Pigments of fungi. Part 49.[1] Structure and biosynthesis of dermocanarin 4, a naphthoquinone-dihydroanthracenone dimer from the fungus *Cortinarius sinapicolor* Cleland

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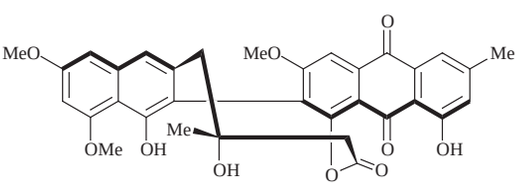
Dermocanarin 4 **3**, a novel naphthoquinone-dihydroanthracenone dimer containing a nine membered lactone ring has been isolated from the fruit bodies of the Australasian fungus *Cortinarius sinapicolor* and the structure and axial stereochemistry have been determined by spectroscopic methods. The biosynthesis of dermocanarin 4 has been studied in intact toadstools by feeding experiments involving [*Me*-^{13}C]methionine, sodium [2-^{13}C]acetate and sodium [1,2-$^{13}C_2$]acetate.

In an earlier paper in this series[1] we reported the isolation and structural elucidation of the dermocanarins 1 **1**, 2 **2** and 3 (a dehydro dimer of **1**), a group of naturally occurring naphthol- and naphthoquinone-anthraquinone dimers that were isolated from the subterranean mycelium of the Australian toadstool *Dermocybe canaria*.[1,2] We describe here the isolation of dermocanarin 4 **3**, a novel naphthoquinone-dihydroanthracenone dimer, from the fruit bodies of the Australasian toadstool *Cortinarius sinapicolor* and the results of isotopic labelling experiments with *C. sinapicolor* that reveal details of the biogenesis of this unique group of fungal metabolites.



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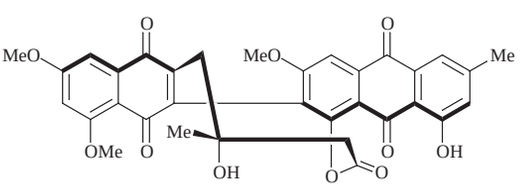

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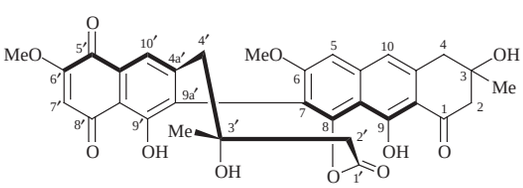

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Results and discussion

Cortinarius sinapicolor is a common species in native forests across Australia and New Zealand.[3] It produces easily recognisable bright mustard-yellow basidiomes that, when young, are extremely glutinous. Photographs of the toadstool have been published but erroneously identified as '*Cortinarius ochraceus*'† by Cole[4] and by Fuhrer[5] but, to date, there have been no reports of its chemistry.

Maceration of the fresh fungus‡ with ethanol gave a deep yellow extract from which several pigments were obtained in pure form by repeated chromatography on silica gel and permeation through Sephadex LH-20. The most polar constituent of the mixture, dermocanarin 4 **3**,§ was obtained as an optically active orange powder, mp 200–203 °C, $[\alpha]_D$ +30 ($CHCl_3$), in a yield of 2.83×10^{-2}% of the fresh weight of the fungus. The other pigments isolated from *C. sinapicolor* do not belong to the dermocanarin class and their structures will be reported elsewhere.

High resolution mass measurement on the molecular ion at *m*/*z* 588 in the mass spectrum of dermocanarin 4 established the molecular formula $C_{32}H_{28}O_{11}$, from which it was evident that the pigment possesses a 'dimeric' octaketide-based structure.[9] The infrared spectrum showed absorptions at ν_{max} 1666 and 1659 cm^{-1}, typical of free and chelated carbonyl groups, at ν_{max} 1625 cm^{-1}, characteristic of a chelated anthracenone carbonyl, and a fourth carbonyl absorption at ν_{max} 1745 cm^{-1} suggesting the presence of ester or lactone functionality. In the electronic spectrum, an absorption maximum at λ 391 nm, which is shifted bathochromically on the addition of base (to 553 nm), is suggestive of a naphthoquinone rather than an anthraquinone chromophore.[10]

The ^{1}H and ^{13}C NMR spectra of dermocanarin 4 (Table 1) give a clear indication that the molecule contains the dihydro-

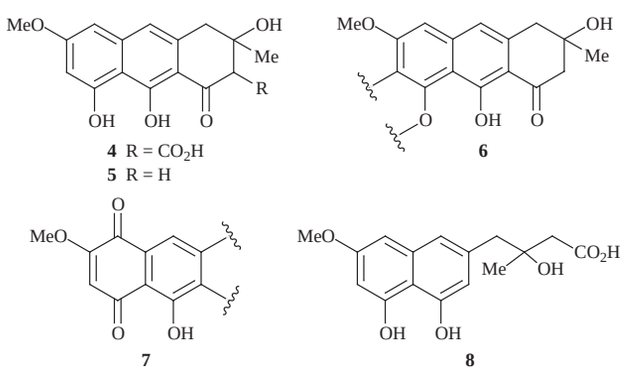
† The taxonomic position of *C. sinapicolor* and its relationship to the confused taxon *C. ochraceus* have recently been discussed in detail by Horak and Wood[3] and by Grgurinovic.[6] It is placed in Sect. Pyromyxae of subgen. *Myxacium* by Moser.[7] Some of the taxonomical implications of the chemistry described herein have been discussed elsewhere.[8]

‡ Toadstools were collected in mixed *Eucalyptus-Leptospermum* forest in the Kinglake National Park, Victoria. Voucher specimens of the material examined are lodged in the Herbarium of the Royal Botanic Garden, Edinburgh, UK under accession number WAT 24272.

§ For consistency and ease of comparison of spectroscopic data, both here and in earlier papers in this series,[1,2] the numbering of the lactone bridge and naphthoquinone ring of dermocanarin 4 is as shown in structure **3**. It is based on the presumption, borne out by results that follow, that each of the two parts of the dermocanarin 4 molecule are derived from a dihydroanthracenone of the type **4**, and are numbered accordingly.

Table 1 ^{13}C (100 MHz) and ^{1}H NMR (400 MHz) data and HMBC correlations ($CDCl_3$) for dermocanarin 4 **3**

Position	δ_C [multiplicity, (*J*/Hz)]	δ_H [multiplicity (*J*/Hz)]	^2J, ^3J {^{13}C–^{1}H}
1	202.8 (t, 5.9)	—	2-H
2	51.7 (t, 129.1)	2.87 (2H, br s)	3-Me, 4-H
3	71.2 (m)	—	3-Me, 4-H
3-Me	29.5 (q, 126.1)	1.49 (s)	—
4	43.5 (t, 131.0)	3.09 (br d, 16.3) (H_A)	2-H, 3-Me, 10-H
		3.21 (br d, 16.3) (H_B)	
4a	138.0 (t, 5.9)	—	4-H
5	104.3 (dd, 161.4 and 5.9)	6.98 (s)	10-H
6	158.5 (m)	—	6-OMe, 5-H
6-OMe	56.3 (q, 145.3)	3.90 (s)	—
7	118.8 (d, 5.9)	—	5-H
8	149.2 (s)	—	—
8a	111.3 (q, 5.9)	—	5-H, 10-H, 9-OH
9	163.5 (d, 4.4)	—	9-OH
9-OH	—	14.48	—
9a	110.4 (m)	—	4-H, 10-H
10	117.2 (d, 162.9)	7.03 (s)	4-H, 5-H
10a	140.9 (s)	—	10-H
1′	169.8 (t, 5.8)	—	2′-H
2′	43.8 (t, 130.6)	2.47 (dd, 13.3, 1.5) (H_A)	3′-Me
		2.57 (d, 13.3) (H_B)	3′-Me
3′	70.7 (m)		2′-H, 3′-Me, 4′-H
3′-Me	32.2 (q, 126.2)	1.28 (s)	3′-OH, 2′-H, 4′-H
4′	47.0 (t, 131.7)	2.34 (d, 13.5) (H_A)	2′-H, 3′-Me, 10′-H
		2.88 (br d, 13.5) (H_B)	
4a′	144.9 (t, 5.1)	—	4′-H
5′	179.2 (dd, 7.3, 4.4)	—	7′-H, 10′-H
6′	161.9 (m)	—	7′-H, 6′-OMe
6′-OMe	56.7 (q, 146.7)	3.93 (s)	—
7′	109.4 (d, 164.3)	6.10 (s)	6′-OMe
8′	190.6 (s)	—	7′-H
8a′	112.9 (q, 5.1)	—	7′-H, 10′-H, 9′-OH
9′	159.0 (d, 4.4)	—	9′-OH
9′-OH	—	12.44	—
9a′	130.0 (m)	—	4′-H, 10′-H, 9′-OH
10′	123.3 (dt, 168.7 and 5.1)	7.77 (s)	4′-H
10a′	130.2 (s)	—	10′-H



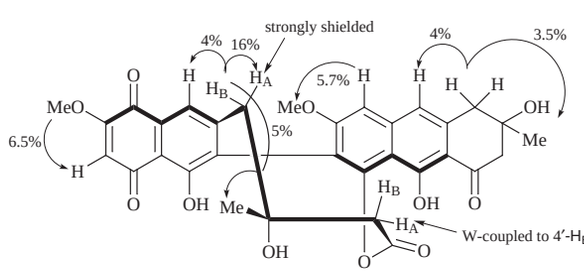


Fig. 1 NMR Evidence for the substitution pattern, relative stereochemistry and preferred conformation of dermocanarin 4 **3**.

anthracenone sub-unit **6**. In total the ^{1}H NMR spectrum of **3** shows the presence of four methylene groups, three aromatic protons, two methoxy groups, two *C*-methyls, two low-field chelated hydroxys, and a quinonoid methine proton. Of these, almost half can be confidently assigned to the protons of the dihydroanthracenone moiety **6** from the chemical shift and HMBC data (Table 1) and the results of differential NOE experiments that are summarised in Fig. 1. From the NOE experiments, it is possible to differentiate between the closely spaced aromatic resonances due to 5-H (δ 6.98) and 10-H (δ 7.03) in the ^{1}H NMR spectrum of **3** and to assign one of the four methylene proton doublets (δ 3.09 and 3.21) to the methylene group at C-4 in **3**. The NOE experiments also locate one (δ 3.90) of the two methoxy groups at C-6 in **3**, one of the *C*-methyls (δ 1.49) at C-3 and places the C-4 methylene group between 10-H (δ 7.03) and the C-3 methyl group (δ 1.49). The strongly hydrogen bonded phenolic hydroxy group at C-9 in **3** resonates at characteristically low field (δ 14.48; *cf.* torosachrysone **5** $\delta_{9\text{-OH}}$ 16.10) whereas there is no resonance in the spectrum of **3** that can be attributed to a free phenolic group at C-8 ($\delta_{8\text{-OH}}$ 9.79 for **5**).[11] This implies that C-8 in **6** is one of the points of connection between this fragment and the remainder of the molecule. The identification of the second point of connection as C-7 follows from the observation that the signal due to 5-H in the spectrum of **3** is a singlet at δ 6.98. This proton is deshielded by 0.47 ppm relative to the corresponding proton in the spectrum of torosachrysone **5**, which is consistent with the presence of a *meta*-aryl substituent (at C-7) in **3**. The ^{13}C chemical shift and multiplicity of the signal due to C-7 (δ 118.8, d, *J* 5.9 Hz) is consistent with the absence of a proton at C-7 as is the observation of correlation in the HMBC spectrum of **3** (Table 1) from C-8a to only 5-H, 10-H and 9-OH. In complementary fashion, C-5 couples only to 5-H and 10-H, C-7 is coupled only to 5-H, and C-8 resonates as a singlet. Thus, the biaryl bond in dermocanarin 4 **3** must stem from C-7 in the dihydroanthracenone moiety **6**.

Fragment **6** accounts for the elements $C_{16}H_{14}O_5$ of the molecular formula $C_{32}H_{28}O_{11}$ of dermocanarin 4 leaving $C_{16}H_{14}O_6$ still to be accommodated. That this does not repre-

sent a second dihydroanthracenone unit is evident from the ^{1}H and ^{13}C NMR spectra (Table 1) and the perceived need to incorporate a naphthoquinone nucleus. Analysis of the remaining NMR data suggests that the two carbonyl groups (δ_C 190.6 and 179.2), the residual chelated phenolic hydroxy (δ_H 12.44), the second methoxy group (δ_H 3.93) and the two remaining aromatic protons (δ_H 6.10 and 7.77; δ_C 109.4 and 123.3) can be brought together in terms of the naphthoquinone substructure **7**.

For example, from the data collected in Table 1 it is clear that the quinone carbonyl groups in **3** must be at C-5′ and C-8′ and in a ring shared with one of the isolated protons (δ 6.10) and the methoxy group (δ 3.93). A differential NOE experiment (Fig. 1) confirms the proximity of the quinonoid methine proton and the methoxy group. Since the phenolic hydroxy group (δ 12.44) is chelated to one quinone carbonyl group and the remaining aromatic proton (δ 7.77), by virtue of its chemical shift, it is clearly *peri* disposed to the other; the remaining carbons, C-4a′ and C-9a′ in **7**, must be the connection points to the rest of the dermocanarin 4 molecule.

The placement of the hydroxy group at C-9′ rather than C-10′ in **3** follows from consideration of the ^{1}H–^{13}C couplings (Table 1). For example, C-8a′ (δ 112.9) is coupled to 7′-H, 9′-OH and 10′-H, while C-10′ is coupled to each of the protons of the C-4′ methylene group.

Fragments **6** and **7** together account for all but the elements $C_5H_8O_2$ of the molecular formula of dermocanarin 4. The spectroscopic data remaining serve to characterise an ester carbonyl group (ν_{max} 1745 cm^{-1}, δ_C 169.8), a *C*-methyl group (δ 1.28), two methylene groups and a tertiary alcohol (δ_C 71.2) (which also takes care of the last oxygen atom). This leads to the same C-1′–C-4′ lactone bridge in **3** that is found in the dermocanarin 1 **1**, 2 **2** and 3.[1]

The connectivity between the fragments **6** and **7** and the lactone bridge in **3** was deduced, again, from the results of 2D NMR experiments. Thus, from the HMBC spectrum of dermocanarin 4 (Table 1), C-4′ is coupled with the protons of the C-4′ methylene group. Since C-10′ also correlates with the C-4′ methylene protons, the carbon terminus of the lactone bridge must connect with C-4′ in **3**. This conclusion is consistent with the results of differential NOE experiments (see below) that place the C-3′ methyl group, one of the C-4′ methylene protons and the aromatic proton at C-10′ in close proximity. Since C-9a′ and C-7, by virtue of their chemical shifts and multiplicities (Table 1), must be connected by the biaryl bond, the lactone bridge must terminate at the C-8 oxygen atom in **3**. Bringing the three fragments together leads to the structure **3** for dermocanarin 4 (no stereochemical detail yet implied).

The preferred conformation of the nine-membered lactone ring and the relative stereochemistry between the biaryl axis and the C-3′ chiral centre in dermocanarin 4 **3** was deduced from the NMR data (Table 1) and the NOE results (Fig. 1) mentioned above. Thus, irradiation of the deshielded component of the C-4′ methylene couplet (4′-H_B, δ 2.88) in **3** affects not only 4′-H_A (δ 2.34) but also the protons of the C-3′ methyl group (δ 1.28) and 10′-H (δ 7.77). These observations, together with the detection of W coupling (J 1.5 Hz) between 4′-H_B and 2′-H_A (δ 2.47), support the conformation, shown in Fig. 1, in which the relative stereochemistry between the C-3′ chiral centre and the biaryl axis in dermocanarin 4 **3** is (3′*R**, 7*R**). Significantly, such a conformation places 4′-H_A directly above the peripheral aromatic ring of the dihydroanthracenone moiety **6**, which is consistent with the high field chemical shift of 4′-H_A (δ 2.34). The conformation depicted in Fig. 1 is supported by the results of molecular modelling experiments.

That dermocanarin 4 is optically active and shows no signal doubling in either the ^{1}H or ^{13}C NMR spectrum proves that, at the limits of detection, the natural product **3** is diastereoisomerically homogeneous and suggests that it exists as a single enantiomer. The absolute configuration at the C-3′ stereogenic centre and at the biaryl axis in dermocanarin 4 **3** is not yet known with certainty but can be tentatively assigned as (3′*R*, 7*R*) from the CD spectrum (Fig. 2) and the NOE data discussed above. Thus, the CD spectrum of dermocanarin 4 **3** (Fig. 2) contains a strong A-type¶ Cotton effect couplet centred close to 275 nm. If a correlation of the sign of the Cotton effects with the axial stereochemistry made previously for 7,7′-linked dihydroanthracenone dimers of the flavomannin type[12] can be extended to the dermocanarins, then the chirality at the stereogenic axis in **3** would be *R* and, consequently, the configuration at C-3′ would also be *R*. The absolute configuration at C-3 in dermocanarin 4 **3** is not known.

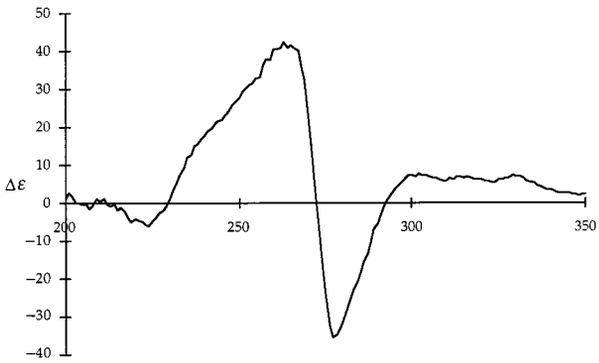


Fig. 2 CD Spectrum (MeOH) of dermocanarin 4 **3**.

Dermocanarin 4 **3** is the first member of the dermocanarin class that has been isolated from the fruit bodies (rather than the vegetative mycelium) of a member of the Basidiomycotina. It is also the first dermocanarin in which the tricyclic sub-unit corresponds to a dihydroanthracenone.

This is the first chemical study of *C. sinapicolor*. Certainly, if the presence of dermocanarin 4 **3** is to be taken as a guide then *C. sinapicolor* has systematic affinities rather to *Dermocybe* subgen. *Icterinula* than *Cortinarius* subgen. *Myxacium*, as is indeed the case proposed by Horak and Wood.[3]

We have also examined specimens of *Cortinarius* (*Myxacium*) *ignotus* from New Zealand, a taxon that is macroscopically identical with *C. sinapicolor*,[13] and specimens of *C. sinapicolor* from Western Australia.‖ Chromatographically, these taxa are chemically indistinguishable from WAT 24272.

The biosynthesis of dermocanarin 4

When young fruit bodies of *Cortinarius sinapicolor* growing in their natural habitat were injected by syringe periodically over ten days with an aqueous solution of sodium [2-^{13}C]acetate (a total of 60 mg per fruit body) dermocanarin 4 **3** was obtained that contained significant levels (Table 2) of isotopic label. The sites and levels of enrichment of ^{13}C in **3** are shown in Table 2. These data are entirely consistent with the formation of dermocanarin 4 **3** by coupling together of the precursors **5** and **8**, themselves assembled (at least formally) by head-to-tail linkage of eight acetate units.

In a parallel experiment *S*-adenosylmethionine was established as the source of the 6- and 6′-*O*-methyl groups in **3** by feeding [*Me*-^{13}C]methionine to the toadstools. The high levels of incorporation of label at these two sites (12.0 and 13.5 atom% enrichment over and above natural abundance) was best

¶ Coupled dihydroanthracenones are divided stereochemically on the basis of their CD spectra into two types, -A and -B.[9] The CD spectrum of an A-type dimer contains an intense Cotton effect couplet close to 275 nm that is negative to longer wavelength and positive to shorter wavelength. The spectrum of a B-type dimer is the inverse. Dimers of the same type have the same axial chirality and the relationship between this and the sign of the Cotton effects has been established in some cases.[12]

‖ The New Zealand and Western Australian material was kindly provided by Professor E. Horak, ETH, Zurich.

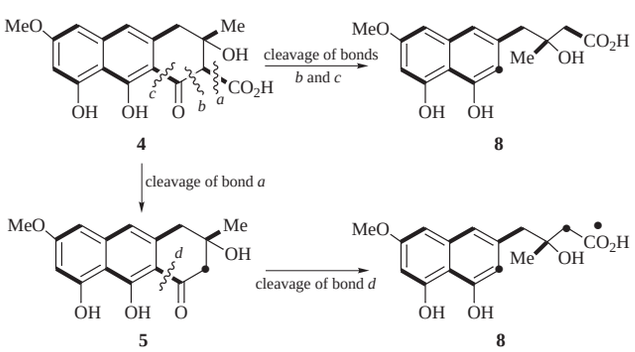
Table 2 ^{13}C NMR data ($CDCl_3$, 100 MHz) for dermocanarin 4 **3** enriched with [2-^{13}C]acetate, [1,2-$^{13}C_2$]acetate and [*Me*-^{13}C]methionine

Carbon	δ/ppm	Atom% enrichment from [2-^{13}C]acetate[a]	$J_{C–C}$ (Hz) from [1,2-$^{13}C_2$]acetate	Carbon	δ/ppm	Atom% enrichment from [2-^{13}C]acetate[a]	$J_{C–C}$ (Hz) from [1,2-$^{13}C_2$]acetate
C-1	202.8	—	54.3	C-1′	169.8	—	54.3
C-9a	110.4	1.4	54.3	C-2′	43.8	1.9	obscured
C-2	51.7	0.9	—	C-3′	71.2	—	obscured
C-3	71.2	—	39.6	3′-Me	32.2	1.4	41.4
3-Me	29.5	1.9	39.6	C-4′	47.0	1.2	44.0
C-4	43.5	1.2	41.1	C-4a′	144.9	—	44.0
C-4a	138.0	—	41.1	C-5′	179.2	0.7	57.2
C-5	104.3	1.4	71.9	C-6′	161.9	—	57.2
C-6	158.5	—	71.9	C-7′	109.4	0.5	57.2
C-7	118.8	0.6	76.3	C-8′	190.6	—	57.2
C-8	149.2	—	76.3	C-8a′	112.9	1.6	64.6
C-8a	111.3	1.6	67.5	C-9′	159.0	—	64.6
C-9	163.5	—	67.5	C-9a′	130.0	0.9	—
C-10	117.2	1.7	57.7	C-10′	123.3	1.4	61.6
C-10a	140.9	—	57.7	C-10a′	130.2	—	obscured
6-OMe	56.3	12.0[b]	—	6′-OMe	56.7	13.5[b]	

[a] Obtained by comparing the intensities of the resonances in both the natural abundance and enriched spectra after normalisation. Figures refer to atom% ^{13}C over and above natural abundance (1.1%). [b] Incorporation of [*Me*-^{13}C]methionine measured from the ^{1}H NMR spectrum of **3** (see text).

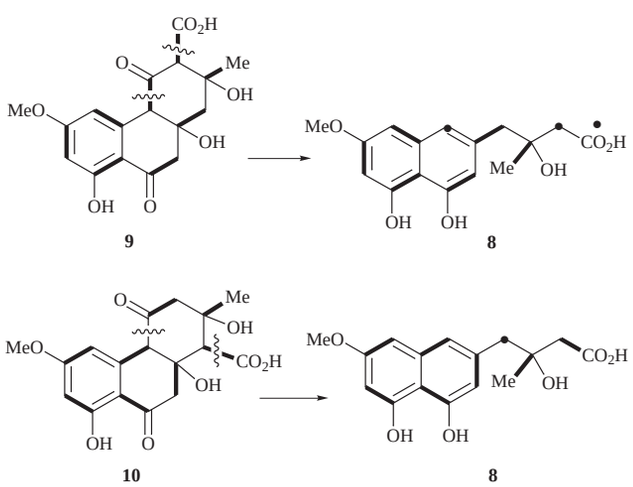
measured by integration of the ^{13}C-satellites (*J* 145.3 and 146.7 Hz) that flank the methoxy singlets at δ 3.90 and 3.93, respectively, in the ^{1}H NMR spectrum of the enriched sample of **3**. The results of the experiments described so far are summarised in Table 2.

The biosynthesis of torosachrysone **5** in fungi is known[11,14] to proceed by way of a β-ketocarboxylic acid of the type **4** (rather than the alternative in which the terminal carboxy group is situated at C-4) by cleavage of bond *a* (Scheme 1) and we have



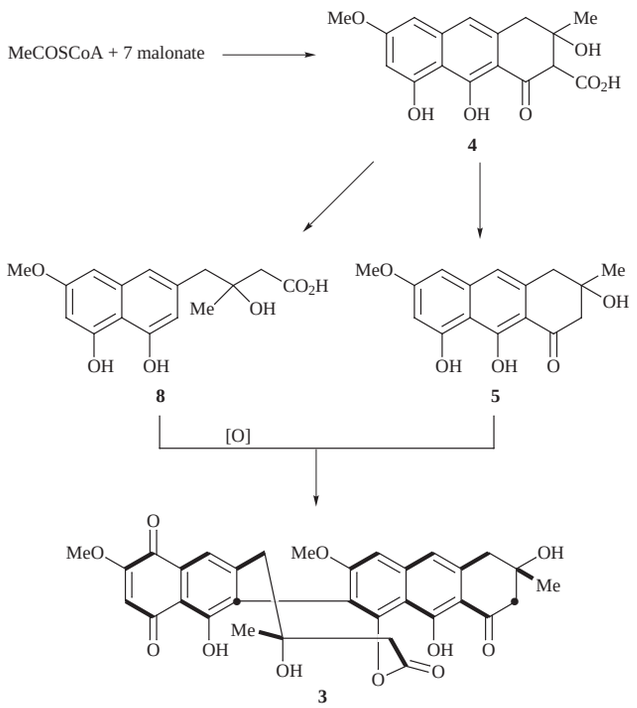
Scheme 1 Alternative routes from the β-ketocarboxylic acid **4** to the naphthalenebutanoic acid **8** showing intact acetate units (—) and disconnected acetate carbons (●).

found that the torosachrysone sub-unit in dermocanarin 4 **3** arises in the same way (*vide infra*). The origin of the building block **8**, which incorporates the naphthalene nucleus together with its pendant 3-hydroxy-3-methylbutanoyl side chain is less obvious. If a linear tricycle is involved (Scheme 1) then **8** could arise either by hydrolytic cleavage of bond *d* in torosachrysone **5** or by sequential cleavage of bonds *b* and *c* in the β-ketocarboxylic acid **4**. Alternatively, if an angular tricycle is involved as in vineomycin biosynthesis (Scheme 2)[15] then the octaketide could fold either in the manner implied in structure **9** or that shown in structure **10**. Whichever case applies, the four alternative routes to **8** shown in Schemes 1 and 2 should be differentiable by the pattern of incorporation of [1,2-$^{13}C_2$]acetate into dermocanarin 4 **3**. Thus, if the position of intact acetate units in the naphthoquinone and lactone bridge in **3** can be determined spectroscopically then the actual precursor to **3**, be it **4**, **5**, **9** or **10**, can be differentiated as shown in Schemes 1 and 2. Accordingly, an aqueous solution of sodium [1,2-$^{13}C_2$]acetate was fed periodically over ten days to each of a small group of young specimens of *C. sinapicolor*, whereafter the toadstools were harvested and dermocanarin 4 **3** was isolated in the usual



Scheme 2 Alternative routes from angular precursors **9** and **10** to the naphthalenebutanoic acid **8** showing intact acetate units (—) and disconnected acetate carbons (●).

way. Inspection of the proton-decoupled ^{13}C NMR spectrum of **3** obtained in this way clearly showed doublets due to intra-acetate ^{13}C–^{13}C coupling flanking all of the natural abundance singlets in the spectrum with the exception of those due to C-2, C-9a′ and the 6- and 6′-*O*-methyl groups. By measuring the ^{13}C–^{13}C coupling constant (*J*, Hz) for each doublet, most (but not all), pairs of carbons in **3** that are directly coupled and therefore derived from the same acetate unit could be determined (Table 2). At this stage, the position of intact acetate units in the naphthoquinone ring in **3**, *i.e.* C-5′–C-6′, C-7′–C-8′, and C-8a–C-9′ with C-9a′ showing no coupling, precludes involvement of the angular precursors **9** and **10**. Option **10** is also invalidated by the fact that C-4a′ and C-4′ in **3** are mutually coupled (*J* 44.0 Hz; Table 2). Unfortunately, signal overlap in some parts of the ^{13}C NMR spectrum made it impossible to measure accurately the coupling constants associated with C-2′, C-3′ and C-10a′ in **3** and C-2′ is critical to the differentiation between the precursors **4** and **5** of **8**. Nevertheless, the incorporation of [1,2-$^{13}C_2$]acetate in **3** was at a sufficient level to enable a two-dimensional INADEQUATE experiment[16] to be performed in a reasonable acquisition time and the results of this, using 50 mg of the enriched natural product **3**, confirm all of the conclusions drawn on the basis of coupling constants (Table 2) and, furthermore, unequivocally identify intact acetate units at C-1′–C-2′, C-3′–3′-Me and C-10′–C-10a′.



Scheme 3 Biosynthesis of dermocanarin 4 **3** showing distribution of label in **3** from sodium [1,2-$^{13}C_2$]acetate.

The location of all of the intact acetate units and the positions of those carbons that have lost their intra-acetate partners in dermocanarin 4 **3** is shown diagramatically in Scheme 3. This pattern confirms that the dihydroanthracenone fragment in **3** arises by decarboxylation (cleavage of bond *a*) of a β-ketocarboxylic acid of the type **4** in the same way that torosachrysone **5** is itself formed in fungi.[11,14] Alternatively, the fact that C-1′ and C-2′ in **3** originate from the same acetate unit precludes the possibility that torosachrysone **5** in a precursor of the 3-hydroxy-3-methylbutanoic acid fragment **8** and proves instead that **8** (or its equivalent) must originate by cleavage of bond *b* in the β-ketocarboxylic acid **4** followed by decarboxylation (hydrolytic cleavage of bond *c*) (Scheme 1).

In summary, the incorporation of [1,2-$^{13}C_2$]acetate into dermocanarin 4 **3** by fruit bodies of *Cortinarius sinapicolor* takes place in a way that is consistent with the biosynthetic pathway shown in Scheme 3 involving the β-ketocarboxylic acid **4** at the branch point in the formation of the major fragments **5** and **8**.

Experimental

General

The melting point was determined on a hot-stage apparatus and is uncorrected. The IR spectrum was recorded using a Perkin-Elmer 983 G spectrophotometer for a sample as a potassium bromide disc. Electronic spectra were recorded on a Varian SuperScan 3 spectrophotometer using ethanolic solutions in 10 mm quartz cells. NMR spectra were recorded with a JEOL JNM-GX-400 spectrometer (^{1}H at 399.65 MHz and ^{13}C at 100.4 MHz) for solutions in $CDCl_3$. Mass spectra were recorded on V. G. Micromass 7070F and JEOL JMS AX505H spectrometers at 70 eV (probe). The specific rotation was measured for a chloroform solution using a Perkin-Elmer 241MC polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. The CD spectrum was obtained using an AVIV 62DS spectrometer for a solution in methanol.

Materials

Thin layer chromatography (TLC) and preparative TLC (PLC) were performed on Merck precoated silica gel 60 F_{254} and Merck Kieselgel 60 GF_{254} (20 g silica gel spread on 20 × 20 cm glass plates), respectively. Visualisation was under UV light (254 or 366 nm). R_f-Values quoted for pure compounds were measured using toluene–ethyl formate–formic acid (50 : 49 : 1) as eluent. Gel permeation chromatography (GPC) employed a column (40 × 3.5 cm) of Sephadex LH-20 suspended in and eluted with methanol.

[*Me*-^{13}C]Methionine (99.6 atom% ^{13}C), sodium [2-^{13}C]acetate (99.5 atom% ^{13}C) and sodium [1,2-$^{13}C_2$]acetate (99.0 atom% ^{13}C) were used as purchased from Sigma-Aldrich. Deuteriochloroform (Cambridge Isotope Laboratories) was washed with water, dried (K_2CO_3), distilled and stored in the dark prior to use.

Cortinarius sinapicolor was collected in the Kinglake National Park, Victoria, Australia from under mixed *Eucalyptus* and *Leptospermum* during May and June 1991 and in subsequent years. Voucher specimens are lodged in the herbarium of the Royal Botanic Garden, Edinburgh, under accession number WAT 24272 and were identified by Dr R. Watling, MBE (Edinburgh) and Professor E. Horak (ETH, Zurich). Specimens of *Cortinarius ignotus* from New Zealand and *C. sinapicolor* from Western Australia were provided by Professor Horak.

Isolation of metabolites from *Cortinarius sinapicolor*

Fresh fruit bodies (1 kg) were macerated in ethanol (2 l) for 2 h at room temperature. The deep yellow extract was concentrated and the aqueous slurry was partitioned, in several portions, between ethyl acetate (500 ml) and water (500 ml). The organic phases were combined, dried and evaporated to afford an orange–brown residue (6.83 g) that was purified by PLC using toluene–ethyl formate–formic acid (50 : 49 : 1) as eluent to give two orange zones (R_f 0.34 and 0.20) and a more polar yellow zone (R_f 0.10). The constituents of the less polar zones will be discussed elsewhere.

The yellow zone (R_f 0.10) was chromatographed repeatedly (PLC, solvent as above) to afford *dermocanarin 4* **3** (283 mg, 2.83×10^{-2}% fr. wt.) as an orange powder, mp 200–203 °C (decomp.) (Found: M^+, 588.1631. $C_{32}H_{28}O_{11}$ requires *M*, 588.1631);** $[\alpha]_D$ +30 (*c* 0.04 in $CHCl_3$); λ/nm 263 ($\Delta\varepsilon$, +46.0), 272.5 (0.0), 279 (−27.4), 292.5 (0.0), 302 (8.3); ν_{max}/cm^{-1} 3446, 1745, 1666, 1650 and 1625; λ_{max}/nm 204 (log ε 4.01), 227 (3.97), 318sh (3.41), 331sh (3.29) and 391 (3.46); *m/z* 588 (M^+, 72%), 570 ($C_{32}H_{26}O_{10}$, 59), 544 (58), 486 (70), 455 (65) and 270 (58); for δ_H and δ_C data see Table 1.

Labelling experiments

(i) [*Me*-^{13}C]methionine. Each of four young specimens were injected using a syringe with an aqueous solution (500 μl) of [*Me*-^{13}C]methionine (0.22 M) on days 1, 4 and 8. The toadstools were harvested on day 13, soaked in ethanol (500 ml) and dermocanarin 4 **3** (6.9 mg) was isolated by chromatography as before. The levels of incorporation of label into the carbons of the two methoxy groups in **3** (Table 2) was measured by integration of the ^{13}C satellites (J_{C-H} 145.3 and 146.7 Hz) that flank the signals at δ 3.90 and 3.93, respectively, in the ^{1}H NMR spectrum of the enriched sample of **3**.

(ii) Sodium [2-^{13}C]acetate. Ten young fruit bodies were each injected with an aqueous solution (150 μl) of sodium [2-^{13}C]acetate (1.2 M) on days, 1, 4 and 6, and finally on day 10. The toadstools were harvested on day 11 and worked up in the usual way to yield dermocanarin 4 **3** (18.4 mg). Enrichment in ^{13}C content over and above natural abundance was measured by

** Repeated attempts to obtain reproducible combustion analysis data for this and other polyphenolic dihydroanthracenone natural products have been hampered by irregular retention of solvents, incomplete combustion and/or chemical degradation (*e.g.* dehydration) during attempts to prepare solvent-free samples.

comparing the peak heights of individual signals in the enriched and natural abundance ^{13}C NMR spectra after normalisation.

(iii) Sodium [1,2-$^{13}C_2$]acetate. Seven sporophores were each injected with an aqueous solution (250 μl) of sodium [1,2-$^{13}C_2$]-acetate (1.2 M) on days 1, 4 and 6 and the toadstools were collected and worked up on day 10. Two other sporophores were similarly fed on days 1, 4, 6 and 10 and were collected on day 11 and worked up along with the other seven sporophores in the usual manner. The results of ^{13}C NMR experiments including a 2D INADEQUATE [16] experiment performed on the sample of dermocanarin 4 **3** (50 mg) obtained in this way are collected in Table 2 and Scheme 3. The 2D INADEQUATE spectrum was recorded over the range δ 25–220 with a data matrix of 64 × 2 K. An average coupling constant, J, of 45 Hz was assumed. A relaxation delay of 1 second was used and 480 scans were accumulated for each t_1 increment. The total acquisition time was *ca.* 2.5 days. Automatic window functions (sine squared) were used and data was zero filled in both dimensions to 128 × 8 K.

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