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DEOXYAUSTROCORTILUTEIN AND DEOXYAUSTROCORTIRUBIN, TETRAHYDROANTHRAQUINONES FROM THE GENUS *CORTINARIUS**

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Key Word Index—*Cortinarius*; *Dermocybe*; Agaricales; pigments; tetrahydroanthraquinones.

Abstract—The hydroxylated tetrahydroanthraquinones deoxyaustrocortilutein and deoxyaustrocortirubin have been isolated from fruit bodies of a red Australian toadstool belonging to *Cortinarius*; the structures are proved by chemical correlation with their co-metabolites austrocortilutein and austrocortirubin.

INTRODUCTION

We have previously recorded in preliminary form [1] the isolation of the first two hydroxylated tetrahydroanthraquinones found in Basidiomycetes. These potent biologically active quinones, austrocortilutein (**1**) and austrocortirubin (**3**), are the major pigments of the fruit bodies of a red toadstool collected in South-Eastern Australia.† In view of the scarcity of pre-anthraquinones such as **1** and **3** in the Higher Fungi and the importance of pigments to the rapidly developing chemotaxonomy of *Cortinarius* [2–4] we have subsequently examined several of the other colouring matters present in this fungus. We report here the isolation and characterisation of a further two tetrahydroanthraquinones which correspond to the 1-deoxy derivatives **2** and **4** of austrocortilutein and austrocortirubin, respectively.‡

RESULTS AND DISCUSSION

Soxhlet extraction of the freeze-dried toadstools with chloroform and thereafter with ethanol gave red-brown solutions which were chromatographed separately. Preparative thin layer chromatography effected preliminary separation of the pre-anthraquinones **1** and **3** from their less abundant, more mobile co-metabolites **2** and **4** which were subsequently purified by further silica gel chromatography.

The less polar of the new tetrahydroanthraquinones, **2** (5.3×10^{-3}%), orange plates, R_f 0.41 [benzene–ethyl formate–formic acid (10:5:3)] showed absorption maxima in the electronic spectrum at 267, 290 and 425 nm consistent with a 5-hydroxy-1,4-naphthoquinone chromophore [5]. This observation, coupled with the presence in the ^{1}H NMR spectrum of a pair of *meta*-coupled aromatic resonances (δ6.62 and 7.16, J = 2.6 Hz) and signals assigned to a *peri*-hydroxy (δ12.35) and to a methoxy group (δ3.90) immediately suggested to us a close relationship between **2** and **1**.

The molecular formula $C_{16}H_{16}O_5$ for **2** was deduced from mass spectral and analytical data and showed that the new pigment corresponded to a deoxy derivative of austrocortilutein (**1**). That the tertiary hydroxy group in **1** had been retained in the deoxy derivative **2** was evident from the ^{1}H NMR spectrum of **2** in which the C-Me resonance persists as a three proton singlet at δ1.41 [*cf* δ1.45 in the spectrum of **1**].

These data are consistent with the 1-deoxyaustrocortilutein structure **2** and, as would be expected when the 1-OH group in austrocortilutein (**1**) is replaced by a hydrogen atom, the aliphatic proton region of the ^{1}H NMR spectrum of **2** proved to be much more complex than the corresponding portion in the spectrum of **1**. Nevertheless, at 400 MHz the spin–spin interactions approach first order and despite overlap of several signals may be interpreted confidently in terms of the conformation **5** for the tetrahydroaromatic ring in **2**. The chemical shifts and multiplicities of the aliphatic protons of **2** are collected in Table 1 while the corresponding coupling constants comprise part of Table 2.

Conclusive proof of the 1-deoxyaustrocortilutein structure (**2**) for the new pigment was obtained by chemical correlation with austrocortilutein (**1**) itself. Thus, exposure of **1** in methanol to H_2/Pd-C resulted in smooth hydrogenolysis of the benzylic hydroxy group and produced orange crystals identical in all respects (m.p., $[\alpha]_D$,

*Part 6 in the series 'Pigments of Fungi'. For Part 5 see *Phytochemistry* (1985) **24**, 2755.

†The fungus has been placed in the subgenus *Democybe* of *Cortinarius* close to *Cortinarius puniceus* Orton and *C. sanguineus* (Wulf. ex Fr.) Fr. (Watling, R., personal communication). Voucher specimens are held in the herbariums of the New South Wales Department of Agriculture Biological and Chemical Research Institute, Rydalmere, NSW (accession number DAR 50092), and the Royal Botanic Garden, Edinburgh, UK (accession number WAT 18086).

‡IUPAC nomenclature rules dictate that in numbering the nucleus of each of the quinones **2** and **4** the left hand ring takes priority. However, for convenience of comparison between spectroscopic data cited here and in ref. [1] we have continued to use for **2** and **4** that numbering system shown for austrocortilutein (**1**). The numbering in **1** is in accord with IUPAC recommendations.

Table 1. Chemical shifts and multiplicities of the protons in the hydroaromatic rings of quinones 2 and 4

H	Chemical shift (δ)* and multiplicity 2	4
1α, 1β	†	†
2α	1.90 *dddd*	1.95 *dddd*
2β	1.65 *ddd*	1.73 *ddd*
3-Me	1.41 *s*	1.43 *s*
4α	†	†
4β	2.55 *ddd*	2.70 *br d*

*The δ values are in ppm downfield from TMS.

†The signals due to H-1α, H-1β and H-4α fall within an envelope from which the individual resonances cannot be discerned: **2** δ2.71–2.78; **4** δ2.88–2.92.

Table 2. Coupling constants of the protons in the hydroaromatic rings of quinones 2 and 4.

Coupling	Coupling constant (J, Hz)* 2	4
1α, 1β	†	†
1α, 2α	4.4	5.5
1α, 2β	8.8	7.9
1α, 4β	2.9	‡
1β, 2α	5.8	5.5
1β, 2β	7.0	7.9
1β, 4β	2.9	‡
2α, 2β	13.6	13.6
2α, 4α	1.8	1.8
4α, 4β	19.4	19.1

*All coupling constants given are those observed: no corrections have been made for non-first-order behaviour.

†Signals due to H-1α and H-1β fall together in an envelope (see footnote, Table 1) which obscures their mutual coupling.

‡Unresolved at 400 MHz.

400 MHz ^{1}H NMR, UV, IR and mass spectra) with the new natural product.

The more polar of the new pre-anthraquinones, **4** (3.5 × 10^{-2} %), $C_{16}H_{16}O_6$, red needles, R_f 0.38 (solvent as for **2**) was identified as a naphthazarin derivative from its electronic and IR spectra. It thus appeared likely that **4** corresponded to a deoxy derivative of austrocortirubin (**3**) and this was supported by comparison of the ^{1}H NMR spectrum of **4** with that of **3** and by close analysis, at 400 MHz, of the aliphatic proton region in the spectrum of the new quinone **4** (Tables 1 and 2). Finally, hydrogenolysis of **3** gave 1-deoxyaustrocortirubin (**4**) which was indistinguishable from the natural product.

Our isolation of deoxyaustrocortilutein (**2**) and deoxyaustrocortirubin (**4**) brings to four the total number of tetrahydroanthraquinones isolated to date from Basidiomycetes. Although a small number of other hydroxylated tetrahydroanthraquinones, e.g. dactylariol (**6**) [6] and altersolanol B (**7**) [7], are known they are restricted in distribution to a group of predacious, ascomycetous conidial fungi [8].

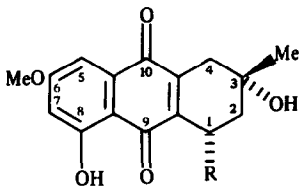
The occurrence of the quinones **2** and **4** as co-metabolites of the major pigments **1** and **3** further supports our suggestion [9] that these pre-anthraquinones represent the products of a new biosynthetic pathway leading from an octaketide progenitor to pigments in this and perhaps in other Australasian cortinars.* Thus, the hydroaromatic rings in austrocortilutein (**1**) and deoxyaustrocortilutein (**2**) (and in their red counterparts **3** and **4**) could arise by stepwise reduction of atrochrysone (**8**). This metabolite has been isolated from a small number of *Cortinarius* toadstools and is a putative precursor of many neutral anthraquinones found in this important group of fungi [9, 10].

EXPERIMENTAL

^{1}H NMR: 400 MHz, $CDCl_3$ with TMS as int. standard; UV: EtOH; mps: uncorr. Prep. TLC: Merck Kieselgel 60 GF_{254} layers (0.1 × 20 × 20 cm) on glass plates. Petrol: bp 60–80°. Combustion analyses were performed by the Australian Microanalytical Service, Melbourne.

Isolation of the quinones **2** *and* **4**. The cinnabar-red fruit bodies, collected from State Forest near Marysville, Victoria during June 1985, were freeze-dried, chopped and a quantity (37.5 g) was extracted continuously (Soxhlet) with $CHCl_3$ and subsequently with EtOH. Evaporation of the $CHCl_3$ extract under red. pres. gave a deep red-brown solid (1.48 g) which was separated by prep. TLC with C_6H_6–HCO_2Et–HCO_2H (50:49:1) (two developments) into zones containing the tetrahydroanthraquinones **1** and **3** (R_f 0.17–0.50) and the tetrahydroanthraquinones **2** and **4** (R_f 0.50–0.64). The mixture of pigments (22 mg) from the more mobile zone was separated by prep. TLC with C_6H_6–HCO_2Et–HCO_2H (70:29:1) (three developments) to give, in order of decreasing R_f: *1-deoxyaustrocortilutein* (**2**), orange plates (from $CHCl_3$–petrol), mp 206–212° after subliming at lower temp. (2 mg, 5.3 × 10^{-3} %) (Found: C, 66.6; H, 5.6. $C_{16}H_{16}O_5$ requires: C, 66.7; H, 5.6%); $[\alpha]_D^{20}$ − 78 ± 5° ($CHCl_3$; *c* 0.051); IR ν_{max}^{KBr} cm^{-1}: 3522, 1652, 1634, 1609, 1580; UV λ_{max}^{EtOH} nm (log ε): 218 (4.42), 267 (4.03), 290 (3.88), 425 (3.51); ^{1}H NMR: δ3.90 (3H, *s*, OMe), 6.62 (1H, *d*, *J* = 2.6 Hz, H-7), 7.16 (1H, *d*, *J* = 2.6 Hz, H-5), 12.35 (1H, *s*, *peri*-OH), other signals are listed in Table 1; EIMS (probe) 70 eV, *m/z* (rel. int.): 289 $[M+H]^+$ (17), 288 $[M]^+$ (100), 246 (13), 245 (31), 244 (19), 243 (21), 231 (22), 203 (14), 91 (10). *1-Deoxyaustrocortirubin* (**4**), red needles (from C_6H_6–petrol), mp 211–216° after subliming at lower temp. (5 mg) (Found: C, 63.3; H, 5.3. $C_{16}H_{16}O_6$ requires: C, 63.15; H, 5.3%); $[\alpha]_D^{20}$ − 59 ± 10° ($CHCl_3$; *c*0.049); IR ν_{max}^{KBr} cm^{-1}: 3452, 1595; UV λ_{max}^{EtOH} nm (log ε): 226 (4.53), 304 (4.01), 472 (3.91), 501 (4.00), 539 (3.84); ^{1}H NMR: δ3.93 (3H, *s*, OMe), 6.20 (1H, *s*, H-7), 12.88 and 13.22 (each 1H, *s*, *peri*-OH), other signals are listed in Table 1; EIMS (probe) 70 eV, *m/z* (rel. int.): 305 $[M+H]^+$ (18), 304 $[M]^+$

*A recent TLC comparison with authentic substances supplied by our laboratory has identified the quinones **1**–**4** as minor pigments in extracts of *Dermocybe splendida* (Horak) from New Zealand and *Dermocybe umbonata* (Cleland) Moser from Australia (Keller, G., personal communication).



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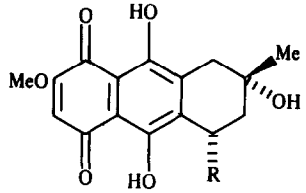
2 R = H



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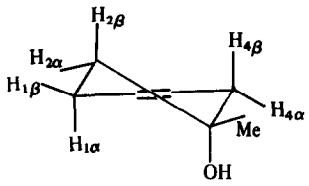

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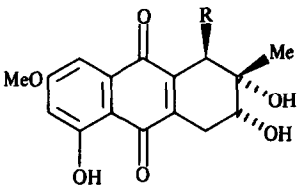
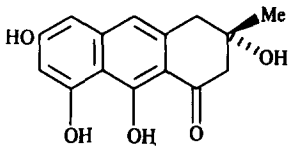


6 R = OH
7 R = H



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(100), 286 (22), 271 (30), 262 (13), 261 (35), 247 (17), 246 (36), 245 (18), 91 (11), 69 (11), 43 (31), 18 (15).

Evaporation of the EtOH extract of the fungus gave, after partitioning between EtOAc and H_2O, a dark red residue (0.64 g) which was purified by chromatography as described above. In this way a further quantity of deoxyaustrocortirubin (**4**) was obtained (8 mg: total 13 mg, 3.5×10^{-2}%).

Hydrogenolysis of the quinones **1** *and* **3**. The quinone (0.1 mmol) in MeOH (15 ml) was exposed to H_2 in the presence of Pd-C (10%, 15 mg) until consumption of H_2 ceased (*ca* 1hr). The catalyst was filtered off and washed with MeOH, and the filtrate was evapd under red. pres. Purification of the residue by prep. TLC with C_6H_6–HCO_2Et–HCO_2H (50:49:1) as eluant (two developments) gave from the quinone **1**, 1-deoxyaustrocortilutein (**2**) (26 mg, 90%) and from the quinone **3**, 1-deoxyaustrocortirubin (**4**) (25 mg, 82%) which were identical in all respects with the natural products described above.

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