# Pigments of fungi. Part 40.[1,2] Dermocanarins 1–3, unique naphthol- and naphthoquinone-anthraquinone dimers that contain a macrocyclic lactone ring from the fungus *Dermocybe canaria* Horak

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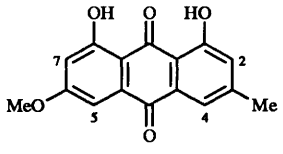
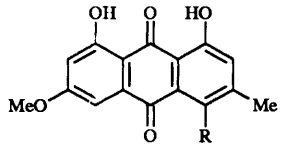
The dermocanarins **4–6** are unique naphthylanthraquinones containing a nine-membered lactone ring that have been isolated as the principal pigments of the subterranian mycelium of the fungus *Dermocybe canaria* and their structures determined by spectroscopic methods. In the fruit bodies of this fungus physcion 8-*O*-β-D-glucopyranoside **12** accompanies the aglycone **1** as the major colouring matters.

In New Zealand *Nothofagus* forests *Dermocybe canaria* is a common species met within a remarkably wide ecological range.[3] The toadstool is readily identified by the brilliant yellow colours of the rather robust fruit bodies[4] and the dense mat of bright yellow mycelium from which the toadstools emanate. Air-dried fruit bodies of *D. canaria* collected by Horak in New Zealand[3] were examined chemically by Keller and Steglich who found that the yellow anthraquinone physcion **1** is the most abundant pigment present, accompanied by lesser amounts of erythroglaucin **2** and the unique aminoanthraquinone **3**.[5]

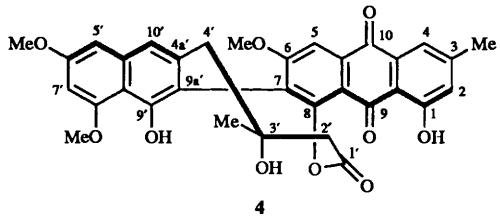
As part of our continuing studies on the constituents of *Dermocybe* species indigenous to Australia[6,7] we have examined the pigments present both in the fresh fruit bodies and in the wild subterranian mycelium of *D. canaria* collected from *Nothofagus* forests in Tasmania, and also examined fresh specimens collected in New Zealand. We report here full details[2] of the isolation, from the mycelium of *D. canaria*, of the first members of a new class of coupled octaketide pigments, the dermocanarins, in which anthraquinone and naphthalene moieties are linked both by a chiral biaryl bond and a lactone bridge. We have also found that in the fruit bodies of *D. canaria* physcion **1** and erythroglaucin **2** are accompanied by their respective 8-*O*-β-D-glucopyranosides. We have been unable to detect 4-aminophyscion **3** in either the mycelium or the fresh fruit bodies of *D. canaria*.



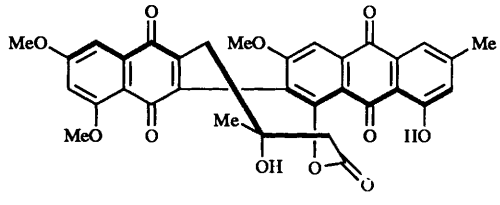
**1**



**2** R = OH
**3** R = $NH_2$



**4**



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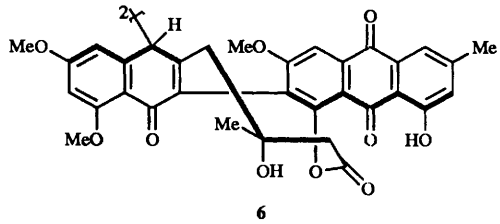


**6**

## Results and discussion

### Pigments from the mycelium

It is rare for chemists to study the constituents of fungal mycelium grown in its natural habitat. However, in the case of *D. canaria*, collection was facilitated by the intense yellow colour and density of the abundant mycelial mat. Extraction with ethanol gave a black extract that was heavily contaminated with lignicaceous material unavoidably leached from the soil debris that inevitably contaminated the mycelium. Nevertheless, after repeated chromatography on silica gel and gel permeation through Sephadex LH-20, the three major yellow pigments, which we have called the dermocanarins 1–3, were isolated in pure form. Unfortunately, no reliable estimate of the concentration in the mycelium of each of the new pigments can be made in view of the contamination mentioned earlier.

Dermocanarin 1 **4**† was obtained as an optically active yellow powder. The molecular formula $C_{33}H_{28}O_{10}$ immediately suggested that dermocanarin 1 belonged to the large group of coupled octaketides that occur in several toxic[8] and medicinal plants,[9] in moulds,[10] and in many fungi belonging to *Dermocybe*, *Cortinarius*, and their allies.[6,11] The infrared spectrum of the pigment **4** showed absorptions at 1667 and 1633 $cm^{-1}$ typical of free and chelated carbonyl groups, respectively, of an anthraquinone,[12] and a third carbonyl absorption at 1754 $cm^{-1}$ that suggested the presence of an ester.[13] The electronic spectrum, with absorption maxima at 235, 272 and 415 nm, supported the presence of an anthraquinone moiety while a pronounced bathochromic shift of the long-wavelength absorption to 510 nm on addition of base suggested that the

† For consistency and ease of comparison of spectroscopic data, both here and in papers to follow, the numbering of the lactone bridge and the naphthalenoid ring in molecules of the dermocanarin type is as shown in structure **4**. It is based on the presumption that these parts of the dermocanarin molecule are derived biogenetically from a dihydroanthracenone of type **10** and are numbered accordingly.

**Table 1** $^1$H NMR data (400 MHz; $CDCl_3$) for the dermocanarins 1 **4**, 2 **5** and 3 **6**

| Nucleus | Chemical shift ($\delta$), multiplicity and coupling constant ($J$/Hz) | | |
|---|---|---|---|
| | **4** | **5** | **6** |
| 2-H | 7.11 (br s) | 7.12 (br s) | 7.11 (br s) |
| 4-H | 7.65 (br s) | 7.64 (br s) | 7.66 (br s) |
| 5-H | 7.90 (s) | 7.83 (s) | 7.92 (s) |
| 3-Me | 2.47 (s) | 2.47 (s) | 2.47 (s) |
| 6-OMe | 4.00 (s) | 3.98 (s) | 4.24 (s) |
| 1-OH | 12.54 (s) | 12.38 (s) | 12.48 (s) |
| 2′-H$^A$ | 2.46 (br d, 13.3) | 2.47 (d, 13.2) | 2.57 (br s) |
| 2′-H$^B$ | 2.54 (d, 13.3) | 2.71 (d, 13.2) | 2.57 (br s) |
| 4′-H$^A$ | 2.39 (d, 13.7) | 1.97 (d, 13.9) | 2.23 (d, 13.5) |
| 4′-H$^B$ | 2.86 (br d, 13.7) | 3.36 (br d, 13.9) | 3.39 (br d, 13.5) |
| 5′-H | 6.71 (d, 2.0) | 7.32 (d, 2.4) | 6.67 (d, 2.2) |
| 7′-H | 6.45 (d, 2.0) | 6.75 (d, 2.4) | 6.17 (d, 2.2) |
| 10′-H | 7.26 (s) | | 4.68 (s) |
| 3′-Me | 1.32 (s) | 1.39 (s) | 1.32 (s) |
| 3′-OH[a] | 3.20 (s) | 3.03 (s) | 2.57 (s) |
| 6′-OMe | 3.90 (s) | 3.96 (s) | 3.78 (s) |
| 8′-OMe | 3.97 (s) | 3.92 (s) | 3.67 (s) |
| 9′-OH | 9.38 (s) | | |

[a] Signal vanished on addition of $D_2O$.

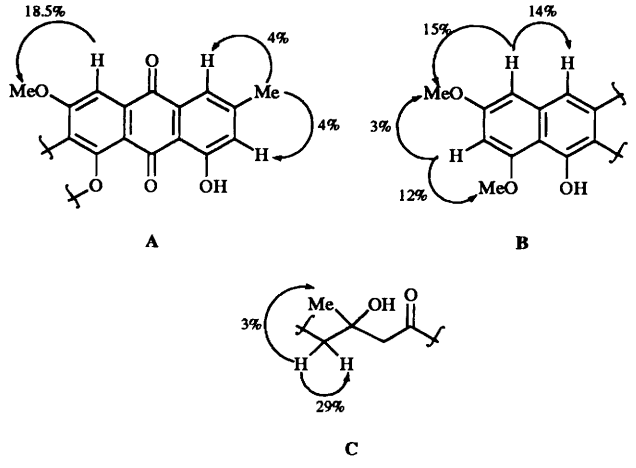



**Fig. 1** Partial structures **A**, **B** and **C** of dermocanarin 1 **4** showing nuclear Overhauser enhancements

anthraquinone nucleus contains a *peri*-hydroxy group.[12] Further details of the substituents in the anthraquinone 'half' of dermocanarin 1 **4** were deduced from the NMR spectra. Thus, the $^1$H NMR spectrum (Table 1) revealed the presence of three aromatic protons, one hydroxy group, one methoxy and one C-methyl group. These substituents could be assembled as depicted in Fig. 1, partial formula **A**, by consideration of the $^{13}$C NMR data (Table 2) and the results of specific proton irradiation and nuclear Overhauser enhancement (NOE) experiments, in the following way. One of the quinone carbonyls, C-10, appears as a triplet ($J$ 4.4 Hz) at $\delta_C$ 182.0 while the other, C-9, resonates at $\delta_C$ 186.6 as a sharp singlet. It follows that protons must occupy both sites *peri* to C-10, while the sites flanking C-9 bear non-hydrogen substituents. Of the two protons that flank C-10, one ($\delta$ 7.65) is broadened by *meta* coupling to the third aromatic proton at $\delta$ 7.11, while the other ($\delta$ 7.90) resonates as a sharp singlet thereby indicating a substituent other than hydrogen at C-7. The results of NOE experiments (Fig. 1, **A**) place the aromatic methyl group ($\delta$ 2.47) at C-3, between the *meta* coupled protons, while the methoxy group ($\delta$ 4.00) must be located at C-6, adjacent to the isolated *peri* proton, 5-H. It remains to differentiate between C-1 and C-8 as the location of the chelated hydroxy group ($\delta$ 12.54) and to confirm C-7 as the site at which the anthraquinone nucleus is connected to the rest of the dermocanarin 1 molecule.

Irradiation of 5-H ($\delta$ 7.90) in **4** simplified several signals in the fully coupled $^{13}$C NMR spectrum, including the one at $\delta_C$ 128.5, which collapsed from a doublet ($J$ 6.1 Hz) to a sharp singlet. The coupling constant implies three-bond coupling and identified the nucleus as C-7, while the chemical shift ($\delta_C$ 128.5) is inconsistent with oxygenation and implies a bond through carbon to the other 'half' of the molecule. The carbon bearing the free phenolic hydroxy group resonates at $\delta_C$ 162.6 as a doublet of doublets ($J$ 4.4 and 3.0 Hz). That the larger coupling was due to a two-bond interaction with the hydroxy proton was confirmed when irradiation at the frequency of that proton ($\delta$ 12.54) caused the $\delta_C$ 162.6 signal to collapse to a doublet ($J$ 3.0 Hz). The smaller coupling was traced in turn to 2-H when irradiation of the signal at $\delta$ 7.11 caused the C-1 signal to collapse to a doublet that then expressed only the larger coupling ($J$ 4.4 Hz). Consistent with these observations, C-8 resonated at $\delta_C$ 152.3 as a sharp singlet.

The partial structure **A** accounts for a fragment $C_{16}H_{10}O_5$ of the molecular formula $C_{33}H_{28}O_{10}$ of dermocanarin 1 **4**. While the remaining elements, $C_{17}H_{18}O_5$, of **4** were strongly suggestive of a dihydroanthracenone sub-unit[11] this proposal was not supported by the remaining data from the $^1$H (Table 1) and $^{13}$C NMR spectra (Table 2). Instead, these data clearly revealed the presence of the substituted naphthalene fragment **B** and the 3-hydroxy-3-methylbutanoyl segment **C** (Fig. 1), as follows. The $^1$H NMR spectrum identified three aromatic protons, two of which are *meta* disposed, two methoxy groups and a phenolic hydroxy group. The two methoxy groups together with the *meta*-coupled aromatic protons were placed in the same ring from the results of difference NOE experiments that are summarized in Fig. 1, partial structure **B**. Enhancement of the isolated aromatic proton ($\delta$ 7.26) upon irradiation of 5′-H ($\delta$ 6.71) located the former at C-10′, while the position of the hydroxy group at C-9′ followed from irradiation experiments in the $^{13}$C NMR spectrum that are detailed as footnotes in Table 2.

The remaining segment **C**, $C_5H_8O_2$, in compound **4** was readily identified from the IR and NMR data as comprising an ester carbonyl group ($\nu_{max}$ 1754 cm$^{-1}$; $\delta_C$ 169.0), two non-adjacent methylene groups, a C-methyl group ($\delta$ 1.32), and an aliphatic hydroxy group ($\delta$ 3.20); the latter two functional groups being situated on the same tertiary centre ($\delta_C$ 70.7). The protons of the C-2′ methylene group appear as an AB quartet ($J$ 13.3 Hz) with components centred at $\delta$ 2.46 and 2.54. Irradiation of these protons caused the ester carbonyl signal ($\delta_C$ 169.0) to collapse from a triplet ($J$ 5.9 Hz) to a singlet and sharpened considerably the signal at $\delta_C$ 70.7 due to C-3′. The protons of the C-4 methylene group also appear as an AB quartet ($J$ 13.7 Hz) with components that differ considerably in chemical shift ($\delta$ 2.39 and 2.86), as discussed further below. The low-field signal in this AB quartet showed strong NOE not only with its geminal neighbour and with the C-3′ methyl protons as shown in Fig. 1 **C** but also with the isolated aromatic proton (10′-H) in the naphthalene ring of compound **4**. This important interaction anchors the C-4′ terminus of the segment **C** to C-4a′ in fragment **B**. The carbon C-9a′, the only site in the naphthalene ring of compound **4** that remains equivocal at this point, resonated as a complex multiplet at $\delta_C$ 111.6, a shift consistent with a bond from C-9a′ to carbon, but not to oxygen. Thus, once it is recognized that the carbonyl carbon C-1′ at the alternative terminus in fragment **C** is part of a lactone bridge, the substructures **A**, **B** and **C** come together unambiguously as the macrocycle **4**.

The assignments made in Tables 1 and 2 and the conclusions

**Table 2** $^{13}C$ NMR data (100 MHz; $CDCl_3$) for the dermocanarins 1 **4**, 2 **5** and 3 **6**

| Nucleus | Chemical shift ($\delta$), multiplicity and coupling constant(s) ($J$/Hz) | | |
|---|---|---|---|
| | **4** | **5** | **6**[a] |
| C-1 | 162.6 (dd, 4.4 and 3.0)[b,c] | 162.6 (dd, 4.0 and 3.0) | 162.6 |
| C-2 | 125.0 (br d, 160.0) | 125.1 (br d, 161.3) | 124.9 |
| C-3 | 148.0 (q, 5.9) | 148.2 (q, 5.9) | 151.2 |
| C-4 | 120.6 (br d, 163.0) | 120.9 (br d, 162.8) | 120.8 |
| C-4a | 132.3 (s) | 132.2 (s) | 132.3 |
| C-5 | 107.2 (d, 165.8) | 107.0 (d, 167.3) | 107.4 |
| C-6 | 163.2 (m)[d] | 161.7 (m) | 163.5 |
| C-7 | 128.5 (d, 6.1)[e] | 125.5 (d, 5.9) | 128.0 |
| C-8 | 152.3 (s) | 151.2 (s) | 151.4 |
| C-8a | 118.1 (d, 5.9)[e] | 118.1 (d, 5.9) | 117.8 |
| C-9 | 186.6 (s) | 186.4 (s) | 186.5 |
| C-9a | 114.4 (q, 6.1)[f] | 114.1 (q, 5.9) | 114.6 |
| C-10 | 182.0 (t, 4.4) | 181.6 (t, 4.4) | 181.8 |
| C-10a | 136.4 (br s)[g] | 137.2 (br s) | 136.3 |
| 3-Me | 22.1 (qt, 127.6 and 4.4) | 22.1 (qt, 127.6 and 4.4) | 22.1 |
| 6-OMe | 56.9 (q, 145.3) | 57.0 (q, 146.7) | 56.8 |
| C-1′ | 169.0 (t, 5.9)[h] | 166.6 (t, 5.9) | 169.9 |
| C-2′ | 43.7 (t, 132.0) | 44.1 (t, 131.1) | 43.6 |
| C-3′ | 70.7 (m)[i] | 71.3 (m) | 72.7 |
| C-4′ | 46.5 (t, 127.7)[i] | 40.6 (t, 132.0) | 47.8 |
| C-4a′ | 134.4 (t, 5.1) | 143.3 (t, 4.4) | 148.0 |
| C-5′ | 99.4 (dt, 161.4 and 4.4) | 104.2 (dd, 167.3 and 4.4) | 109.3 |
| C-6′ | 157.3 (m) | 162.2 (m) | 162.1 |
| C-7′ | 98.1 (dd, 158.5 and 5.9) | 104.6 (dd, 160.0 and 5.9) | 95.4 |
| C-8′ | 158.4 (m) | 164.8 (m) | 163.0 |
| C-8a′ | 109.9 (qd, 5.9 and 3.0)[j] | 114.3 (t, 5.9) | 114.2 |
| C-9′ | 151.5 (d, 5.9)[k] | 178.8 (s) | 179.7 |
| C-9a′ | 111.6 (m)[j] | 142.9 (dd, 5.9 and 4.4) | 143.5 |
| C-10′ | 121.0 (dq, 161.4 and 5.9) | 185.7 (m) | 43.3 |
| C-10a′ | 137.4 (br s) | 135.8 (br s) | 136.7 |
| 3′-Me | 31.6 (q, 127.6)[i] | 33.9 (q, 128.4) | 32.9 |
| 6′-OMe | 55.4 (q, 148.2) | 56.5 (q, 146.8) | 55.4 |
| 8′-OMe | 56.2 (q, 146.7) | 56.0 (q, 145.2) | 56.2 |

[a] Assignments made by analogy with compounds **4** and **5**. [b] Doublet ($J$ 4.4) on irradiation of 2-H. [c] Doublet ($J$ 3.0) on irradiation of 1-OH. [d] Quartet ($J$ 4.4) on irradiation of 5-H. [e] Singlet on irradiation of 5-H. [f] Triplet ($J$ 6.1) on irradiation of 2-H. [g] Sharp singlet on irradiation of 5-H. [h] Singlet on irradiation of 2′-$H_2$. [i] Sharpened on irradiation of 2′-$H_2$. [j] Quartet ($J$ 5.9) on irradiation of 9′-OH. [k] Singlet on irradiation of 9′-OH.

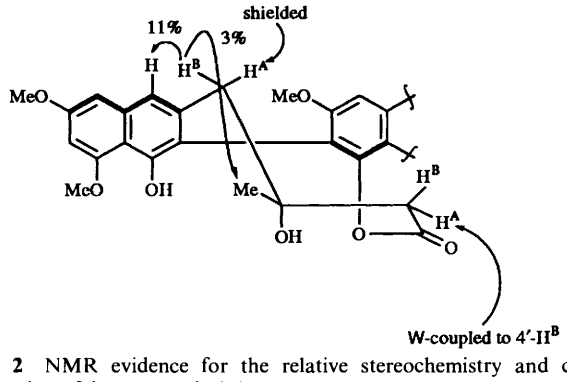



**Fig. 2** NMR evidence for the relative stereochemistry and conformation of dermocanarin 1 **4**

made above are fully supported by the results of 2D $^1H$–$^{13}C$ homonuclear chemical-shift correlation (COSY) experiments. While the absolute stereochemistry of dermocanarin 1 **4** is not yet known, the relative configuration and conformation depicted in structure **4** is consistent with several observations (Fig. 2) including the strong NOE between 4′-$H^B$, 10′-H, and the protons of the C-3′ methyl group, which indicates a close spatial relationship between these nuclei. The signal due to 4′-$H^B$ is also broadened by W-type coupling that was traced to 2′-$H^A$ by irradiation at the resonance frequency of the latter nucleus, whereupon 4′-$H^B$ appeared as a sharp doublet. It was noted earlier that the methylene proton 4′-$H^A$ is considerably shielded compared with its geminal neighbour 4′-$H^B$ and consequently resonates at $\delta$ 2.39. Examination of molecular models suggested that in the conformation depicted in structure **4**, and emphasized in Fig. 2, 4′-$H^A$ is forced into the shielding zone above the left-hand aromatic ring of the anthraquinone moiety. With the ring in this conformation, 4′-$H^B$, 10′-H and the C-3′ methyl group are all in close proximity.

Dermocanarin 2 **5**, which is less mobile on silica gel than its co-metabolite **4**, was obtained after extensive chromatography as an optically active orange–yellow powder. The molecular formula $C_{33}H_{26}O_{11}$ was immediately indicative of a close relationship between compounds **5** and **4**. Comparison of the $^1H$ and $^{13}C$ NMR data obtained for the two pigments (Tables 1 and 2, respectively) corroborated this relationship and, further, established in compound **5** precisely the same partial structures **A** and **C** as were found in dermocanarin 1 **4**. This is best recognized by comparison of the $^{13}C$ NMR data owing to carbons C-1 to C-10a, the C-3 methyl, the 6-*O*-methyl carbon, the carbons C-1′ to C-4′, and the C-3′ methyl group in the spectra (Table 2) of compounds **4** and **5**. The difference between the dermocanarins 1 **4** and 2 **5** must therefore reside in the naphthalene moiety.

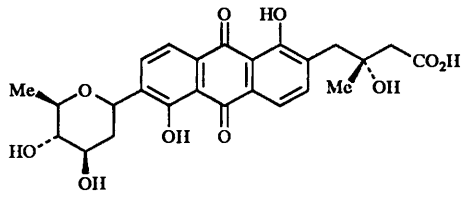
The $^1H$ NMR spectrum of dermocanarin 2 **5** exhibits, in addition to those signals assigned to the partial structures **A** and **C**, two methoxy resonances ($\delta$ 3.92 and 3.96) and a pair of *meta*-coupled aromatic protons ($\delta$ 6.75 and 7.32). Significantly, the phenolic hydroxy group ($\delta$ 9.38) and the isolated aromatic proton ($\delta$ 7.26) observed in the spectrum of

compound **4** are absent in the spectrum of compound **5** and their place is taken by two quinonoid carbonyl groups ($\delta_C$ 178.8 and 185.7). This pointed to the coupled naphthoquinone–anthraquinone formulation **5**. The location of the biaryl linkage and the C-terminus of the lactone bridge at C-9a′ and C-4a′, respectively, in the naphthoquinone nucleus followed from the multiplicity and long-range coupling experienced by each of the naphthoquinonoid carbonyl carbons C-10′ and C-9′. The former resonates as a multiplet at $\delta_C$ 185.7 and correlates ($^1H$–$^{13}C$ long-range COSY) with 5′-H ($\delta$ 7.32) and with both protons of the C-4′ methylene group ($\delta$ 1.97 and 3.36). On the other hand, C-9′ appears as a sharp singlet at $\delta_C$ 178.8 and displays no long-range coupling whatsoever. It seems very likely that dermocanarin 2 **5** occupies a conformation similar to that shown in Fig. 2 for compound **4**, and for the same reasons. It is of interest that 4′-$H^B$ in compound **5** is deshielded ($\Delta\delta$ 0.5 ppm) with respect to its counterpart in compound **4** in accord with its proximity in the former to the anisotropic influence of the C-10′ carbonyl group.

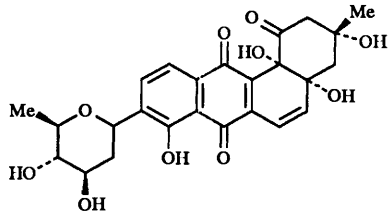
The least stable and least abundant yellow pigment isolated from the mycelium of *D. canaria*, dermocanarin 3, was assigned the 'tetrameric' structure **6** by comparison of its spectroscopic data with those of **4** and **5**. The $^1H$ and $^{13}C$ NMR spectra of compound **6** (Tables 1 and 2, respectively) display resonances assignable to 27 protons and 33 carbon atoms. The FAB mass spectrum of compound **6** exhibits an $[M + 1]^+$ ion at *m/z* 1167 consistent with the molecular formula $C_{66}H_{54}O_{20}$. The presence in the NMR spectra of signals for half this number of carbon and hydrogen atoms suggested that dermocanarin 3 must consist of two identical halves each containing the elements $C_{33}H_{27}O_{10}$. Comparison of the $^1H$ and $^{13}C$ NMR data for the dermocanarins 1 **4** and 2 **5** with the corresponding data for compound **6** revealed the continuing presence in the latter of the same anthraquinone and lactone sub-units **A** and **C**, respectively, together with a naphthalene ring that still bears the C-6′ and C-8′ methoxy groups and the *meta*-coupled protons 5′-H and 7′-H. The rest of the naphthalenoid ring consists of a carbonyl group ($\delta_C$ 179.7) and a methine carbon that resonates at $\delta_C$ 43.3. This chemical shift and that of the attached proton ($\delta$ 4.68) strongly support its presence in a naphthalenone ring of the type shown in structure **6**. The location of the carbonyl group at C-9′ and consequently the methine carbon at C-10′ was deduced from the chemical shift ($\delta$ 6.67) of 5′-H in **6**, which is very close to its position in the spectrum of dermocanarin 1 **4** and is not consistent with its position *peri* to a carbonyl group. Since C-10′ bears only one hydrogen atom it is the logical point at which the two identical halves of the dermocanarin 3 molecule are joined.

While structure **6** satisfies all of the spectroscopic criteria that we have been able to assemble it represents the keto tautomer of a binaphthol. We suggest that in this case the considerable steric strain engendered by the central biaryl linkage renders $sp^3$ hybridization at C-10′ thermodynamically more stable than the $sp^2$ alternative.

The dermocanarins 1–3 represent the first members of a completely new class of fungal coupled-octaketide pigments.[11] The presence of the macrocyclic lactone ring and the naphthalenoid sub-unit render them unique among coupled anthraquinones and coupled pre-anthraquinones.[14] The 3-hydroxy-3-methylbutanoyl side-chain is rare in natural products; interestingly, it is found intact in vineomycinone $B_2$ **7**, an antibiotic anthraquinone glycoside isolated from a *Streptomyces* species.[15] It has been shown by labelling experiments that the side-chain in compound **7** arises by cleavage of the tetracyclic precursor **8**.[16] In the case of the dermocanarins it seems reasonable to suggest that the naphthalene ring and the elements of the lactone bridge arise from a progenitor of the dihydroanthracenone type. For




**7**




**8**

example, cleavage of torosachrysone 8-*O*-methyl ether **10**, or of its putative precursor **9**,[17,18] could yield the carboxylic acid **11** capable of subsequent esterification with physcion **1** and biaryl coupling to yield dermocanarin 1 **4** (Scheme 1). The dihydroanthracenone **10** has been isolated from several toadstools belonging to the genera *Cortinarius*[19,20] and *Tricholoma*,[21] while physcion **1** is a minor constituent of the mycelium (Experimental section) and the major pigment present in the fruit bodies of *Dermocybe canaria*.[5] Experiments designed to shed further light on the biogenesis of the dermocanarins and on their absolute stereochemistry are in progress and will be reported in due course.

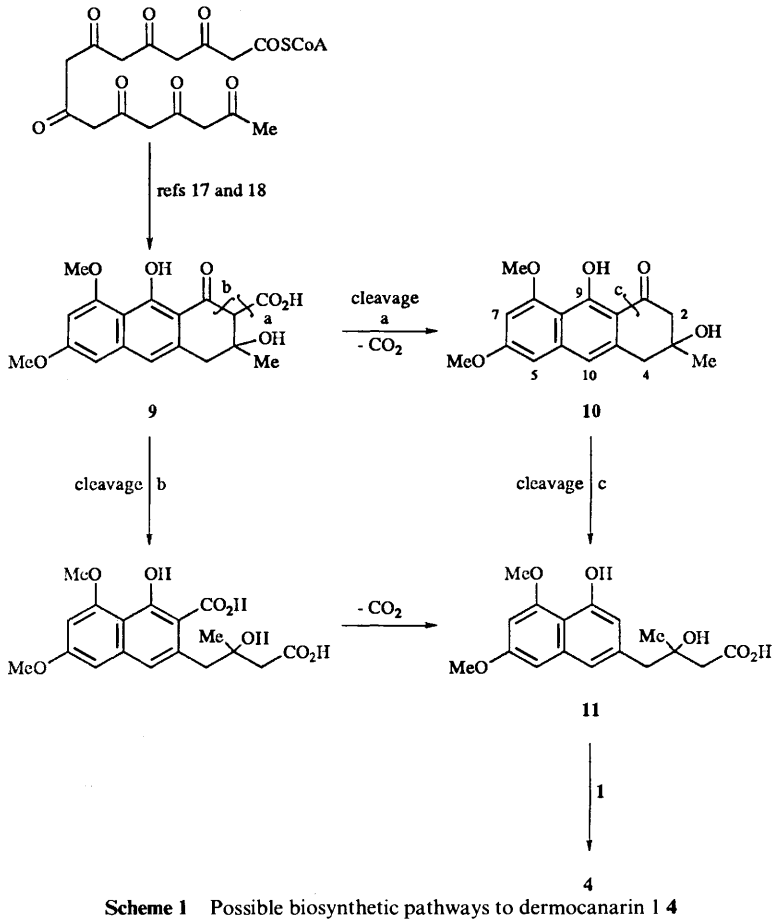
**Pigments from the fruit bodies**

Extraction of the fresh fruit bodies of *D. canaria*, collected both in Tasmania and in New Zealand, with ethanol gave a rich golden yellow extract, which was partitioned between ethyl acetate and water. In agreement with the work of Keller and Steglich[5] we found that the predominant pigments in the organic phase are physcion **1** ($4.5 \times 10^{-2}$% yield based on the fresh weight of the fungus) and erythroglaucin **2** ($2 \times 10^{-3}$% yield). Minor components (TLC) were the dermocanarins 1–3; no trace of 4-aminophyscion **3** could be detected.

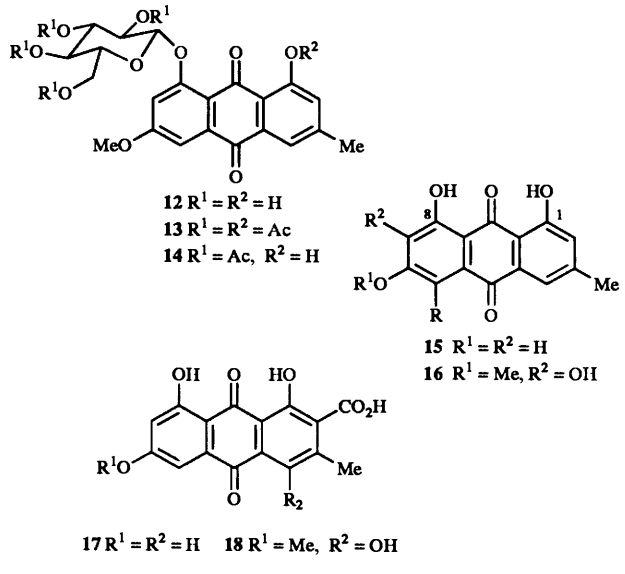
The content of the intensely yellow aqueous phase was purified by repeated gel permeation through Sephadex LH-20 in methanol to afford physcion 8-*O*-β-D-glucopyranoside **12** as an orange powder in $5.6 \times 10^{-2}$% yield from the fresh weight of the fungus. The glucoside **12** was identified from the NMR spectra of the natural product itself and of the penta- and tetra-acetyl derivatives **13** and **14**, respectively. The location of the glucoside at C-8 rather than C-1 followed unequivocally from 2D $^1H$–$^{13}C$ COSY experiments on the tetra-acetate **14**. Thus, C-2 ($\delta_C$ 125.0) correlates with 2-H ($\delta$ 7.08), the C-3 methyl protons ($\delta$ 2.45), 4-H ($\delta$ 7.58), and the free phenolic hydroxy group ($\delta$ 12.94) establishing that these nuclei occupy the same ring. Furthermore, C-8 in compound **14**, which resonates at $\delta_C$ 159.8, correlates both with 7-H ($\delta$ 7.09) and the anomeric proton at $\delta$ 5.16.

This is the first report of a fungal anthraquinone monosaccharide in which the sugar is attached to the C-8 hydroxy group of the quinone. Emodin **15** and dermocybin **16** occur in fruit bodies of *Dermocybe sanguinea* principally as the alternative 1-*O*-β-D-glucopyranosides,[22] while the tentative suggestion that endocrocin **17** and cinnarubin **18** occur in *D. schaefferi* and *Cortinarius bulliardi*, respectively, as their 8-*O*-β-D-glucopyranosides still requires ratification.[11] The physcion glucoside **12** was known previously only from higher plants.[12,14]

The water-soluble fraction from *Dermocybe canaria* also afforded a very small quantity of a second, red glycoside, which




**Scheme 1** Possible biosynthetic pathways to dermocanarin 1 **4**




on acidic hydrolysis afforded erythroglaucin **2**. Although lack of material precluded further study, it seems reasonable to suggest that this pigment is erythroglaucin 8-*O*-β-D-glucopyranoside.

## Experimental

Mps were determined on a Kofler hot-stage apparatus, IR spectra for samples as potassium bromide discs on a Perkin-Elmer 983 G spectrophotometer, UV spectra in ethanol 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 (with *J* values in Hz), mass spectra on a V. G. Micromass 7070F spectrometer (EI probe, 70 eV) or on a JEOL JMS AX505H (FAB ionization, thioglycerol matrix), and optical rotations on a Perkin-Elmer 241M polarimeter at 22 °C ($[\alpha]_D$-values are given in units of $10^{-1}$ deg cm$^2$ g$^{-1}$). CD spectra were obtained using a Cary 61 spectropolarimeter for solutions in methanol. 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 eluent. Column chromatography used Merck Kieselgel 60 silica gel. Gel permeation chromatography (GPC) employed Pharmacia Sephadex LH-20 suspended in and eluted with methanol (unless stated otherwise). Solutions were routinely dried over $Na_2SO_4$ prior to concentration under reduced pressure (water-bath; 35 °C). Light petroleum refers to the fraction of distillation range 40–60 °C.

*Dermocybe canaria* was collected in the Mount Field National Park, Tasmania, during April 1987 and in the Craigieburn Forest Park, Canterbury, New Zealand, during June 1991. The Tasmanian material was identified by direct comparison with *D. canaria* type (E. Horak, personal communication); voucher specimens of the New Zealand material are lodged in the herbarium of the Royal Botanic Gardens, Edinburgh, UK, under accession number WAT 22915 and were identified by Dr. R. Watling.

### Isolation of pigments from *Dermocybe canaria* mycelium

Fresh mycelium (~ 250 g) was soaked in ethanol (3 dm$^3$) for 2 h. The black extract was evaporated to dryness and the residue was partitioned between ethyl acetate (3 × 400 cm$^3$) and water

(300 $cm^3$). The aqueous phase was discarded and the black organic extracts were combined, dried and evaporated. The residue was dissolved in chloroform and applied to the top of a short column (6 × 10 cm) of silica gel. Elution with toluene–ethyl formate–formic acid (50:49:1) gave an eluate that was washed with aqueous buffer solution (pH 7.2; 3 × 200 $cm^3$) and evaporated to dryness. The pale brown residue (760 mg) was filtered through a short column of Sephadex LH-20 in methanol–acetone (2:1). The filtrate was evaporated and the dark yellow residue (186 mg) was further purified by PLC [toluene–ethyl formate–formic acid (50:49:1)] and GPC through Sephadex LH-20 to afford, in order of increasing polarity on silica gel: (i) physcion **1** ($R_f$ 0.80) (22 mg) as orange coloured needles, mp 207–208 °C (from EtOAc–light petroleum), identical in all respects with an authentic sample; (ii) *dermocanarin 1* **4**, a yellow powder ($R_f$ 0.70) (26 mg), mp 215–218 °C (from EtOH at −20 °C) (Found: $M^+$, 584.1681. $C_{33}H_{28}O_{10}$ requires M, 584.1682); $[\alpha]_D$ +27 (*c* 0.5, $CHCl_3$); CD $\lambda_{max}$/nm ($\Delta\varepsilon$) 240 (+25.52), 255 (+7.92), 267 (+12.30), 280 (+3.52), 297 (+7.0), 305 (0.0) and 340 (−1.76); $\nu_{max}/cm^{-1}$ 3413, 1754, 1667, 1633 and 1578; $\lambda_{max}$/nm 214 (log $\varepsilon$ 4.87), 235 (4.70), 272 (4.50), 302sh (4.21), 344 (3.97) and 415 (3.85); $\lambda_{max}$(EtOH + NaOH)/nm 510 (log $\varepsilon$ 3.77); *m/z* 584 ($M^+$, 54%), 542 ($C_{31}H_{26}O_9$, 73), 540 ($C_{32}H_{28}O_8$, 38), 326 (25), 284 (27), 226 (100) and 42 (53); $\delta_H$: Table 1; $\delta_C$: Table 2; (iii) *dermocanarin 2* **5**, an orange–yellow powder ($R_f$ 0.45) (28 mg), mp 235–240 °C (from EtOH at −20 °C) (Found: $M^+$, 598.1475. $C_{33}H_{26}O_{11}$ requires M, 598.1475); $[\alpha]_D$ +32 (*c* 0.5, $CHCl_3$); CD $\lambda_{max}$/nm ($\Delta\varepsilon$) 235 (+8.03), 250 (+3.65), 257 (+5.11), 273 (−4.40), 279 (0.0), 283 (+5.11), 302 (−0.73), 308 (0.0), 320 (+1.46) and 340 (+0.73); $\nu_{max}/cm^{-1}$ 3457, 1771, 1651, 1633 and 1593; $\lambda_{max}$/nm 216 (log $\varepsilon$ 4.80), 272 (4.51), 302sh (4.21) and 420 (3.82); (EtOH + NaOH)/nm 515 (log $\varepsilon$ 3.75); *m/z* 598 ($M^+$, 4%), 556 (58), 555 (38), 554 (100), 540 (20) and 514 (24); $\delta_H$: Table 1; $\delta_C$: Table 2; and (iv) dermocanarin 3 **6**, a yellow powder ($R_f$ 0.10) (8 mg), mp 142–146 °C (from $CHCl_3$–light petroleum); $[\alpha]_D$ +100 (*c* 0.5, $CHCl_3$); $\nu_{max}/cm^{-1}$ 3457, 1757, 1651, 1632 and 1538; $\lambda_{max}$/nm 216 (log $\varepsilon$ 4.83), 272 (4.68) and 420 (3.80); *m/z* (FAB) 1167 [$(M + 1)^+$, 4%] and 584 (100); *m/z* (EI) 584 ($C_{33}H_{28}O_{10}$, 3%) and 542 (25); $\delta_H$: Table 1; $\delta_C$: Table 2.

### Isolation of pigments from *Dermocybe canaria* fruit bodies

Fresh fruit bodies (217 g) were chopped, and soaked in ethanol (2.5 $dm^3$) for 16 h. The solvent was evaporated off and the brown-yellow residue (780 mg) was partitioned between ethyl acetate (3 × 300 $cm^3$) and water (300 $cm^3$). The deep yellow organic phase was evaporated and the residue was purified by repeated PLC using toluene–ethyl formate–formic acid (50:49:1) and toluene–carbon tetrachloride (10:4) as eluents to afford physcion **1** (98 mg, 4.5 × $10^{-2}$% fr.wt), identical in all respects with an authentic sample, and erythroglaucin **2** (4.5 mg, 2 × $10^{-3}$% fr.wt) as red microneedles, mp 200–205 °C (from EtOAc) (lit.,[12] 205–206 °C) (Found: $M^+$, 300.0632, Calc. for $C_{16}H_{12}O_6$: M, 300.0634); $\nu_{max}/cm^{-1}$ 3432, 1596 and 1568; $\lambda_{max}$/nm 230 (log $\varepsilon$ 4.43), 254 (4.18), 275 (4.15), 303 (3.95), 456sh (3.95), 479sh (4.02), 489 (4.07), 508 (3.96) and 522 (3.92); *m/z* 300 ($M^+$, 100%); $\delta_H$ 2.36 (3 H, s, 3-Me), 3.95 (3 H, s, 6-OMe), 6.71 (1 H, d, *J* 2.4, 7-H), 7.14 (1 H, br s, 2-H), 7.41 (1 H, d, *J* 2.6, 5-H), 12.37 (1 H, s, 1-OH), 12.46 (1 H, s, 8-OH) and 13.38 (1 H, s, 4-OH).

The orange-yellow aqueous phase was evaporated to dryness and the residue was purified by GPC through Sephadex LH-20 in methanol to afford physcion 8-*O*-β-D-glucopyranoside **12** (121 mg, 5.6 × $10^{-2}$% fr.wt) as an orange coloured powder, mp 225–240 °C (lit.,[12] 230–232 °C); $\nu_{max}/cm^{-1}$ 3460–3340, 1675 and 1620; $\lambda_{max}$/nm (MeOH) 220 (log $\varepsilon$ 4.51), 270 (4.40) and 420 (3.96); and a small quantity (~5 mg) of a red powder that is discussed further below.

### Hydrolysis of physcion 8-*O*-β-D-glucopyranoside 12

The glucoside **12** (5 mg) in 50% aq. ethanol (10 $cm^3$) containing conc. hydrochloric acid (1 drop) was heated on a steam-bath for 15 min. On cooling, the solution was concentrated, and extracted with ethyl acetate (3 × 10 $cm^3$). The aqueous layer was discarded and the organic phase was concentrated and purified as described above to yield physcion **1** (2.5 mg), identical in all respects with an authentic sample.

Similarly, hydrolysis of the red powder obtained from the aqueous solution from *D. canaria* and mentioned above afforded erythroglaucin **2**, identical by TLC and mass spectroscopy with the material described earlier.

### 1-*O*-Acetylphyscion 8-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside) 13

Prepared from compound **12** (40 mg) with pyridine (5 $cm^3$) and acetic anhydride and purified by PLC [toluene–ethyl formate–formic acid (50:49:1)] to afford title compound **13** (43 mg, 66%), mp 170–174 °C (from EtOAc–hexane) (lit.,[23] 169–170 °C); $\delta_H$ 2.05, 2.06, 2.10 and 2.12 (each 3 H, s, MeCO), 2.52 (3 H, s, 1-OAc), 2.48 (3 H, s, 3-Me), 3.92 (1 H, m, 5′-H), 3.96 (3 H, s, 6-OMe), 4.23 (2 H, d, *J* 4.0, 6′-$H_2$), 5.09 (1 H, d, *J* 8.0, 1′-H), 5.16 (1 H, t, *J* 9.6, 4′-H), 5.31 (1 H, t, *J* 9.6, 3′-H), 5.51 (1 H, dd, *J* 9.6 and 8.0, 2′-H), 6.98 (1 H, d, *J* 2.6, 7-H), 7.19 (1 H, br s, 2-H), 7.50 (1 H, d, *J* 2.6, 5-H) and 7.97 (1 H, br s, 4-H).

### Physcion 8-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranoside) 14

The penta-acetate **13** (40 mg) was suspended in water (10 $cm^3$) at 0 °C and pyridine was added dropwise (~1 $cm^3$ in total) until the solid had dissolved.[24] The red solution was stirred at room temperature for 45 min, acidified with cold aq. phosphoric acid (1 mol $dm^{-3}$; 20 $cm^3$) and extracted with ethyl acetate (3 × 50 $cm^3$). The extracts were combined, dried, and evaporated, and the orange coloured residue was purified by PLC [toluene–ethyl formate–formic acid (75:24:1)] to afford the *tetra-acetate* **14** (40 mg) as orange coloured needles, mp 240–246 °C (from EtOH–hexane) (lit.,[25] 235 °C) (Found: C, 58.6; H, 4.8. $C_{30}H_{30}O_{14}$ requires C, 58.6; H, 4.9%); $\nu_{max}/cm^{-1}$ 3404, 1714, 1677 and 1622; $\lambda_{max}$/nm 223 (log $\varepsilon$ 4.53), 273 (4.40) and 412 (3.95); $\delta_H$ 2.05, 2.06, 2.10 and 2.11 (each 3 H, s, MeCO), 2.45 (3 H, s, 3-Me), 3.92 (1 H, m, 5′-H), 3.98 (3 H, s, 6-OMe), 4.25 (2 H, m, 6′-$H_2$), 5.16 (1 H, d, *J* 8.1, 1′-H), 5.19 (1 H, t, *J* 9.8, 4′-H), 5.34 (1 H, t, *J* 9.8, 3′-H), 5.46 (1 H, dd, *J* 9.8 and 8.0, 2′-H), 7.08 (1 H, br s, 2-H), 7.09 (1 H, d, *J* 2.6, 7-H), 7.58 (1 H, br s, 4-H), 7.60 (1 H, d, *J* 2.6, 5-H) and 12.94 (1 H, s, 1-OH); $\delta_C$ 20.6 and 20.8 (each 2 × *Me*CO), 22.1 (3-Me), 56.2 (6-OMe), 62.2 (C-6′), 68.4 (C-4′), 70.8 (C-2′), 72.5 (C-5′), 72.6 (C-3′), 100.0 (C-1′), 106.8 (C-5), 111.4 (C-7), 114.8 (C-9a), 116.6 (C-8a), 120.4 (C-4), 125.0 (C-2), 132.6 (C-4a), 137.7 (C-10a), 147.5 (C-3), 159.8 (C-8), 163.2 (C-1), 165.0 (C-6), 170.2, 170.4, 172.3 and 173.4 (4 × Me*C*O), 182.6 (C-10) and 187.2 (C-9).

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