# Pigments of Fungi, Part 27. New Xanthorin Derivatives from a Fungus of the Genus Dermocybe

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*J. Nat. Prod.*, **1992**, 55 (4), 517-520• DOI:
10.1021/np50082a023 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 55, No. 4, pp. 517-520, April 1992*



# PIGMENTS OF FUNGI, PART 27.[1] NEW XANTHORIN DERIVATIVES FROM A FUNGUS OF THE GENUS *DERMOCYBE*

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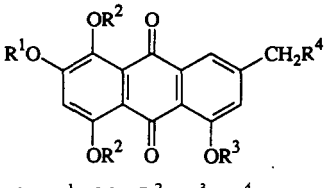
ABSTRACT.—The hydroxylated anthraquinones **2** and **4** were isolated from an indigenous Australian mushroom of the genus *Dermocybe*, and their structures were established by spectroscopic and chemical methods.

Hydroxylated anthraquinones occur in great variety in the fruiting bodies of fungi belonging to the closely related genera *Cortinarius* and *Dermocybe*. [The rank of *Dermocybe* remains a matter for debate. In line with most recent chemical publications (1), and consistent with Moser (2), we have elected to treat *Dermocybe* as a genus. Other authorities regard *Dermocybe* as a sub-genus of *Cortinarius*.] More than thirty pigments of this type are now known from European, North American, and Scandinavian species where their presence has assumed considerable taxonomic importance (1). To date, comparatively little study has been made of *Dermocybe* species indigenous to the Southern Hemisphere; nevertheless, there are now clear indications that the secondary metabolism of Australasian dermocybes is sufficiently different from that of their Northern Hemisphere counterparts to warrant systematic chemical study (3–7).



**1** $R^1$=Me, $R^2$=$R^3$=$R^4$=H
**2** $R^1$=$R^3$=Me, $R^2$=$R^4$=H
**3** $R^1$=$R^2$=$R^3$=Me, $R^4$=H
**4** $R^1$=$R^3$=Me, $R^2$=H, $R^4$=OH
**5** $R^1$=$R^2$=$R^3$=Me, $R^4$=OH
**6** $R^1$=$R^2$=$R^3$=H, $R^4$=OH

We report here the isolation from a diminutive, yellow-brown Australian *Dermocybe* mushroom [WAT 22963 (Cortinariaceae)] of two anthraquinone pigments and describe their identification as **2** and **4**, two new derivatives of the known natural product xanthorin (1,5,8-trihydroxy-6-methoxy-3-methyl-9,10-anthracenedione) [**1**] (8–10).

## RESULTS AND DISCUSSION

Fresh fruiting bodies of WAT 22963 were chopped and macerated in EtOH overnight. Subsequent operations served to concentrate the extracts, distribute the pigments between EtOAc and $H_2O$, and concentrate the coloring matters in the organic phase. Preparative tlc then afforded two red pigments, each in pure form.

The chromatographically more mobile pigment (57 ppm fresh wt) was obtained as red needles to which the molecular formula $C_{17}H_{14}O_6$ was assigned by hreims ($[M]^+$ *m/z* 314.0791). The electronic spectrum, with absorption maxima at 467, 496, and 532 nm, was suggestive of a 1,4-dihydroxylated anthraquinone (11). The $^1$H-nmr spectrum revealed the presence of two chelated hydroxyls (δ 13.43 and 13.98), two OMe groups (δ 3.99 and 4.07), an isolated aromatic proton (δ 6.71), two meta-coupled ($J$=0.8 Hz) aromatic protons (δ 7.17 and 7.86), and an aromatic C-Me group (δ 2.53). These

[1]For Part 26, see M. Gill and A. Giménez, *J. Nat. Prod.*, in press.

data are consistent with, but do not uniquely define, the anthraquinone structure **2** for this red pigment.

The arrangement of the substituents in each individual peripheral ring in the anthraquinone nucleus of **2** was established by differential nOe experiments. Thus, irradiation of the protons of the higher field OMe group (δ 3.99) caused enhancement (8%) of only the isolated aromatic proton resonance (δ 6.71). On the other hand, irradiation of the protons of the alternative OMe group (δ 4.07) caused enhancement (6%) in the signal (δ 7.17) from one of the two meta-coupled aromatic protons. This result places one OMe together with the isolated aromatic proton and both chelated OH groups in the same peripheral ring, with the second OMe, the meta-coupled protons, and the C-Me group in the other. In accord with this conclusion, irradiation of the C-Me group (δ 2.53) enhanced the signals from both of the meta-coupled aromatic protons.

The connectivity between the two ends of the anthraquinone **2** was established unequivocally by direct chemical correlation with the tri-*O*-methyl ether **3** of xanthorin [**1**] isolated from *Dermocybe splendida* (10). Treatment of the red pigment **2** with $Me_2SO_4$ and $K_2CO_3$ in $Me_2CO$ gave the corresponding di-*O*-methyl ether, $C_{19}H_{18}O_6$, which was indistinguishable chromatographically and spectroscopically from the quinone **3** obtained from xanthorin [**1**] by permethylation under the same conditions. Xanthorin-1-*O*-methyl ether [**2**] is a new natural product.

The more polar red pigment (86 ppm fresh wt) was obtained as red needles, $C_{17}H_{14}O_7$, $[M]^+$ *m/z* 330.0737, containing one oxygen atom more than its co-metabolite **2**. The electronic spectrum (λ max 467, 495, and 531 nm) suggested that this second anthraquinone possesses the same chromophore as **2**, which is in full accord with the $^1$H-nmr data. Thus, singlets at δ 13.96, 13.41, 6.72, 4.10, and 3.99 reveal the presence of two chelated hydroxyls, an isolated aromatic proton, and two OMe groups, respectively, while the appearance of a pair of meta-coupled doublets ($J = 0.8$ Hz) at δ 7.48 and 7.97 shows that the extra oxygen atom is not attached directly to the anthraquinone nucleus. Its location as an OH substituent on the 3-Me group is evident by the replacement of the three-proton singlet (δ 2.53) observed in the spectrum of **2** with a two-proton signal at δ 4.89 in the spectrum of its more polar counterpart. The formula **4**, therefore, emerges for this new natural product.

The arrangement of the substituents in each individual peripheral ring of the anthraquinone nucleus in **4** was verified by nOe experiments that parallelled those performed previously on xanthorin-1-*O*-methyl ether [**2**].

The correct connectivity between the two ends of the anthraquinone **4** was proved by chemical correlation with the (new) permethyl ether **5** of the known insect pigment **6** (12). Accordingly, an authentic sample of ω,5-dihydroxyemodin [**6**] isolated from the ericoccid *Eriococcus coriaceus* (12) was methylated by using $Me_2SO_4/K_2CO_3$ in $Me_2CO$. The methyl ether **5**, $C_{19}H_{18}O_7$, so obtained was identical in all respects with the di-*O*-methyl ether of the new natural product **4**.

ω-Hydroxyxanthorin-1-*O*-methyl ether [**4**] is only the second example from within the large group of *Cortinarius* and *Dermocybe* pigments of an anthraquinone bearing an ω-OH group. The other is fallacinol, which occurs along with its ω-*O*-acetyl derivative in *Dermocybe cinnabarina* (13).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—$^1$H-nmr spectra were measured at 399.65 MHz on a JEOL JNM-GX 400 spectrophotometer for solutions in $CDCl_3$. Ei (70 eV) mass spectra were obtained wth a V.G. Micromass 7070F spectrometer. Uv spectra were recorded with a Varian SuperScan 3 spectrophotometer for solutions in EtOH. Preparative tlc was performed on layers

(20 × 20 × 0.1 cm) of Merck Kieselgel $GF_{254}$ Si gel with toluene-$HCO_2Et$-$HCO_2H$ (50:49:1) as eluent.

FUNGUS MATERIAL.—Mushrooms were collected in July 1990 from mixed *Eucalyptus* forest on the western slopes of Mount Slide in the Kinglake National Park, Victoria, Australia. A voucher specimen is lodged in the herbarium of the Royal Botanic Garden, Edinburgh, UK, under accession number WAT 22963.

TAXONOMIC DIAGNOSIS OF WAT 22963.—*Basidia* 4-spored, 4.8–5.2 × 17.5–22 μm excl. sterigmata (1.3–1.8 mm long). Clavate with basal pedicel, hyaline becoming honey-yellow with age. *Basidiospores* 6–6.5 × (3.5)–4 μm elliptic sometimes broadly so or very slightly amygdaliform in side-view, elliptic in face-view with broad apex, verruculose to strongly verrucose at apex and appearing as if forming a cap; pale golden brown in $H_2O$, slightly darker in ammoniacal solutions, relatively thick-walled, germ-pore absent, small but distinct hyaline apiculus present. *Cheilocystidia* forming an irregular margin 11–15 × 5.5–6.5 μm, irregularly clavate, bluntly fusoid to elongate vesiculose, pale to strongly pigmented tawny orange; *pleurocystidia* absent. *Hymenophoral trama* regular, of broad to very broad hyphae ranging from 12–14 μm in mediostratum to 20–25 μm in lateral stratum delimited by a narrow zone of parallel hyphae 3–4 μm broad immediately beneath the hymenial/subhymenial cells; hymenium with dispersed amorphous collections of rich orange-brown to rich tawny material in ammoniacal solutions. *Pileipellis* a cutis 5–6 cells deep of radially arranged broadly ellipsoid to shortly cylindric, branched, tawny, relatively thick-walled, generally smooth hyphae 28–33 × (11)–22 μm broad with membranal pigmentation; velar cells on pileipellis of purplish date, branched, clamp-connected hyphae 6.5–8.7 μm broad; *pilocystidia* absent. *Context* of similar cells to pileus or more variable and slightly longer, less pigmented cells with paler membranal pigment, differentiated at top by smaller ellipsoid cells flattened in a radial plane, and at base with filamentous hyphae 3–4 μm broad supporting hymenium. *Stipitipellis* with an outer layer of filamentous, red-brown, flexuous hyphae 7.5–9 μm broad often adhering together overlying a layer of less pigmented, more regular, cylindric cells; outer layer with velar hyphae similar in all respects to those on pileus. Clamp-connections throughout.

The species is placed close to *Dermocybe vinicolor* Horak (15).

EXTRACTION AND ISOLATION.—Fresh mushrooms (7 g) were chopped and soaked in EtOH (150 ml) at room temperature for 12 h. The extract was evaporated, and the residue was partitioned between EtOAc (3 × 50 ml) and $H_2O$ (50 ml). The dried ($Na_2SO_4$) organic layers were evaporated, and the residue (26 mg) was purified by preparative tlc to afford the anthraquinones **2** (0.4 mg, 57 ppm fresh wt, $R_f$ 0.55) and **4** (0.6 mg, 86 ppm fresh wt, $R_f$ 0.20).

*Xanthorin-1-O-methyl ether* [**2**].—Red needles ($CHCl_3$, −20°): mp 205–209°; found $[M]^+$ *m/z* 314.0791 ($C_{17}H_{14}O_7$ requires 314.0790); uv λ max (log ε) 221 (2.81), 230 (2.77), 236 (2.77), 256 (2.84), 294 (2.40), 467 (2.34), 496 (2.40), 532 (2.20) nm; $^1H$ nmr δ 2.53 (s, 3-Me), 3.99 (s, 6-OMe), 4.07 (s, 1-OMe), 6.71 (s, H-7), 7.17 (d, $J$ = 0.8 Hz, H-2), 7.86 (d, $J$ = 0.8 Hz, H-4), 13.43 and 13.98 (each s, 5-OH, 8-OH); ms *m/z* $[M]^+$ 314 (100), 296 (48), 268 (29), 253 (22), 225 (12), 115 (14), 77 (11), 15 (12).

ω-*Hydroxyxanthorin-1-O-methyl ether* [**4**].—Red needles ($CHCl_3$, −20°): mp 220–223°; found $[M]^+$ *m/z* 330.0737 ($C_{17}H_{14}O_7$ requires 330.0739); uv λ max (log ε) 222 (2.75), 230 (2.72), 236 (2.73), 257 (2.82), 293 (2.39), 467 (2.31), 495 (2.36), 531 (2.16) nm; $^1H$ nmr δ 3.99 (s, 6-OMe), 4.10 (s, 1-OMe), 4.89 (d, $J$ = 4.4 Hz, 3-$CH_2$), 6.72 (s, H-7), 7.48 (d, $J$ = 0.8 Hz, H-2), 7.97 (d, $J$ = 0.8 Hz, H-4), 13.41 and 13.96 (each s, 5-OH, 8-OH), ω-OH obscured; ms *m/z* $[M]^+$ 330 (77), 312 (48), 284 (33), 269 (21), 241 (14), 115 (14), 77 (15).

1,5,6,8-TETRAMETHOXY-3-METHYL-9,10-ANTHRACENEDIONE [**3**].—*From xanthorin* [**1**].—A mixture of xanthorin (1 mg), anhydrous $K_2CO_3$ (200 mg), and $Me_2SO_4$ (1 drop) in dry $Me_2CO$ (1 ml) was heated under reflux for 4 h. The reaction was cooled, the products were partitioned between EtOAc (20 ml) and $H_2O$ (6 × 20 ml), and the colorless aqueous phase was discarded. The organic phase was dried ($Na_2SO_4$) and evaporated, and the residue in MeOH was filtered through a column containing Sephadex LH-20. Crystallization from EtOAc/hexane gave the anthraquinone **3** (1 mg) as yellow needles: mp 188–189° [lit. (14) 185–186°]; found $[M]^+$ *m/z* 342.1104 ($C_{19}H_{18}O_6$ requires 342.1103); uv λ max (log ε) 226 (2.94), 256 (2.76), 280 (2.65), 406 (2.27) nm; $^1H$ nmr δ 2.44 (s, 3-Me), 3.93 (s, OMe), 3.96 (s, 3 × OMe), 6.77 (s, H-7), 7.02 (br s, H-2), 7.51 (br s, H-4); ms *m/z* $[M]^+$ 342 (100), 327 (98).

*From xanthorin-1-O-methyl ether* [**2**].—A mixture of **2** (0.4 mg), anhydrous $K_2CO_3$ (200 mg) and $Me_2SO_4$ (1 drop) in dry $Me_2CO$ (1 ml) was heated under reflux for 4 h, and the mixture was worked up and purified as before. Gel permeation gave the anthraquinone **3** (0.4 mg) identical with material described above.

ω-HYDROXY-1,5,6,8-TETRAMETHOXY-3-METHYL-9,10-ANTHRACENEDIONE [**5**].—*From ω,5-dihydroxyemodin* [**6**].—Compound **6** (5 mg) was converted to **5** under the same conditions used to obtain **3** from **1**. The reaction was cooled, and the products were distributed between $CH_2Cl_2$ (20 ml) and $H_2O$ (6 × 20 ml). The organic phase was dried ($Na_2SO_4$) and evaporated, and the residue was purified by preparative tlc to afford the anthraquinone **5** (1.7 mg) as yellow needles (EtOAc/hexane): mp 157–158° (dec); found $[M]^+$ *m/z* 358.1052 ($C_{19}H_{18}O_7$ requires 358.1052); ir ν max 1666 $cm^{-1}$; uv λ max (log ε) 228 (2.64), 256 (2.45), 281 (2.40), 410 (3.06) nm; $^1H$ nmr δ 3.83 (s, OMe), 3.94 (s, 3 × OMe), 5.22 (s, 3-$CH_2$), 6.78 (s, H-7), 7.70 (br s, H-2), 7.90 (br s, H-4); ms *m/z* $[M]^+$ 358 (11), 327 (100).

*From ω-hydroxyxanthorin-1-O-methyl ether* [**4**].—Anthraquinone **4** (0.6 mg) was converted to **5** under the same conditions used to obtain **5** from **6**, and the mixture was worked up and purified as before. Preparative tlc gave the anthraquinone **5** (0.4 mg) identical with material described above.

## ACKNOWLEDGMENTS

We thank Professor D.W. Cameron, The University of Melbourne, for an authentic sample of ω,5-dihydroxyemodin, and Mr. T. May, National Herbarium of Victoria, for help in the location and collection of fungi. Generous financial support was received from the Australian Research Council.

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*Received 23 July 1991*