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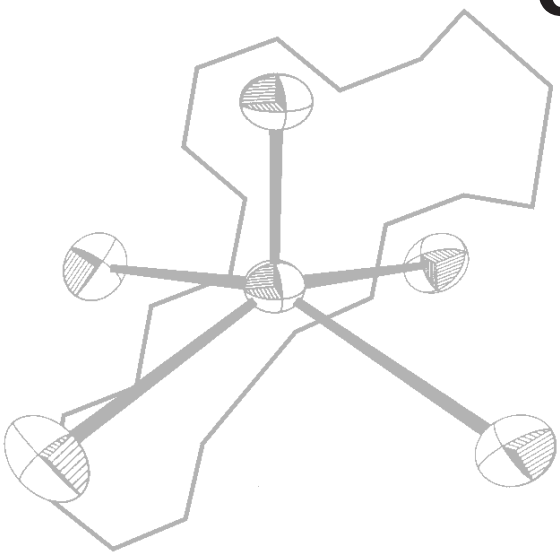

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Pigments of Fungi. LXIII† Synthesis of (1*S*,3*R*)- and (1*R*,3*S*)-Austrocortilutein and the Enantiomeric Purity of Austrocortilutein in some Australian *Dermocybe* Toadstools

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The naturally occurring tetrahydroanthraquinones (1*S*,3*R*)- and (1*R*,3*S*)-austrocortilutein (1b) and (1d), respectively, are synthesized for the first time in enantiomerically pure form by Diels–Alder cycloaddition between the functionalized butadiene derivative (4) and the corresponding monochiral *trans*-1,3-dihydroxy-1,2,3,4-tetrahydro-5,8-naphthoquinone (5a) or (5b), themselves derived from citramalic acid. Separation of the four stereoisomeric austrocortiluteins by using h.p.l.c. over a chiral stationary phase reveals that the enantiomeric purity of the (1*S*,3*S*)- and (1*R*,3*R*)-quinones (1a) and (1c) varies from species to species whereas the (1*S*,3*R*)-isomer (1b) is, in the five cases examined, enantiomerically pure.

Keywords. Fungi; pigments; toadstools; quinones; Australian; *Dermocybe*; cycloaddition.

Introduction

All four stereoisomers of 1,3,8-trihydroxy-6-methoxy-3-methyl-1,2,3,4-tetrahydro-9,10-anthraquinone (austrocortilutein) are known as natural products.[1] Thus, the (1*S*,3*S*)- and (1*S*,3*R*)-diastereoisomers (1a) and (1b) were first isolated from the Australasian toadstool *Dermocybe splendida*[2] and have later been found in several other, closely related, *Dermocybe* species.[1] The (1*R*,3*R*)- and (1*R*,3*S*)-stereoisomers (1c) and (1d) are less common, being found so far only in *Dermocybe* spp. WAT 20934 and WAT 21567,[3] respectively.‡ The relative stereochemistry between the C 1 and C 3 hydroxy groups in (1*S*,3*S*)-austrocortilutein (1a) was determined from the ^{1}H n.m.r. spectrum and the absolute configuration followed by chemical degradation to the butanolide (2) and an X-ray crystal structure analysis of the acetonide derivative.[2] The other austrocortiluteins were correlated directly with (1a) and by way of their 1-deoxy derivatives.[2,4]

We recently reported the first total synthesis of the (1*S*,3*S*)- and (1*R*,3*R*)-austrocortilutein enantiomers (1a) and (1c) beginning from the (*R*)- and (*S*)-enantiomers (3a) and (3b) of citramalic acid.[5] We have now extended this methodology to encompass the two remaining natural products in this series, namely (1*S*,3*R*)- and (1*R*,3*S*)-austrocortilutein (1b) and (1d), and have developed a chromatographic method for determining the stereochemical purity of both the synthetic and natural compounds.

Results and Discussion

The Synthesis of (1 S*,3* R*)- and (1* R*,3* S*)-Austrocortilutein*

Our strategy for the synthesis of the *trans*-1,3-dihydroxytetrahydroanthraquinones (1b) and (1d) is shown in retrosynthetic terms in Scheme 1. Thus, we saw as the final step in the synthetic process a Diels–Alder cycloaddition between the silyloxy diene (4) and each of the hitherto unknown *trans*-1,3-dihydroxytetahydro-5,8-naphthoquinones (5a) and (5b). The quinones (5a) and (5b) could themselves arise by stereoselective reduction and oxidative demethylation of the corresponding chiral tetralones (6a) and (6b), which could arise directly from the known *cis*-1,3-dihydroxytetahydronaphthalenes (7a) and (7c).[5] Accordingly, the (1*R*,3*R*)-dihydroxytetrahydronaphthalene (7a) was prepared from (*S*)-citramalic acid (3b) precisely as described before.[5] Oxidation of the (1*R*,3*R*)-diol (7a) in dichloromethane by using tetrapropylammonium perruthenate[6] and 4-methylmorpholine *N*-oxide in the presence of molecular sieves gave the new (3*R*)-tetralone (6a), $[\alpha]_D$ –15 (*c*, 1.55 in $CHCl_3$), in 85% yield. Alternatively, and somewhat more efficiently, oxidation of the diol (7a) with the Dess–Martin periodinane[7] in aqueous dichloromethane gave the chiral tetralone (6a) in 88% yield.

The spectroscopic data are in full accord with the tetralone structure (6a) for the product. Thus, the mass spectrum shows a molecular ion at *m/z* 236 that corresponds from

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‡ The code refers to the accession number under which voucher specimens are held in the herbarium of the Royal Botanic Garden, Edinburgh.

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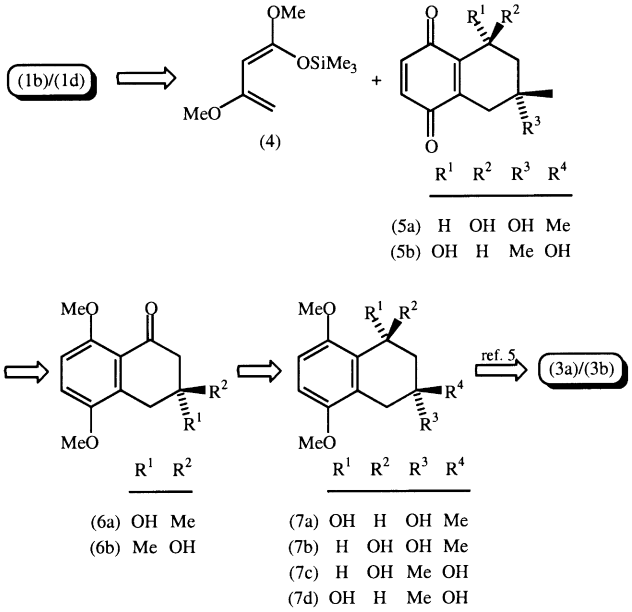
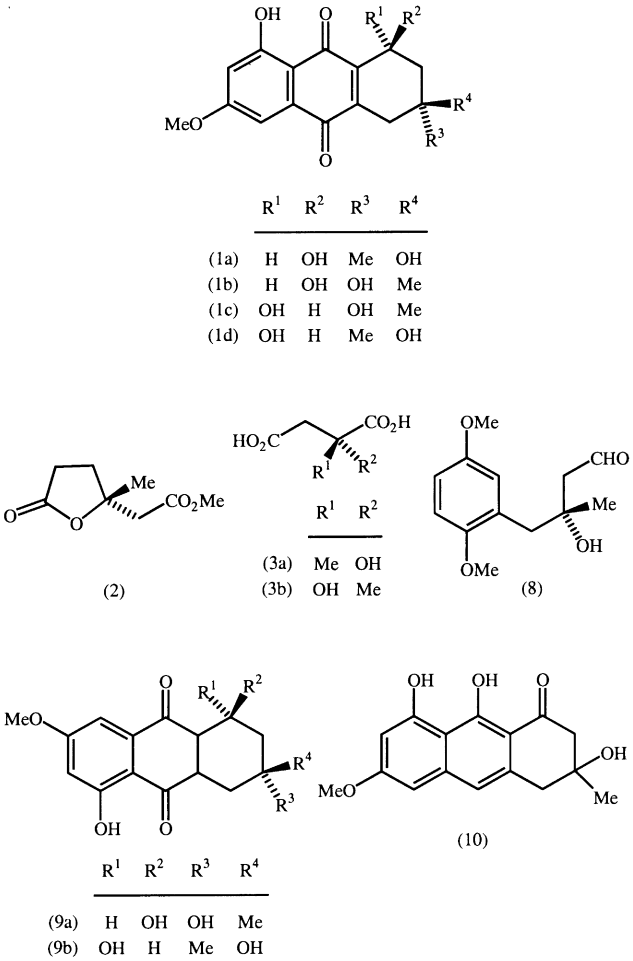
high-resolution mass measurement and combustion analysis data to the molecular formula $C_{13}H_{16}O_4$. The infrared spectrum of (6a) shows a strong ketone carbonyl stretch at 1670 cm^{-1} and in the ^1H n.m.r. spectrum an AB quartet (*J* 15.7 Hz), with components centred at δ 2.71 and 2.77, can be assigned to the protons of the C 2 methylene group. These data confirm that the benzylic hydroxy group in (7a) has been oxidized. The tetralone (6a) is a stable crystalline solid, m.p. 162–164°C, which can be stored at –18°C for several weeks without significant decomposition.

The next step was to reduce the carbonyl group in the (*R*)-tetralone (6a) stereoselectively so as to generate a preponderance of the (1*S*,3*R*)-diol (7b). In order to preserve valuable monochiral intermediates during the optimization of the conditions necessary to effect this transformation efficiently, we first used the isochiral ketone (6a,b).† Thus, treatment of the ketone (6a,b) with lithium borohydride in tetrahydrofuran gave a near quantitative yield of a mixture of the desired (1*S**,3*R**)-diol (7b,d) and the (1*R**,3*R**)-diol (7a,c) in a ratio of 1 : 2. The diastereomeric diols were conveniently separated by using flash pad chromatography and their relative stereochemistry was readily determined from the respective ^1H n.m.r. spectra.[2]

A much more satisfactory outcome was obtained when the tetralone (6a) was exposed to tetramethylammonium borohydride in the presence of acetic acid.[8] Under these conditions tetramethylammonium triacetoxyborohydride is generated, which is a much more stereoselective reagent than borohydride itself. Firstly, tetramethylammonium borohydride was prepared in 75% yield by reaction of sodium borohydride with tetramethylammonium hydroxide. Subsequent treatment of the (*R*)-tetralone (6a) in a mixture of acetonitrile and acetic acid (2 : 1) at 0°C with this reagent gave, exclusively, (1*S*,3*R*)-1,3-dihydroxy-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (7b), $[\alpha]_D$ +49 (*c*, 1.53 in $CHCl_3$), in 85% yield. The mass spectrum of the product (7b) confirmed the molecular formula $C_{13}H_{18}O_4$ that makes it isomeric with the (1*R*,3*R*)-diol (7a). The *trans*-relative stereochemistry between the C 1 and C 3 hydroxy groups in (7b) was evident from the ^1H n.m.r. spectrum and by comparison of it with the corresponding data from the (1*R*,3*R*)-diol (7a).[5] Of particular significance, the axial proton at C 2 in the spectrum of (7b) appears as a double doublet due to geminal coupling (*J* 13.5 Hz) to H 2*eq* and vicinal coupling (*J* 8.5 Hz) to H 1. The magnitude of the vicinal coupling constant indicates a *trans*-diaxial relationship between H 2*ax* and H 1 in a preferred half-chair conformation that places the hydroxy group at C 1 in an equatorial configuration (Fig. 1). The corresponding proton (H 2*ax*) in the spectrum of the (1*R*,3*R*)-diol (7a) appears as a double doublet with coupling constants of 14.5 and 5.0 Hz. In support of the structure (7b), the equatorial proton at C 2 in the spectrum of (7b) appears as a double doublet of doublets due to geminal coupling with H *ax*, vicinal coupling (*J* 6.8 Hz) with H 1 and W-coupling (*J* 2.2 Hz) with H 4*eq*. The hydroxy groups at C 1 and C 3 in (7b) must therefore be *trans*-disposed and, with the C 3 stereochemistry inherited from the (*R*)-tetralone (6a), the absolute configuration of the product (7b) must be (1*S*,3*R*).



Scheme 1. Route to the (1*S*,3*R*)- and (1*R*,3*S*)-austrocortiluteins (1b) and (1d) from citramalic acid.



† This was prepared in the same way as the (*R*)-tetralone (6a) starting from isochiral diol (7a,c).[5]

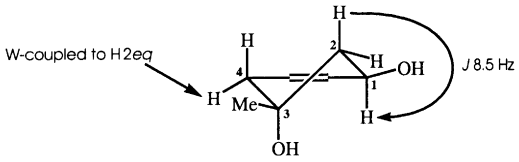


Fig. 1. Preferred half-chair conformation of the tetrahydroaromatic ring in the (1*S*,3*R*)-diol (7b).

The stereospecificity of the hydride reduction process and the stereochemistry of the product (7b) can be rationalized in terms of a mechanism involving chelation control through a cyclic transition state (Fig. 2) during delivery of hydride to the *re* face of the carbonyl group in (6a). Consequently, the involvement of the C 3 hydroxy group is critical in securing the required stereochemistry at the developing C 1 stereogenic centre.

As a potentially shorter route to *trans*-diols of the type (7c) and (7d) we briefly explored the possibility of proceeding directly from the aldehyde (8), the immediate precursor of the *cis*-diol (7a), to the *trans*-diol (7b) by using a Friedel–Crafts reaction that is sterically rather than chelation controlled. Unfortunately, exposure of the aldehyde (8) to both aluminium trichloride and boron trifluoride caused irrevocable damage to the molecule and this route was abandoned in favour of the oxidation–reduction process.

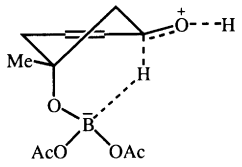


Fig. 2. Transition state in the reduction of the (3*R*)-tetralone (6a).

Oxidative demethylation of the (1*S*,3*R*)-tetrahydronaphthalene (7b) by using ammonium cerium(IV) nitrate in aqueous acetonitrile gave (1*S*,3*R*)-1,3-dihydroxy-1,2,3,4-tetrahydro-5,8-naphthoquinone (5a) in good yield as a yellow gum, $[\alpha]_D$ +163 (*c*, 1.09 in EtOH). High-resolution mass measurement of the molecular ion at *m/z* 208 in the mass spectrum of (5a) confirmed the formula $C_{11}H_{12}O_4$. In the ^{1}H n.m.r. spectrum of the new quinone (5a) there are no signals attributable to methoxy resonances while in the ^{13}C n.m.r. spectrum the carbon atoms of the quinone carbonyl groups resonate at δ 189.0 and 187.5.

In the next step the (1*S*,3*R*)-tetrahydro-5,8-naphthoquinone (5a) and 1,3-dimethoxy-1-trimethylsilyloxybuta-1,3-diene (4)[5,9] were stirred together in benzene at room temperature during 12 h. The mixture of cycloadducts was diluted with water and stirred in the presence of air for a further 12 h. The products were separated by chromatography and a yellow zone that separated was identified as a mixture of (1*S*,3*R*)-austrocortilutein (1b) and its regioisomer (9a). The combined yield of the quinones (1b) and (9a) was 47%, with the former predominating in a ratio of 87 : 13 as determined by integration of the phenolic hydroxy resonances at δ 12.14 (1b) and 12.21 (9a) in the ^{1}H n.m.r. spectrum of the mixture. The remainder of the spectrum of (9a) is very similar to that of (1b), as might be expected. Fractional crystallization of the mixture from chloroform gave (1*S*,3*R*)-austrocortilutein (1b) as fine yellow needles, $[\alpha]_D$ +293 (*c*, 0.10 in $CHCl_3$), in 30% yield from (5a). H.p.l.c. analysis (see below) of (1b) proves that the synthetic material is enantiomerically pure and this, together with other physical, chiroptical and spectroscopic data (Experimental section) are in full accord with the identity of the synthetic quinone (1b) and (1*S*,3*R*)-austrocortilutein as it was isolated from *Dermocybe splendida*.[2] Since the structure of the natural quinone (1b) is known unequivocally,[2] the (predictable) regiochemical outcome of the Diels–Alder reaction between (5a) and (4) is confirmed. This is the first synthesis of (1*S*,3*R*)-austrocortilutein (1b) in monochiral form.

We now turned to the last of the four austrocortilutein stereoisomers, namely, (1*R*,3*S*)-austrocortilutein (1d). The synthesis of the enantiomerically pure (1*S*,3*S*)-dihydroxy-1,2,3,4-tetrahydronaphthalene (7c) from (*R*)-citramalic acid (3a) was described before.[5] The chemistry connecting the diol (7c) with (1*R*,3*S*)-austrocortilutein (1d) mirrors that discussed above and need not be reiterated in detail here. The spectroscopic properties of the individual intermediates proved to be indistinguishable, with the notable exception of their specific rotation, with those of their counterparts in the enantiomeric series.

Briefly, the (1*S*,3*S*)-1,3-dihydroxytetrahydronaphthalene (7c) was oxidized with the Dess–Martin periodinane to the (*S*)-tetralone (6b), $[\alpha]_D$ +14 (*c*, 1.53 in $CHCl_3$), reduction of which with tetramethylammonium borohydride gave the *trans*-diol (7d), $[\alpha]_D$ –48 (*c*, 1.56 in $CHCl_3$), in 85% yield. Oxidative demethylation of (7d) gave (1*R*,3*S*)-1,3-dihydroxy-1,2,3,4-tetrahydro-5,8-naphthoquinone (5b) as a yellow gum, $[\alpha]_D$ –166 (*c*, 1.07 in EtOH), which reacted with 1,3-dimethoxy-1-trimethylsilyloxybuta-1,3-diene (4) to give a mixture of (1*R*,3*S*)-austrocortilutein (1d) and its isomer (9b) in a ratio of 87 : 13, by ^{1}H n.m.r. spectroscopy. Fractional crystallization of the mixture from chloroform gave pure (1*R*,3*S*)-austrocortilutein (1d) as fine yellow needles, $[\alpha]_D$ –265 (*c*, 0.18 in $CHCl_3$), that was indistinguishable in all respects from the natural product.[3]

The Enantiomeric Purity of Austrocortilutein

The likely biosynthetic progenetor of the austrocortiluteins is torosachrysone (10), both enantiomers of which occur, usually in anisochiral mixtures, in *Dermocybe* and *Cortinarius*.[10–13] Consequently, to this point we have been unsure as to the enantiomeric purity of the various austrocortiluteins that occur in *Dermocybe*. With authentic samples of austrocortilutein now available in all four stereochemical modifications we developed a chromatographic method for their analysis.

We have used two chiral h.p.l.c. systems: the first (A) employed a Chiralpak OD column (0.46 by 25 cm; Daicel Chemical Industries) with ethanol/hexane (2 : 3) as eluent, and the second (B) used a Chiralpak AD column (0.46 by 25 cm; Daicel Chemical Industries) with different combinations of ethanol and hexane as eluent. The retention times of the four synthetic austrocortiluteins (1a–d) in both systems A and B are collected in Table 1. The chromatograms obtained for

Table 1. H.p.l.c. retention times (min) for the four stereoisomers (1a–d) of austrocortilutein[A]

Compound	Retention time			
	System A[B] 2 : 3 EtOH/hexane	System B[C] 100% EtOH	2 : 1 EtOH/hexane	1 : 1 EtOH/hexane
(1*S*,3*S*)-Austrocortilutein (1a)	18.5	26.2	40.1	49.9
(1*S*,3*R*)-Austrocortilutein (1b)	30.9	22.4	26.3	33.4
(1*R*,3*R*)-Austrocortilutein (1c)	25.0	32.0	40.1	49.9
(1*R*,3*S*)-Austrocortilutein (1d)	18.6	28.5	46.9	62.3

[A] Conditions are given in the Experimental section. [B] A Daicel chiralpak OD column was used. [C] A Daicel chiralpak AD column was used.

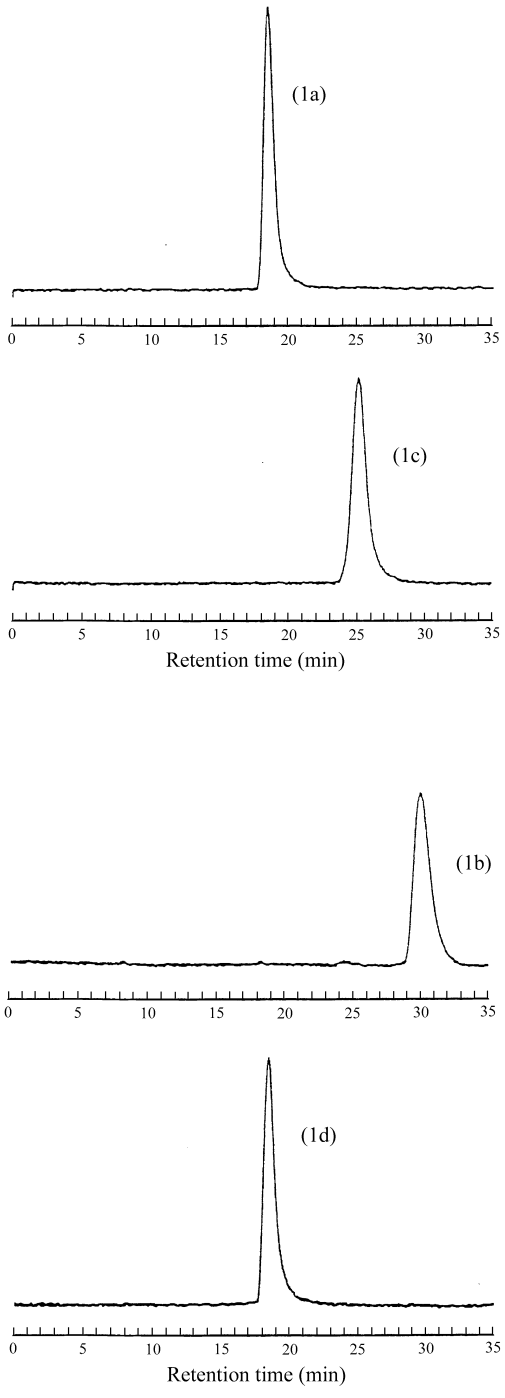


Fig. 3. H.p.l.c. traces (system A) for: (*a*) (1*S*,3*S*)- and (1*R*,3*R*)-austrocortilutein (1a) and (1c); (*b*) (1*S*,3*R*)- and (1*R*,3*S*)-austrocortilutein (1b) and (1d).

the two enantiomeric pairs of synthetic quinones by using system A are shown in Figs 3*a*,*b*. These traces show not only that each enantiomer is well resolved from its antipode but also that all synthetic samples are enantiomerically pure at the levels of detection.

As was mentioned earlier (1*S*,3*S*)- and (1*S*,3*R*)-austrocortiluteins (1a) and (1b), respectively, were originally isolated from the ethanolic extracts of *Dermocybe splendida*[2] while the (1*R*,3*R*)- and (1*R*,3*S*)-austrocortiluteins (1c) and (1d), respectively, were obtained from the Australasian *Dermocybe* sp. WAT 20934.[3] H.p.l.c. analysis of these four natural products using system B gave the enantiomeric excesses shown in the first two rows of Table 2. In the final three rows of Table 2 are the results obtained with samples of (1*S*,3*S*)- and (1*S*,3*R*)-austrocortilutein, (1a) and (1b), recently obtained from three closely related *Dermocybe* species collected in Tasmania. The results of the analysis show that while (1*S*,3*S*)-austrocortilutein (1a) from *D. splendida* is enantiomerically pure, the same pigment from the Tasmanian species is anisochiral, varying in purity between 48 and 34% e.e. depending on the species. Similarly, (1*R*,3*R*)-austrocortilutein (1c) is present in *Dermocybe* sp. WAT 20934 in only 72% e.e. In contrast to the variability observed in the *cis*-1,3-diol systems (1a) and (1c), all results so far obtained with the *trans*-diastereoisomers (1b) and (1d) indicate high enantiomeric purity.

Table 2. Stereochemistry and enantiomeric excess (e.e.)[A] of austrocortilutein stereoisomers (1a–c) from *Dermocybe*

Species	Major diastereoisomer	e.e. (%)	Minor diastereoisomer	e.e. (%)
Dermocybe splendida	(1*S*,3*S*)	100	(1*S*,3*R*)	100
Dermocybe sp. WAT 20934	(1*R*,3*R*)	72	(1*S*,3*R*)	100
Dermocybe sp. 950426A1	(1*S*,3*S*)	34	(1*S*,3*R*)	100
Dermocybe sp. 940417A1	(1*S*,3*S*)	36	(1*S*,3*R*)	100
Dermocybe sp. 950509A0	(1*S*,3*S*)	48	(1*S*,3*R*)	100

[A] Determined by h.p.l.c. analysis using system B (see Experimental section).

At this stage and with so few species examined it is not possible to speculate on the significance, or otherwise, of these observations. However, it is pertinent to note that, as in the case of torosachrysone (10) itself,[13] these chiral compounds are being formed in some organisms with what is much less than the level of stereocontrol that one is led to associate with natural systems.

With a sensitive method now available for determining the stereochemical composition of these and related pig-

ments, we are in a position to examine the extracts of other related fungi and build up a more extensive picture of the distribution and stereochemistry of tetrahydroanthraquinones in *Dermocybe* and *Cortinarius* and to evaluate their taxonomic significance.

Experimental

General Methods and Materials

General details are given in Part LXI.[14] High-performance liquid chromatography (h.p.l.c.) was performed on an ISCO model 2350 with an ISCO UA-6 u.v.–visible detector, an optical flow cell operating at 280 nm and a Spectra-Physics SP 270 integrator. System A used a Chiralpak OD column (0.46 by 25 cm; 10 μm particle size), while system B used a Chiralpak AD column (0.46 by 25 cm;10 μm particle size), both used as purchased from Daicel Chemical Industries, Tokyo.

Dermocybe sp. 950426A1, 940417A1 and 950509A0 were collected by Dr D. A. Ratkowski, School of Agricultural Science, The University of Tasmania, Hobart. The codes refer to the accession numbers under which specimens are held in the herbarium in Hobart.

Synthesis of (1*S*,3*R*)- and (1*R*,3*S*)-Austrocortilutein (1b) and (1d), Respectively

(R)-3-Hydroxy-5,8-dimethoxy-3-methyl-1,2,3,4-tetrahydronaphthalen-1-one (6a) and (S)-3-Hydroxy-5,8-dimethoxy-3-methyl-1,2,3,4-tetrahydronaphthalen-1-one (6b)

(i) Tetrapropylammonium perruthenate (7.4 mg, 0.021 mmol) was added in one portion to a mixture of the (1*R*,3*R*)-diol (7a) (100 mg, 0.42 mmol), 4-methylmorpholine *N*-oxide (74 mg, 1.5 equiv.) and powdered molecular sieves (200 mg, 4 Å) in dichloromethane (4 ml). The suspension was stirred at room temperature for 24 h and filtered through a pad of silica with ethyl acetate as eluent. The solvents were evaporated and the residue was purified by using flash pad chromatography with a gradient solvent system of light petroleum/ether to give the (R)-*tetralone* (6a) (84 mg, 85%) as colourless needles from ethyl acetate, m.p. 162–164°C {isochiral (6a,b) m.p. 157–159°C}, $[\alpha]_D$ –15 (*c*, 1.55 in $CHCl_3$) {the (S)-*tetralone* (6b) $[\alpha]_D$ +14 (*c*, 1.53 in $CHCl_3$)} (Found: C, 66.3; H, 6.9. $C_{13}H_{16}O_4$ requires C, 66.1; H, 6.8%). ν_{max} 3479, 2963, 1670, 1586, 1478, 1458, 1433, 1278, 1257, 1072 cm^{-1}. δ_H (300 MHz) 1.40 (3H, s, 3-Me), 2.00 (1H, m, 3-OH), 2.71 (1H, d, *J* 15.7 Hz, H 2), 2.77 (1H, d, *J* 15.7 Hz, H´2), 2.91 (1H, d, *J* 17.8 Hz, H 4), 3.14 (1H, d, *J* 17.8 Hz, H´4), 3.79 and 3.84 (each 3H, s, 2×OMe), 6.80 and 6.98 (each 1H, d, *J* 9.0 Hz, H 6, H 7). δ_C (75 MHz) 29.2, 37.7, 54.1, 55.9, 56.3, 70.8, 110.5, 116.0, 122.0, 131.5, 150.7, 154.0, 196.3. Mass spectrum *m/z* 236 (M^+, 87%), 218 (33), 203 (71), 178 (100), 177 (31), 163 (65), 148 (25), 121 (30), 120 (42), 77 (28).

(ii) A solution of the Dess–Martin periodinane (454 mg, 1.07 mmol) in dichloromethane (15 ml) was added dropwise to a vigorously stirred solution of (1*R*,3*R*)-diol (7a) (170 mg, 0.714 mmol) and water (19 μl, 1.1 mmol) in dichloromethane (20 ml) over 5 min. The cloudy mixture was stirred at room temperature for 6 h and poured into saturated aqueous sodium hydrogen carbonate (30 ml) containing sodium thiosulfate (1.5 g) and stirred at room temperature for 20 min. The organic phase was separated and washed with saturated sodium hydrogen carbonate (2×30 ml), water (2×30 ml), dried and evaporated. The residue was crystallized from ethyl acetate to give the (*R*)-tetralone (6a) (149 mg, 88%), identical to material described above.

Tetramethylammonium Borohydride

An aqueous solution of tetramethylammonium hydroxide (25%, 170 ml, 0.47 mol) was added to sodium borohydride (17.6 g, 0.47 mol) and the mixture was diluted with deionized water and evaporated to dryness. The colourless solid was suspended in ethanol (95%, 170 ml) and filtered. The filter cake was suspended in ethanol (70 ml) and filtered; this process was repeated a further 10 times. The product was dried at 80°C under vacuum (0.1 mmHg) overnight to give tetramethylammonium borohydride (31.5 g, 75%) as a colourless microcrystalline solid.

(1S,3R)-5,8-Dimethoxy-3-methyl-1,2,3,4-tetrahydronaphthalene-1,3-diol (7b) and (1R,3S)-5,8-Dimethoxy-3-methyl-1,2,3,4-tetrahydronaphthalene-1,3-diol (7d)

Tetramethylammonium borohydride (150 mg, 1.69 mmol) was added to acetic acid (7 ml) at 18°C. After 10 min, acetonitrile (7 ml) was added and the mixture was cooled to 0°C. A solution of the (*R*)-tetralone (6a) (99.1 mg, 0.416 mmol) in acetonitrile (7 ml) was added dropwise over 5 min and the reaction mixture was allowed to warm to room temperature during 18 h. The mixture was diluted with sodium potassium tartrate (0.5 M, 20 ml) and the products were extracted into dichloromethane (5×40 ml). The combined extract was washed with cold dilute sodium hydrogen carbonate (2×40 ml), dried and evaporated and the residue was purified by flash chromatography with ether as eluent to give the *(1S,3R)-diol* (7b) (85 mg, 85%) as colourless needles from ethyl acetate, m.p. 108–110°C {isochiral (7b,d) m.p. 105–107°C} (Found: C, 65.6; H, 7.7. $C_{13}H_{18}O_4$ requires C, 65.5; H, 7.6%), $[\alpha]_D$ +49 (*c*, 1.53 in $CHCl_3$) {the *(1R,3S)-diol* (7d), $[\alpha]_D$ –48 (*c*, 1.56 in $CHCl_3$)}. ν_{max} 3542, 2961, 1481, 1256 cm^{-1}. δ_H (300 MHz) 1.39 (3H, s, 3-Me), 1.79 (1H, dd, *J* 13.5, 8.5 Hz, H 2*ax*), 2.26 (1H, ddd, *J* 13.5, 6.8, 2.2 Hz, H 2*eq*), 2.66 (1H, d, *J* 17.5 Hz, H 4*ax*), 2.80 (1H, dd, *J* 17.5, 2.2 Hz, H 4*eq*), 3.75 and 3.83 (each 3H, s, 2×OMe), 4.01 (1H, m, OH), 5.19 (1H, m, H 1), 6.71 (2H, m, H 6, H 7). δ_C (75 MHz) 30.2, 37.7, 42.7, 55.6, 55.7, 65.2, 69.8, 107.0, 109.1, 124.8, 127.6, 151.7, 151.9. Mass spectrum *m/z* 238 (M^+, 19%), 205 (100), 202 (20), 187 (32), 18 (34).

Reduction of (6a,b) with Lithium Borohydride

A solution of lithium borohydride in tetrahydrofuran (2 M, 450 μl, 0.9 mmol) was added dropwise to a solution of the isochiral tetralone (6a,b) (84 mg, 0.36 mmol) in tetrahydrofuran (10 ml). The solution was stirred at room temperature for 3 h and diluted with ethyl acetate (20 ml) and water (20 ml). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2×30 ml). The combined extract was evaporated to give a colourless gum that was purified by flash chromatography, with ether as eluent, to afford a mixture containing the *cis*-diol (7a,c) (56 mg, 66%) and the *trans*-diol (7b,d) (28 mg, 33%), each of which was identical by ^1H n.m.r. spectroscopy with materials described above.

(1S,3R)-1,3-Dihydroxy-3-methyl-1,2,3,4-tetrahydro-5,8-naphthoquinone (5a) and (1R,3S)-1,3-Dihydroxy-3-methyl-1,2,3,4-tetrahydro-5,8-naphthoquinone (5b)

A solution of ammonium cerium(IV) nitrate (139 mg, 0.254 mmol) in water (4 ml) was added over 5 min to a solution of the dimethyl ether (7b) (30.2 mg, 0.127 mmol) in acetonitrile (4 ml). The mixture was stirred at room temperature for 5 min and diluted with water (20 ml). The product was extracted into dichloromethane (5×20 ml), dried (Na_2SO_4) and evaporated. Gel permeation chromatography (Sephadex LH-20) of the residue gave the *(1S,3R)-naphthoquinone* (5a) (18.1 mg, 69%) as a yellow gum (Found: $M^{+\bullet}$, 208.0733. $C_{11}H_{12}O_4$ requires $M^{+\bullet}$, 208.0736), $[\alpha]_D$ +163 (*c*, 1.09 in EtOH) {the *(1R,3S)-naphthoquinone* (5b), $[\alpha]_D$ –166 (*c*, 1.07 in EtOH)}. δ_H (300 MHz) 1.42 (3H, s, 3-Me), 1.68 (1H, dd, *J* 13.4, 9.3 Hz, H 2*ax*), 2.24 (1H, ddd, *J* 13.4, 6.6, 2.4 Hz, H 2*eq*), 2.42 (1H, dd, *J* 19.5, 2.9 Hz, H 4*ax*), 2.65 (1H, br d, *J* 19.5 Hz, H 4*eq*), 3.46 (1H, s, OH), 5.04 (1H, m, H 1), 6.73 (2H, m, Ar–H). δ_C (75 MHz) 30.5, 36.7, 41.8, 64.5, 69.0, 136.6, 136.7, 140.3, 140.6, 187.5, 189.0. Mass spectrum *m/z* 208 (M^+, 3%), 190 (77), 151 (36), 150 (65), 148 (100), 147 (45).

(1S,3R)-Austrocortilutein (1b) and (1R,3S)-Austrocortilutein (1d)

A solution of 1,3-dimethoxy-1-trimethylsilyloxybuta-1,3-diene (4) (21.2 mg, 0.105 mmol) and the napthoquinone (5a) (18.1 mg, 0.087 mmol) in benzene (5 ml) was stirred at room temperature for 12 h. Water (6 ml) was added and the mixture was stirred vigourously for 12 h in the presence of air. The mixture was diluted with dichloromethane (25 ml) and washed with water (2×15 ml), dried (Na_2SO_4) and evaporated. The residue was purified by flash pad chromatography with toluene/ethyl formate/formic acid (50 : 49 : 1) to give a mixture (12.4 mg, 47%) of (1*S*,3*R*)-austrocortilutein (1b) and its

regioisomer (9a) in an 87 : 13 ratio. Fractional crystallization from chloroform gave (1*S*,3*R*)-austrocortilutein (1b) (7.9 mg, 30%) as orange-yellow needles, m.p. 162–165°C (lit.[2] 162–164°C), $[\alpha]_D$ +293 (*c*, 0.10 in $CHCl_3$) [lit.[2] +288 (*c*, 0.10 in $CHCl_3$)] {(1*R*,3*S*)-austrocortilutein (1d), $[\alpha]_D$ –265 (*c*, 0.18 in $CHCl_3$), lit.[3] –262 (*c*, 0.18 in $CHCl_3$)} (Found: $M^{+\bullet}$, 304.0946. Calc. for $C_{16}H_{16}O_6$: $M^{+\bullet}$, 304.0946). λ_{max} 221 (log ε 4.49), 270 (4.02), 284sh (3.78), 428 nm (3.46). ν_{max} 1665, 1631, 1596 cm^{-1}. δ_H (300 MHz) 1.47 (3H, s, 3-Me), 1.75 (1H, dd, *J* 13.5, 9.1 Hz, H 2*ax*), 2.31 (1H, ddd, *J* 13.5, 6.6, 2.6 Hz, H 2*eq*), 2.55 (1H, dd, *J* 19.6, 2.5 Hz, H 4*ax*), 2.80 (1H, ddd, *J* 19.6, 2.6, 1.8 Hz, H 4*eq*), 3.91 (3H, s, 6-OMe), 4.00 (1H, m, 1-OH), 5.18 (1H, m, H 1), 6.62 (1H, d, *J* 2.6 Hz, H 7), 7.17 (1H, d, *J* 2.6 Hz, H 5), 12.14 (1H, s, *peri*-OH). Mass spectrum *m/z* 304 (M^+, 4%), 286 (60), 271 (30), 269 (22), 268 (100).

^{1}H n.m.r. for (9a): δ_H (300 MHz) 1.47 (3H, s, 3-Me), 1.75 (1H, dd, *J* 13.5, 9.1 Hz, H 2*ax*), 2.31 (1H, ddd, *J* 13.5, 6.6, 2.6 Hz, H 2*eq*), 2.55 (1H, dd, *J* 19.6, 2.5 Hz, H 4*ax*), 2.84 (1H, ddd, *J* 19.6, 2.6, 1.8 Hz, H 4*eq*), 3.91 (3H, s, 6-OMe), 4.00 (1H, m, 1-OH), 5.18 (1H, m, H 1), 6.62 (1H, d, *J* 2.6 Hz, H 7), 7.17 (1H, d, *J* 2.6 Hz, H 5), 12.21 (1H, s, *peri*-OH).

H.P.L.C. Experiments

Synthetic Quinones (1a–d)

A solution (40 µl) of each of the quinones (1a–d) in ethanol (0.05 mg ml^{-1}) was chromatographed at room temperature over Chiralpak OD (system A) and Chiralpak AD (system B) columns by using ethanol or combinations of ethanol and hexane (Table 1) as eluent at a flow rate of 0.5 ml min^{-1}. Retention times are collected in Table 1; chromatograms of (1*S*,3*S*)- and (1*R*,3*R*)-austrocortilutein (1a) and (1c) are shown in Fig. 3*a*, while those of (1*S*,3*R*)- and (1*R*,3*S*)-austrocortilutein (1b) and (1d) are shown in Fig. 3*b*.

Fungal Metabolites

Fresh fruit bodies of *Dermocybe splendida* and *Dermocybe* sp. WAT 20934 were collected, extracted with ethanol and purified by preparative thin-layer chromatography as described before.[2,3] The yellow zones corresponding to austrocortilutein were isolated, dissolved in ethanol (*c*. 0.05 mg ml^{-1}) and analysed by using h.p.l.c. system B. Similarly, air-dried specimens (*c*. 1 g) of *Dermocybe* sp. 950426A1, 940417A1 and 950509A0 were extracted with ethanol and the solvent was removed in vacuum. The residue was partitioned between ethyl acetate (20 ml) and water (10 ml) and the organic phase was dried (Na_2SO_4) and evaporated. Preparative thin-layer chromatography using toluene/ethyl formate/formic acid (50 : 49 : 1) gave two yellow zones that were analysed further by h.p.l.c. system B. The enantiomeric excess (e.e.) of each sample is shown in Table 2.

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References

[1] Gill, M., *Nat. Prod. Rep*., 1994, **11**, 62.
[2] Gill, M., Smrdel, A. F., Strauch, R. J., and Begley, M. J., *J. Chem. Soc., Perkin Trans. 1*, 1990, 1583.
[3] Gill, M., Gimenez, A., Jhingran, A. G., Smrdel, A. F., and Qureshi, A., *Phytochemistry*, 1992, **31**, 947.
[4] Gill, M., and Smrdel, A. F., *Phytochemistry*, 1987, **26**, 2999.
[5] Gill, M., and Ten, A., *Aust. J. Chem.*, 2000, in press.
[6] Griffith, W. P., and Ley, S. V., *Aldrichimica Acta*, 1990, **23**, 13.
[7] Dess, D. B., and Martin, J. C., *J. Am. Chem. Soc*., 1991, **113**, 7277; Ireland, R. E., and Liu, L., *J. Org. Chem*., 1993, **58**, 2899; Meyer, S. D., and Schreiber, S. L., *J. Org. Chem*., 1994, **59**, 7549.
[8] Evans, D. A., Chapman, K. T., and Carreira, E. M., *J. Am. Chem. Soc*., 1988, **110**, 3560, and references cited therein.
[9] Savard, J., and Brassard, P., *Tetrahedron*, 1984, **40**, 3455.
[10] Gill, M., Gimenez, A., Jhingran, A. G., and Smrdel, A. F., *Phytochemistry*, 1989, **28**, 2647.
[11] Eagle, S. N., Gill, M., Saubern, S., and Yu, J., *Nat. Prod. Lett.*, 1993, **2**, 151.
[12] Gill, M., *Nat. Prod. Rep*., 1996, **13**, 513.
[13] Gill, M., Saubern, S., and Yu, J., *Aust. J. Chem.*, 2000, in press.
[14] Gill, M., Raudies, E., and Yu, J., *Aust. J. Chem*., 1999, **52**, 989.