pigments are derived biosynthetically from nonaketide precursors and therefore represent the first pigments of this type to be found in *Dermocybe*.

Results and Discussion

The fragile fruit bodies of *Dermocybe sanguinea* (Wulf. ex Fr.) Wünche sensu Cleland[9] grow singly or in small groups in mixed *Eucalyptus* forest, usually among grass. They are distinguished by their blood-red cap and thin red–orange fibrilous stipe. Pictures of this rare toadstool have been published,[10] and it is recognised there and elsewhere[11] that the Northern and Southern Hemisphere species are not the same. A preliminary chromatographic analysis of an ethanolic extract of *Dermocybe sanguinea* (sensu Cleland) revealed the presence of at least seven pigments, six of which have subsequently been isolated and characterised.

Whole fresh fruit bodies, collected near Marysville, Victoria, were homogenised and soaked in ethanol. The residue remaining after evaporation of the solvent was partitioned between ethyl acetate and water and the organic phase was evaporated to dryness. The red residue was separated by short column chromatography into a red–orange mobile zone and a green, less mobile zone. The principal constituents of the less mobile zone are discussed first.

The pigments in this green zone were further purified by careful PLC to afford two clearly discernible green fractions. The major fraction gave the new green pigment (**8**), which we have named dermochrysone, as needles, m.p. 189–192 °C, in a yield of $1.4 \times 10^{-2}\%$ of the fresh weight of the fungus. Dermochrysone (**8**) is optically active, exhibiting a specific rotation $[\alpha]_D$ + 28° in chloroform.

The molecular formula $C_{18}H_{18}O_6$ followed from the presence in the mass spectrum of a molecular ion at *m/z* 330 and from supporting combustion analysis data. The electronic spectrum, by virtue of its similarity to those of torosachrysone (**9**) and atrochrysone (**10**),[12] was immediately suggestive of a dihydroanthracenone chromophore. A close relationship to torosachrysone (**9**) was further revealed on inspection of the ^{1}H NMR spectrum which shows lowfield singlets at δ 15.99 and 9.76, typical of the chelated phenolic hydroxy groups in molecules of this type. Other resonances in the spectrum which are consistent with the presence of a dihydroanthracenone nucleus included a broadened aromatic singlet at δ 6.84 (10-H), a pair of *meta* coupled doublets at δ 6.54 and 6.48 (5-H and 7-H, respectively), and a singlet at δ 4.02 (3-OH) which disappeared on addition of D_2O. These signals, together with a three proton singlet at δ 3.88 characteristic of an aromatic methoxy, and two AB quartets with components centred at δ 3.02 and 3.14 (*J* 15.6 Hz, 4-H_2), and δ 2.84 and 2.94 (*J* 17.6 Hz, 2-H_2), take care of all but five of the hydrogen atoms present in

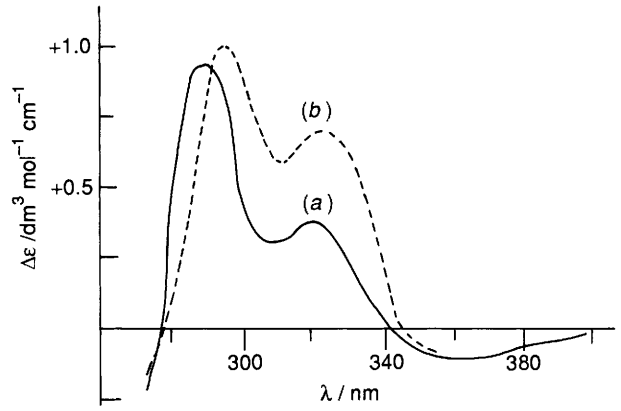
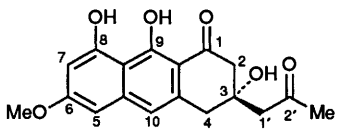
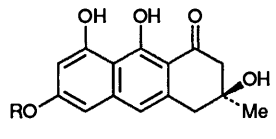


Figure 1. CD spectra: (*a*), dermochrysone (**8**) from *D. sanguinea*; (*b*), (*R*)-(−)-atrochrysone *ent*-(**10**) from *Cortinarius odoratus*;[13] in MeOH.

the molecular formula of dermochrysone. The remaining protons are accounted for by a two proton resonance at δ 2.78 (1′-H_2) and a three proton singlet at δ 2.19 (2′-Me). Notably, a three proton singlet (δ 1.45) characteristic of the C-3 methyl group in torosachrysone (**9**) is absent from the spectrum of dermochrysone (**8**).



(**8**)



(**9**) R = Me
(**10**) R = H

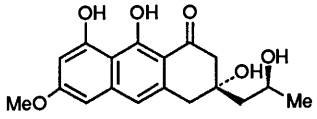
Collectively, the data discussed so far provide convincing evidence for structure (**8**) (no stereochemistry yet implied) for this unique dihydroanthracenone. Further support was gained from the IR spectrum in which carbonyl absorptions at 1692 and 1635 cm^{-1} are consistent with the presence of a ketone group and a conjugated chelated carbonyl, respectively.

The ^{13}C NMR spectrum of dermochrysone and 2D ^{1}H-^{13}C COSY experiments fully support structure (**8**). Thus, the ^{13}C NMR spectrum reveals the presence of two carbonyl carbons (δ 209.9 and 200.9), three methylene groups (δ 50.7, 49.3, and 41.4), and a C-methyl group (δ 31.6). In the 2D ^{1}H-^{13}C (long range) COSY spectrum, the aliphatic ketone carbon (δ 209.9) correlates over two bonds with the protons of both the methylene group at δ 2.78 and the methyl group at δ 2.19. In turn, the conjugated carbonyl carbon (δ 200.9) correlates with those components of the AB quartet centred at δ 2.84 and 2.94, in full accord with the assignments made above. Furthermore, a resonance at δ 71.5, clearly arising from a hydroxylated, quaternary carbon shows correlation through two bonds both with the methylene protons at δ 2.78 and with the components of both AB systems. These correlations confirm that the C_3 side chain in dermochrysone must emanate from the tertiary, hydroxylated carbon (C-3). Further support for the location of the C_3 chain in (**8**) was obtained from the mass spectrum. Thus, the base peak in the mass spectrum of dermochrysone appears at *m/z* 272 and corresponds by high resolution mass measurement to an ion with the composition $C_{15}H_{12}O_6$. This ion may arise by concerted loss of the elements of acetone by way of a McLafferty rearrangement.

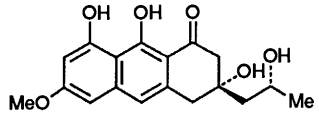
As mentioned previously, dermochrysone (**8**) is optically active. The absolute configuration at C-3 in the dihydroaromatic ring may be assigned as (*S*) by comparison (Figure 1) of the CD spectrum of (+)-dermochrysone (**8**) with the data recorded for (*R*)-(−)-atrochrysone *ent*-(**10**).[13] Furthermore, CD data which have been reported previously[12] for (*S*)-(−)-torosachrysone (**9**) and (*S*)-(+)-atrochrysone (**10**) fully support the stereochemical assignment made here for (+)-dermochrysone (**8**).

Dermochrysone is a new natural product and represents the first dihydroanthracenone which apparently emanates from a nonaketide precursor.

The minor green zone obtained from the PLC purification of the less mobile fraction was found to be a mixture of two dihydroanthracenones in an approximately equimolar ratio. Thus, the ^{1}H NMR spectrum showed doubling of some of the most characteristic resonances associated with the dihydroanthracenone nucleus. For example, signals arising from four discrete phenolic hydroxy groups appeared at δ 16.02, 16.01, 9.81, and 9.80, and the protons of two methyl groups appeared at δ 1.26 and 1.23, each as a doublet (*J* 6.5 Hz). Other signals, such as an aromatic proton singlet at δ 6.89, and *meta* coupled doublets at δ 6.54 and 6.46, did not resolve into two sets of signals. Other resonances in the ^{1}H NMR spectrum (see Experimental section), together with the mass spectrum which exhibited a single molecular ion at *m/z* 332 ($C_{18}H_{20}O_6$) led to the conclusion that this minor green pigment is a mixture of the diastereoisomeric alcohols (**11**) and (**12**), epimeric at the stereogenic centre in the side chain. We have appended the



(**11**)



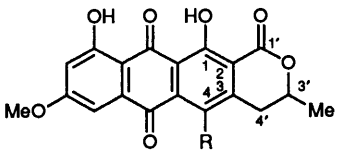
(**12**)

name dermochrysonol to this pair of alcohols and have made the assumption that they share a common absolute stereochemistry at the tertiary centre with (*S*)-dermochrysone (**8**). The small quantity of the dermochrysonols (0.2 mg, 3 × 10^{-4}% fr. wt.) and difficulties encountered in trying to separate the individual diastereoisomers made the unequivocal assignment of structure impossible and has prevented further progress being made with these compounds.

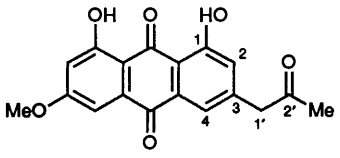
Three components of the more mobile, red–orange zone were separated from each other only after extensive chromatography. In this way, it proved possible to purify and characterise three new natural products: the major pigment of the toadstool, which we have called dermolactone, a pink–red metabolite, 4-hydroxydermolactone, and a yellow pigment which we have called dermoquinone. The novel anthraquinone structures (**13**), (**14**), and (**15**), respectively, have been assigned to these pigments principally from the spectroscopic data, as follows.

Dermolactone (**13**) was obtained as needles, m.p. 268–271 °C, in a yield of 2.1 × 10^{-2}% of the fresh weight of the fungus. This orange pigment is optically active, $[\alpha]_D$ +22° ($CHCl_3$), and exhibits an abundant molecular ion at *m/z* 354 in the mass spectrum. High resolution mass measurement and combustion analysis data established the molecular formula $C_{19}H_{14}O_7$ for dermolactone.

The presence of a 1,8-dihydroxyanthraquinone chromophore was suggested by the long wavelength UV absorption at 450 nm, and supported when addition of sodium hydroxide caused a characteristic bathochromic shift to 516 nm.[14] The IR spectrum of the pigment showed absorption at 3 430 cm^{-1} assignable to a chelated hydroxy group, and strong quinonoid carbonyl bands at 1 675 cm^{-1} (free) and 1 621 cm^{-1} (chelated). A third carbonyl absorption, at 1 726 cm^{-1}, is consistent with the presence of an ester or a δ-lactone ring. The ^{1}H NMR spectrum revealed low field singlets at δ 13.35 and 12.36 which may be assigned to chelated phenolic hydroxy groups, three aromatic protons consisting of a broad singlet at δ 7.64 and a pair of *meta* coupled



(13) R = H
(14) R = OH



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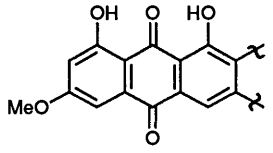

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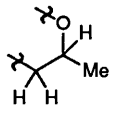


(17)

doublets at δ 7.35 and 6.73, and a methoxy resonance at δ 3.95. These data are fully consistent with the partial structure (**16**) for dermolactone.

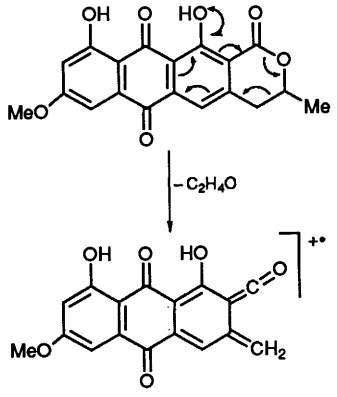
The remaining elements of the dermolactone structure must accommodate both the inferred carbonyl functionality (ν_{max} 1 726 cm^{-1}), and further ^1H NMR data, discussed below, which define the partial structure (**17**).

Thus, two and one proton multiplets at δ 3.05 and 4.70, respectively, constitute an ABX pattern. The one proton multiplet at δ 4.70 is further coupled to a three proton doublet at δ 1.55 (*J* 6.6 Hz). Irradiation of this three proton doublet caused collapse of the one proton resonance at δ 4.70 to a doublet of doublets (*J* 5.1 and 3.1 Hz), while irradiation of the AB multiplet at δ 3.05 caused collapse of the same methine resonance to a quartet (*J* 6.6 Hz) and simultaneously increased the height of the aromatic singlet at δ 7.64. These irradiation experiments and others confirmed the presence of a Me-CH(O)-CH_2 moiety and, furthermore, established the relative disposition of this moiety with respect to the A ring in dermolactone (**13**). Once the third carbonyl group is incorporated between partial formulae (**16**) and (**17**) a δ-lactone structure emerges for dermolactone.

The connectivity between rings A, B, and C in (**13**) was confirmed by examination of the fully proton coupled ^{13}C NMR spectrum. Most notable from these data is the fact that one of the quinone carbonyl carbon resonances appears as a triplet (δ 181.4, *J* 4.4 Hz) due to three bond coupling to two *peri* hydrogens (4-H and 5-H). Thus, all the spectroscopic data collected for dermolactone are consistent with structure (**13**) for this pigment.

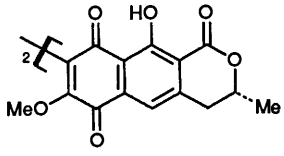
In the mass spectrum of dermolactone (**13**) the base peak appears at *m/z* 310 ($C_{17}H_{10}O_6$ by high resolution mass measurement) and may be considered to originate from the molecular ion, *m/z* 354, *via* a concerted elimination of the elements of acetaldehyde as shown in Scheme 1.

Consistent with structure (**13**), dermolactone is optically active {$[\alpha]_D$ +22° ($CHCl_3$)}. The CD spectrum of the pigment is recorded in the Experimental section. The δ-lactone ring in (**13**) is reminiscent of similar functionality found in the carcinogenic naphthoquinone xanthomegnin (**18**), and several closely related pigments from the moulds *Aspergillus sulphureus* and *A. melleus*.[15] The same ring system is found in its simplest form in the mould metabolite mellein (**19**), a co-metabolite of



Scheme 1. Principal mass spectral fragmentation of dermolactone (**13**).

xanthomegnin in *A. melleus*.[15] The absolute stereochemistry of (**18**) and (**19**) is known from the results of degradative studies from which (*R*)-(−)-β-hydroxybutyric acid was ultimately obtained,[16,17] however, the absolute configuration of dermolactone (**13**) is not known at this stage.



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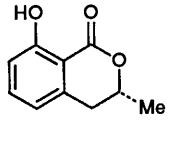


(19)

The pink–red pigment (**14**), $C_{19}H_{14}O_8$ (high resolution mass spectrometry), was obtained as a powder, m.p. 220–228 °C, in a yield of 4.5 × 10^{-3}% of the fresh weight of the fungus. Like dermolactone (**13**) this pink metabolite is optically active, $[\alpha]_D$ +92° ($CHCl_3$), and a close relationship between the two was immediately apparent on inspection of the respective ^1H NMR spectra. Thus, while resonances characteristic of the δ-lactone ring, an aromatic methoxy, and a pair of *meta* coupled aromatic protons are common to both spectra, the isolated aromatic proton resonance (δ 7.64) in the spectrum of dermolactone (**13**) is replaced in the spectrum of (**14**) by a third lowfield signal (δ 13.46) consistent with the presence of a hydroxy group at C-4. This accords with the molecular formula of (**14**) and with the UV spectrum, which shows long wavelength absorption (λ_{max} 455sh, 477, 500, and 538 nm) typical of a 1,4-dihydroxy-9,10-anthraquinone chromophore.[14] Interestingly, the presence of a hydroxy group at C-4 in (**14**) modifies the conformation adopted by the δ-lactone ring and increases the magnetic non-equivalence of the benzylic methylene protons, which now resonate at δ 2.75 (dd, *J* 17.6 and 3.0 Hz) and δ 3.38 (dd, *J* 17.6 and 11.8 Hz).

The third anthraquinone isolated from *D. sanguinea* was the yellow dermoquinone (**15**), m.p. 195–200 °C, which was obtained in a yield of 6 × 10^{-4}% of the fresh weight of the fungus. A molecular formula $C_{18}H_{14}O_6$ is consistent with a molecular ion at *m/z* 326 in the mass spectrum, and a 1,8-dihydroxy-9,10-anthraquinone chromophore was evident from the UV spectrum (λ_{max} 433 nm, shifted to 514 on addition of base).[14] The ^1H NMR data, collected in Figure 2, are in full accord with structure (**15**) for dermoquinone, a molecule which would seem to arise, at least formally, *via* dehydration and tautomerisation from dermochrysone (**8**).

The structure (**15**) corresponds to the (uncharacterised) 'optically inactive ketone, $C_{18}H_{14}O_6$' derived by mild oxidation of nalgiovensin (**20**) a metabolite of *Penicillium nalgiovensis*.[18] *P. nalgiovensis* is an organism known only from the manufacture of Ellischaurer cheese in Czechoslovakia.[14]

A cursory examination of the major pigments from *Dermo-*

Table 1. ^{13}C NMR data ($CDCl_3$) and enrichments for dermochrysone (**8**) isolated from *D. sanguinea* after impregnation with sodium [1-^{13}C]acetate.

Carbon	Chemical shift (δ)	Atom% enrichment[a]	Carbon	Chemical shift (δ)	Atom% enrichment[a]
C-1	200.9	0.9	C-7	101.1	—
C-1′	50.7	—	C-8	159.8	0.8
C-2	49.3	—	C-8a	108.0	—
C-2′	209.9	0.8	C-9	165.9	1.3
C-3	71.5	0.7	C-9a	108.1	—
C-4	41.4	—	C-10	117.6	—
C-4a	134.7	0.7	C-10a	141.0	1.3
C-5	99.8	—	6-OMe	55.5	—
C-6	163.6	0.5	2′-Me	31.6	—

[a] Refers to ^{13}C content over and above natural abundance (1.1%).

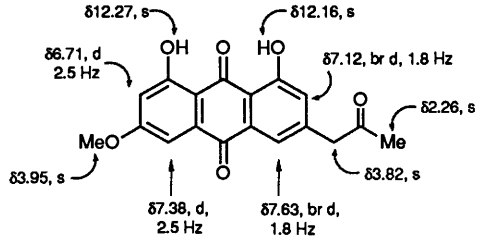
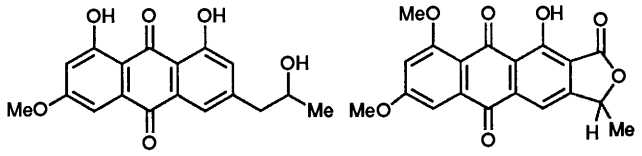


Figure 2. ^1H NMR data ($CDCl_3$) for dermoquinone (**15**).



(20) (21)

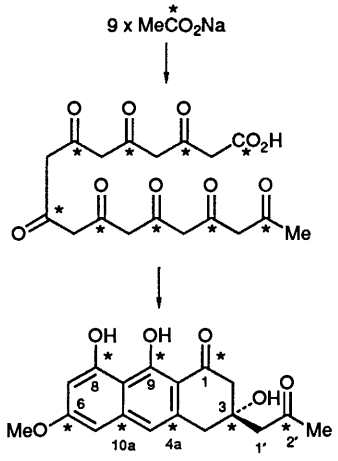
cybe sanguinea (sensu Cleland), namely dermochrysone (**8**) and dermolactone (**13**), suggests a probable common biogenesis from a nonaketide precursor. In order to firmly establish the biogenetic origins of the pigments present in the fruit bodies of *D. sanguinea*, sodium [1-^{13}C]acetate was administered to toadstools growing in their natural environment.

Fruit bodies of *D. sanguinea* are fragile and we harboured serious doubts as to whether they were capable of surviving the impregnation procedure. Nevertheless, three young carpophores were supplied *via* syringe, each with 500 μl of a 2.44M aqueous solution of sodium [1-^{13}C]acetate. The fruit bodies survived and were allowed to grow for a further 3 days after which time they were harvested and the pigments (**8**) and (**13**) were isolated from the extracts as described previously.

All resonances in the ^{13}C NMR spectra of dermochrysone (**8**) and dermolactone (**13**) have been unequivocally assigned from examination of fully proton coupled spectra, and by analogy with the corresponding spectra obtained for torosachrysone (**9**)[12] and austrocorticin (**21**).[19] All assignments have been corroborated by 2D ^1H-^{13}C COSY experiments.

The ^{13}C NMR spectrum of dermochrysone (**8**) isolated from the feeding experiments shows specific incorporation at carbon atoms 1, 2′, 3, 4a, 6, 8, 9, and 10a. Enrichment in ^{13}C content at these sites was measured by comparison of the intensities of resonances in both the natural abundance and enriched spectra after normalisation.[20] These data are collected in Table 1.

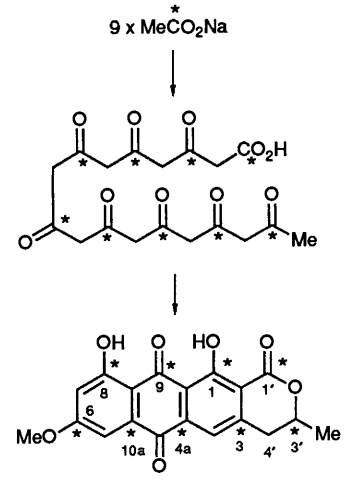
The specific incorporation of label from sodium [1-^{13}C]-acetate into the pigment (**8**) is entirely consistent with the formation of dermochrysone from a nonaketide precursor, itself assembled (at least formally) by head-to-tail linkage of acetate units as depicted in Scheme 2.



Scheme 2. Incorporation of sodium [1-^{13}C]acetate into dermochrysone (**8**).

The ^{13}C NMR spectrum of dermolactone (**13**) isolated from the feeding experiment shows specific incorporation at carbon atoms 1, 1′, 3, 3′, 4a, 6, 8, 9, and 10a. Enrichment in ^{13}C content at these sites was quantified in the usual way and these data are collected in Table 2.

The specific sites of incorporation of label from sodium [1-^{13}C]acetate into pigment (**13**) are fully consistent with the formation of dermolactone from a nonaketide precursor, itself assembled by head-to-tail linkage of acetate units as depicted in Scheme 3.

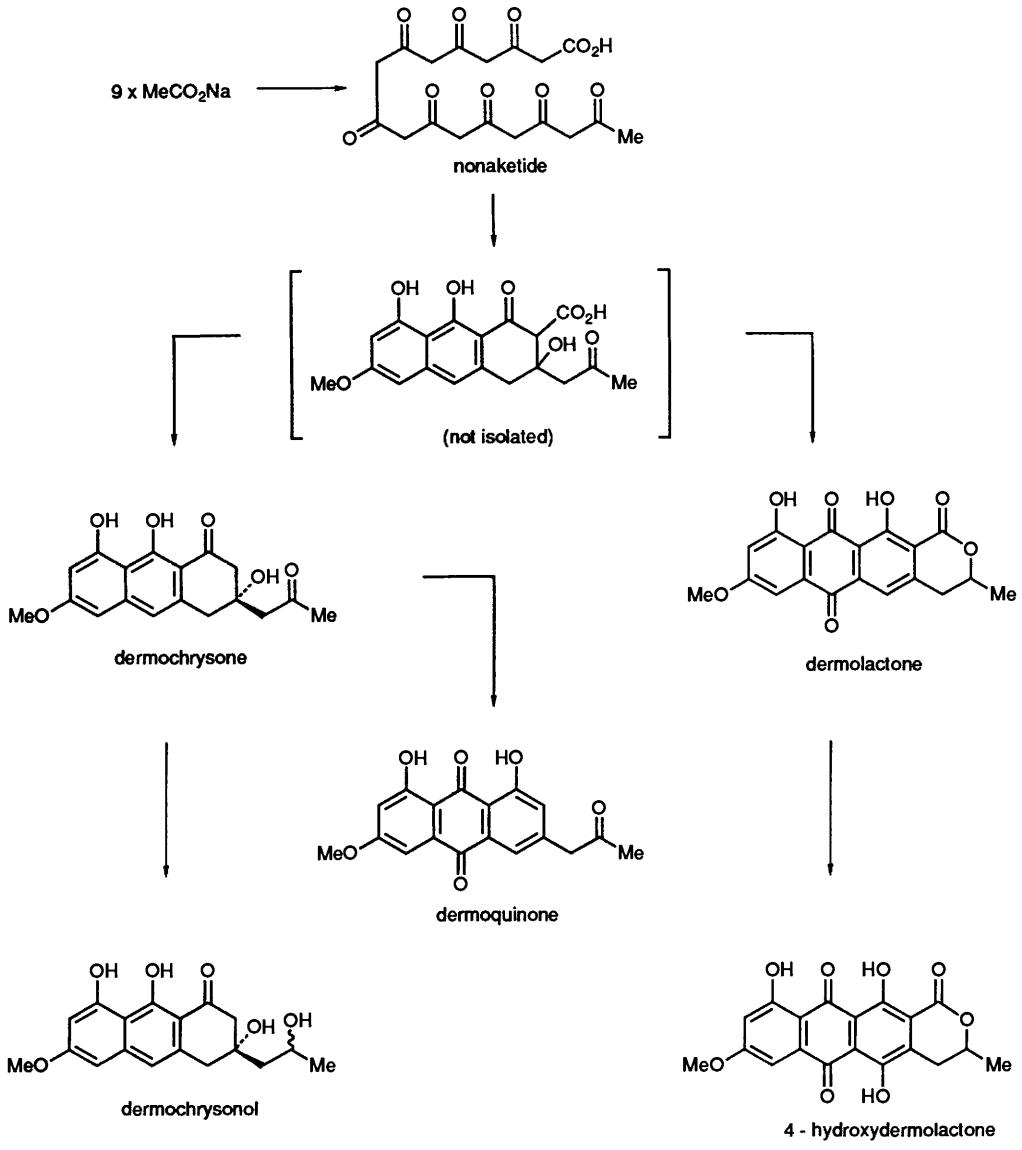


Scheme 3. Incorporation of sodium [1-^{13}C]acetate into dermolactone (**13**).

Table 2. ^{13}C NMR data ($CDCl_3$) and enrichments for dermolactone (**13**) isolated from *D. sanguinea* after impregnation with sodium [1-^{13}C]acetate.

Carbon	Chemical shift (δ)	Atom% enrichment[a]	Carbon	Chemical shift (δ)	Atom% enrichment[a]
C-1	164.1	0.7	C-7	107.3	—
C-1′	166.7	1.1	C-8	165.6	0.6
C-2	117.1	—	C-8a	110.2	—
C-3	148.7	0.9	C-9	189.3	0.4
C-3′	74.5	1.3	C-9a	116.7	—
C-4	117.3	—	C-10	181.4	—
C-4′	36.2	—	C-10a	134.3	0.3
C-4a	136.9	0.3	6-OMe	56.2	—
C-5	108.7	—	3′-Me	20.6	—
C-6	166.7	1.1			

[a] Refers to ^{13}C content over and above natural abundance (1.1%).

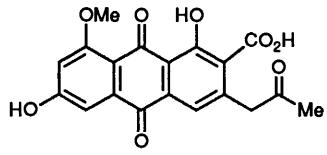


Scheme 4. Possible biosynthetic relationships between pigments of *Dermocybe sanguinea* (sensu Cleland).

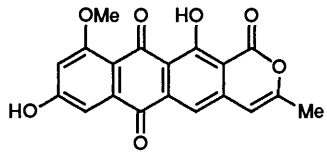
On the basis of these results, a series of biosynthetic relationships between pigments of *Dermocybe sanguinea* (sensu Cleland) may be proposed as depicted in Scheme 4.

The pigments (**8**) and (**11**)–(**15**) are the first nonaketide derived metabolites to be found in *Dermocybe*. Three anthraquinones, leprolutein (**22**), anhydroleprolutein (**23**), and leprovenetin (**24**),

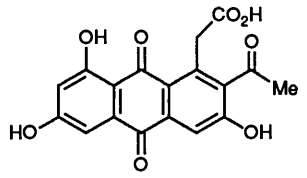
for which a nonaketide origin has been suggested,[6] have been isolated in trace amounts from toadstools belonging to *Cortinarius* subgenus *Leprocybe*.[8] As well as establishing a new pathway for pigment production in *Dermocybe*, our studies invalidate the link between the Australian taxon and *Dermocybe sanguinea* (Wulf. ex Fr.) Wünche in which an octaketide pathway is clearly operational.



(22)



(23)



(24)

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, IR spectra as potassium bromide discs on a Perkin-Elmer 983G spectrophotometer, UV spectra in ethanol (unless stated otherwise) on a Varian SuperScan 3 spectrophotometer, ^1H and ^{13}C NMR spectra on a JEOL JNM-GX-400 spectrometer operating at 399.65 MHz (^1H) and 100.40 MHz (^{13}C) for solutions in deuteriochloroform (unless stated otherwise), mass spectra on a V.G. Micromass 7070F spectrometer (EI probe, 70 eV), and optical rotations on a Perkin-Elmer 241MC polarimeter at 22 °C. CD spectra were obtained using a Cary 61 spectropolarimeter for solutions in methanol (unless stated otherwise). All TLC and preparative TLC (PLC) was performed on Merck Kieselgel 60 GF_{254}; R_F values quoted for pure compounds were measured using toluene–ethyl formate–formic acid (50:49:1) as eluant. Column chromatography used Merck Kieselgel 60 silica gel. Gel permeation chromatography employed Pharmacia Sephadex LH-20 suspended in and eluted with methanol. Solutions were routinely dried over Na_2SO_4 prior to concentration under reduced pressure (water bath; 35 °C). Sodium [1-^{13}C]acetate (99.5 atom% ^{13}C) was used as purchased from Sigma–Aldrich.

Voucher specimens of *Dermocybe sanguinea* (sensu Cleland) are lodged in the herbarium of the Royal Botanic Garden, Edinburgh, U.K., under accession number WAT20931.

Isolation of Pigments from Dermocybe sanguinea (*sensu Cleland*).—Fresh fruit bodies were collected from mixed *Eucalyptus* forest along the Island Hop track near Marysville, Victoria, during June–July, 1988. A quantity (66 g) was finely chopped and soaked in ethanol (600 ml) at room temperature for 3 h. The extract was evaporated and the red residue (0.28 g) was partitioned between ethyl acetate (3 × 100 ml) and water (150 ml). The dried (Na_2SO_4) organic layer was evaporated to dryness and the red residue (45 mg) was dissolved in chloroform (8 ml). The solution was applied to a short column (3.5 × 3.0 cm) of silica gel and eluted with toluene–ethyl formate–formic acid (50:49:1) to afford two fractions: a red–orange fraction which eluted first (18 mg) and a green fraction (12 mg) which followed. Each fraction was further purified separately.

The Less Mobile Fraction.—The components of the green fraction (12 mg) were separated by PLC using toluene–ethyl formate–formic acid (50:49:1) into two zones, each of which was further purified by gel permeation. The less polar zone gave (S)-(+)-*dermochrysone* (**8**) (R_F 0.33) (9.2 mg, 1.4 × 10^{-2}% fr. wt.) as green prisms, m.p. 189–192 °C from methanol at −20 °C (Found: C, 64.5; H, 5.5. $C_{18}H_{18}O_6$. 1/2 MeOH requires C, 64.2; H, 5.8%; Found: M^+, 330.1103. $C_{18}H_{18}O_6$ requires M, 330.1103); $[\alpha]_D$ +28° (*c* 0.14 in $CHCl_3$); CD 275 ($\Delta\varepsilon$ − 0.40 dm^3 mol^{-1} cm^{-1}), 280 (0.0), 290 (+0.93), 308 (+0.17), 315 (+0.36), 340 (−0.04), and 400 nm (0.0); ν_{max} 3 473, 1 692, and 1 635 cm^{-1}; λ_{max} 278 (log ε 4.55), 318 (4.01), and 397 nm (4.30); *m/z* 330 (M^+, 38%), 312 ($C_{18}H_{16}O_5$, 20), and 272 ($C_{15}H_{12}O_5$, 100); δ_H 2.19 (3 H, s, 2′-Me), 2.78 (2 H, br s, 1′-H_2), 2.84 and 2.94 (each 1 H, d, *J* 17.6 Hz, 2-H_2), 3.02 and 3.14 (each 1 H, d, *J* 15.6 Hz, 4-H_2), 3.88 (3 H, s, 6-OMe), 4.02 (1 H, s, 3-OH), 6.48 (1 H, d, *J* 2.4 Hz, 7-H), 6.54 (1 H, d, *J* 2.4 Hz, 5-H), 6.84 (1 H, br s, 10-H) 9.76 (1 H, s, 8-OH), and 15.99 (1 H, br s, 9-OH); δ_C, see Table 1.

The molar polar zone contained a mixture of the *dermochrysonols* (**11**) and (**12**) (R_F 0.24) (0.4 mg, 6 × 10^{-4}% fr. wt.) as dark green needles, m.p. 155–160 °C from methanol at −20 °C (Found: M^+, 332. $C_{18}H_{20}O_6$ requires M, 332); ν_{max} 3 455, 1 642, and 1 595 cm^{-1}; λ_{max} 222, 275, 322, 335sh, and 405 nm; *m/z* 332 (M^+, 10%), 314 (42), and 270 (100); δ_H 1.23 and 1.26 (each 3 H, d, *J* 6.5 Hz, 2′-Me), 1.65–1.85 (4 H, m, 2′-H_2), 2.70–3.26 (8 H, m, 2-H_2 and 4-H_2), 3.84 (6 H, s, 6-OMe), 6.46 (2 H, d, *J* 2.2 Hz, 7-H), 6.54 (2 H, br s, 5-H), 6.89 (2 H, br s, 10-H), 9.80 and 9.81 (each 1 H, s, 8-OH), and 16.01 and 16.02 (each 1 H, s, 9-OH).

The More Mobile Fraction.—The components of the red–orange fraction (18 mg) were separated by PLC [toluene–ethyl formate–formic acid (140:60:1), three consecutive developments] to afford in order of decreasing polarity: (+)-*dermolactone* (**13**) (R_F 0.62) (14 mg, 2.1 × 10^{-2}% fr. wt.) as orange needles, m.p. 268–271 °C from ethyl acetate–formic acid at −20 °C (Found: C, 64.6; H, 4.0. $C_{19}H_{14}O_7$ requires C, 64.4; H, 4.0%); $[\alpha]_D$ +22° (*c* 0.07 in $CHCl_3$); CD($CHCl_3$) 248 ($\Delta\varepsilon$ + 0.45 dm^3 mol^{-1} cm^{-1}), 258 (−0.97), 275 (0.0), 295 (+0.6), 320 (−0.20), and 400 nm (0.0); ν_{max} 3 430, 1 726, 1 675, and 1 621 cm^{-1}; λ_{max}($CHCl_3$) 278sh (log ε 4.22), 287 (4.27), and 450 nm (3.95); λ_{max}($CHCl_3$ + OH^-) 516 nm (3.81); *m/z* 354 (M^+, 86%) and 310 ($C_{17}H_{10}O_6$, 100); δ_H 1.55 (3 H, d, *J* 6.6 Hz, 3′-Me), 3.05 (2 H, m, 4′-H_2), 3.95 (3 H, s, 6-OMe), 4.70 (1 H, m, 3′-H), 6.73 (1 H, d, *J* 2.6 Hz, 7-H), 7.35 (1 H, d, *J* 2.6 Hz, 5-H), 7.64 (1 H, br s, 4-H), 12.36 (1 H, s, 1-OH), and 13.35 (1 H, s, 8-OH); δ_C 20.6 (q, *J* 129.1 Hz, 3′-Me), 36.2 (t, *J* 135.0 Hz, C-4′), 56.2 (q, *J* 145.7 Hz, 6-OMe), 74.5 (d, *J* 150.0 Hz, C-3′), 107.3 (ddd, *J* 162.8, 8.8, and 5.8 Hz, C-7), 108.7 (dd, *J* 167.0 and 6.0 Hz, C-5), 110.2 (q, *J* 5.1 Hz, C-8a), 116.7 (m, C-9a), 117.1 (m, C-2), 117.3 (dt, *J* 168.7 and 3.0 Hz, C-4), 134.3 (s, C-4a), 136.9 (s, C-10a), 148.7 (m, C-3), 164.1 (d, *J* 3.4 Hz, C-1), 165.6 (t, *J* 5.1 Hz, C-8), 166.7 (m, C-6 and C-1′), 181.4 (t, *J* 4.4 Hz, C-10), and 189.3 (s, C-9), (+)-4-*hydroxydermolactone* (**14**) (R_F 0.66) (3.0 mg, 4.5 × 10^{-3}% fr. wt.) as a red powder, m.p. 220–228 °C (decomp.) from chloroform–light petroleum (b.p. 40–60 °C) (Found: M^+, 370.0689. $C_{19}H_{14}O_8$ requires M, 370.0689); $[\alpha]_D$ +92° (*c* 0.08 in $CHCl_3$); ν_{max} 3 400, 1 723, 1 670, and 1 645 cm^{-1}; λ_{max}($CHCl_3$) 227 (log ε 4.40), 254 (4.15), 275 (4.02), 300 (3.70), 455sh (3.96), 477 (4.02), 500 (4.05), and 538 nm (3.90); *m/z* 370 (M^+, 18%),

328 (33), 326 (57), 324 (68), 57 (93), and 44 (100); δ_H 1.57 (3 H, d, *J* 6.2 Hz, 3′-Me), 2.75 (1 H, dd, *J* 17.6 and 3.0 Hz, 4′-H_{eq}), 3.38 (1 H, dd, *J* 17.6 and 11.8 Hz, 4′-H_{ax}), 3.96 (3 H, s, 6-OMe), 4.62–4.68 (1 H, m, 3′-H), 6.77 (1 H, d, *J* 2.5 Hz, 7-H), 7.43 (1 H, d, *J* 2.5 Hz, 5-H), 12.36 (1 H, s, 1-OH), 13.05 (1 H, s, 8-OH), and 13.46 (1 H, s, 4-OH), and *dermoquinone* (**15**) (R_F 0.74) (0.4 mg, 6.1 × 10^{-4}% fr. wt.) as orange needles, m.p. 195–200 °C from methanol at −20 °C (Found: M^+, 326. $C_{18}H_{14}O_6$ requires *M*, 326); ν_{max} 3 422, 1 705, 1 680, and 1 620 cm^{-1}; λ_{max} 225 (log ε 4.27), 266 (4.03), 287 (3.98), and 433 nm (3.79); λ_{max} (EtOH + OH^-) 514 nm (3.70); *m*/*z* 326 (M^+, 30%) and 284 (100); δ_H 2.26 (3 H, s, 2′-Me), 3.82 (2 H, s, 1′-H_2), 3.95 (3 H, s, 6-OMe), 6.71 (1 H, d, *J* 2.5 Hz, 7-H), 7.12 (1 H, br d, *J* 1.8 Hz, 2-H), 7.38 (1 H, d, *J* 2.5 Hz, 5-H), 7,63 (1 H, br d, *J* 1.8 Hz, 4-H), 12.16 (1 H, s, 1-OH), and 12.27 (1 H, s, 8-OH).

Feeding Experiments.—Three young fruit bodies of *Dermocybe sanguinea* (sensu Cleland) were each impregnated once using a syringe (*ca.* 10 a.m.) with an aqueous solution of sodium [1-^{13}C]acetate (500 μl, 2.44M, 99.5 atom% ^{13}C). On day 4 (*ca.* 4 p.m.), the toadstools were picked, finely chopped and soaked in ethanol (250 ml). Dermochrysone (**8**) (5 mg) and dermolactone (**13**) (3 mg) were isolated as described above. The ^{13}C NMR spectrum of dermochrysone showed specific incorporation of label as detailed in Table 1, and the ^{13}C NMR spectrum of dermolactone showed specific incorporation of label as detailed in Table 2.

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