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Phytochemistry, Vol. 24, No. 11, pp. 2755–2758, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

ANTHRAQUINONES FROM THE GENUS *CORTINARIUS*

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(*Received* 1 *March* 1985)

Key Word Index—*Cortinarius*; *Dermocybe*; basidiomycete; anthraquinones.

Abstract—The anthraquinones 6-methylxanthopurpurin-3-methyl ether, xanthorin, and 1,4-dihydroxy-2-methoxy-7-methyl-9,10-anthraquinone (austrocortinin) have been isolated from fruit bodies of a red Australian toadstool belonging to the genus *Cortinarius*; austrocortinin is reported for the first time as a natural product.

INTRODUCTION

Anthraquinones are found in great variety in toadstools of the genus *Cortinarius* [1] and their occurrence and distribution has proved particularly useful in differentiating infrageneric taxa [2]. While several of the quinones isolated to date such as emodin, physcion, erythroglaucin and endocrocin are also found elsewhere in nature, others such as dermocybin (**1**) [3], dermolutein (**3**) [4], cinnalutein (**4**) [5], dermorubin (**5**) [4] and cinnarubin (**6**) [5] are restricted to *Cortinarius* and in particular to the subgenus *Dermocybe*. The abundance and ease of extraction of these and related* pigments, coupled with their efficient chromatographic separation and rapid identification has led to their use as chemotaxonomic markers during mycological studies of European [7], South American [8] and North American [9] varieties of *Cortinarius* toadstools.

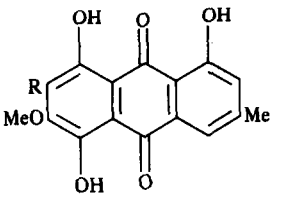
In view of the importance of anthraquinones to the systematics of *Cortinarius* we have examined the anthraquinone content of a red Australian member of this group† and report here the presence of three hydroxylated anthraquinones, **2**, **7** and **8**, which have not previously been reported in *Cortinarius* or in any other genus of *Basidiomycetes*; further, the anthraquinone **8** is a new natural product. We have made a preliminary report elsewhere of the presence in this red toadstool of the novel tetrahydroanthraquinones **9** and **10** [10].

RESULTS AND DISCUSSION

Extraction of fresh, cinnabar-red sporophores of the fungus with alcohol gave a deep red solution which was concentrated, extracted with petrol to remove a lipid fraction consisting largely of triolein (trioleylglycerol) and then with ethyl acetate to remove pigments from the aqueous phase. Preliminary column chromatography separated the less polar anthraquinones from the more

*Many of the fungal anthraquinones are present as, or are accompanied by, their 1-β-D-glucopyranoside derivatives [5, 6].

†Taxonomic difficulties exist within the genus *Cortinarius* and there is a marked confusion in the nomenclature in the subgenus *Dermocybe*. The taxonomy of *Cortinarius* in Australia is severely underdeveloped and the Australian taxa are basically unknown. Voucher specimens of the fungus discussed here are lodged at the herbariums of the NSW Department of Agriculture Biological and Chemical Research Institute, Rydalmere, NSW (accession number DAR 50092) and the Royal Botanic Gardens, Edinburgh, U.K. The fungus has been placed in the subgenus *Dermocybe* close to *Cortinarius* (*Dermocybe*) *sanguineus* (Wulf. ex Fr.) Fr. and *Cortinarius* (*Dermocybe*) *puniceus* Orton [Watling, R., personal communication].



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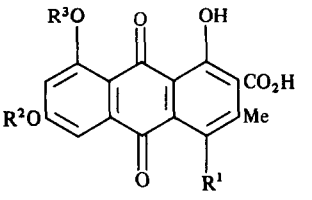
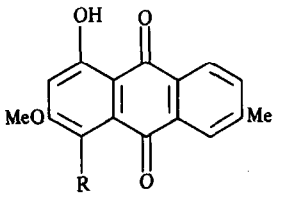


3 $R^1 = R^2 = H$, $R^3 = Me$
4 $R^1 = R^3 = H$, $R^2 = Me$
5 $R^1 = OH$, $R^2 = H$, $R^3 = Me$
6 $R^1 = OH$, $R^2 = Me$, $R^3 = H$



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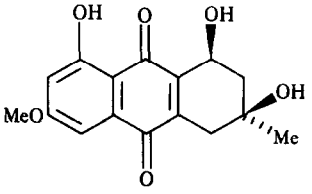

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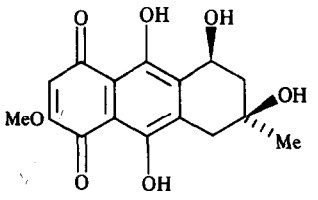

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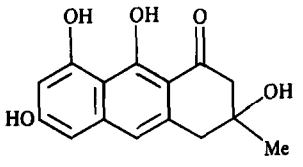

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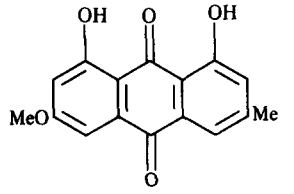

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polar, hydroxylated tetrahydroanthraquinones **9** and **10**, and the individual anthraquinones were subsequently isolated by preparative TLC.

The most abundant anthraquinone, orange crystals, $C_{16}H_{12}O_4$, formed a mono-acetate and exhibited visible and IR spectra indicative of a 1-hydroxyanthraquinone structure [11]. From the ^{1}H NMR spectrum [12] the pigment was identified as 6-methylxanthopurpurin-3-methyl ether (**7**) and this was confirmed by direct comparison with an authentic sample isolated from *Alternaria solani* [12].

The ^{1}H NMR spectrum of a second anthraquinone, **8**, red crystals ($C_{16}H_{12}O_5$), here named austrocortinin, revealed in addition to methyl and methoxyl proton singlets and signals from aromatic protons in a 1,2,4-arrangement, an isolated aromatic proton (δ6.70) and two *peri*-hydroxyl groups (δ13.46 and 13.58). Strong carbonyl absorption at 1593 cm^{-1} in the IR spectrum supported a 1,4-dihydroxyanthraquinone structure which was consistent with visible absorption at λ_{max} 458 sh, 482 and 514 nm. The identity of austrocortinin (**8**) was confirmed by direct comparison both of the pigment itself and its diacetate derivative with synthetic materials [13].

A second red anthraquinone, $C_{16}H_{12}O_6$, was identified as xanthorin (**2**) from its ^{1}H NMR spectrum and by comparison with a synthetic sample prepared from emodin [14].

6-Methylxanthopurpurin-3-methyl ether (**7**) and xanthorin (**2**) have not previously been reported from higher fungi. Quinone **7** occurs in various ascomycetous conidial

fungi of the genera *Alternaria* and *Phomopsis* [1] while xanthorin (**2**) is a lichen constituent [15, 16]. A synthesis of austrocortinin (**8**) has been described [13] but this is the first report of its occurrence as a natural product.

The anthraquinones **2**, **7** and **8** are isolated from fresh sporophores in yields of 0.5×10^{-3}, 0.5×10^{-2} and 0.8×10^{-3}%, respectively. Significantly, none of the typical *Dermocybe* anthraquinones [7–9] are present in the Australian species, a result which accords with a TLC survey of several Australian *Cortinarii* [Watling, R., personal communication] and suggests the possibility of an alternative mode of octaketide biosynthesis in this, and perhaps other, Australian *Cortinarius* toadstools. The anthraquinones **7**, **2** and **8** are most likely derived biogenetically in this organism from their tetrahydroanthraquinone cometabolites **9** and **10**, respectively, by dehydration (for **7** and **8**) and by partial dehydration followed by oxidation (for **2**). Thus, this prospective pathway complements earlier proposals that the final step in anthraquinone elaboration in higher fungi involves oxidation of the corresponding anthrone [17, 18]. It is attractive to speculate that both pathways may share a common precursor in the form of the *Cortinarius* metabolite atrochrysone (**11**) [18] or its equivalent.

That the anthraquinones described here do not arise from their tetrahydroanthraquinone cometabolites as artefacts of the isolation and purification procedure is evidenced by the following observations. The pure tetrahydroanthraquinones **9** (2.2×10^{-2}% fr. wt) and **10** (4.1×10^{-2}%) are not aromatized under the conditions used for their isolation. Thus they are recovered unchanged even when heated to reflux in ethanol, in ethyl acetate and in chloroform. Furthermore, the relative proportion of 6-methylxanthopurpurin-3-methyl ether (**7**), austrocortinin (**8**) and xanthorin (**2**) isolated is not consistent with their chemical derivation from the quinones **9** and **10**. Thus, when the dehydration of tetrahydroquinones **9** and **10** is achieved in the presence of mineral acid [10] both quinones undergo facile aromatisation and afford, from austrocortilutein (**9**), a mixture (*ca* 93:7) of 6-methylxanthopurpurin-3-methyl ether (**7**) and physcion (**12**), and from austrocortirubin (**10**), a mixture (*ca* 95:5) of austrocortinin (**8**) and xanthorin (**2**). It is notable that physcion is not detected in extracts of the fungus. Finally, anthraquinones **2**, **7** and **8** and tetrahydroanthraquinones **9** and **10** are detected in chloroform extracts of quick frozen, freeze-dried sporophores in the same relative proportions as are isolated with ethanol.

EXPERIMENTAL

^{1}H NMR: 99.55 MHz, $CDCl_3$ (unless stated otherwise) with TMS as internal standard; UV: EtOH; mps: uncorr. CC: Merck Kieselgel 60 (0.015–0.04 mm); prep. TLC: Merck Kieselgel 60 GF_{254} layers (0.1 × 20 × 20 cm) on glass plates. Petrol refers to light petroleum bp 60–80°. Combustion analyses were performed by the Australian Microanalytical Service, Melbourne.

Isolation of the constituents of the Cortinarius *toadstool.* The cinnabar-red fruit bodies (400 g) were collected from Yarra State Forest, Marysville, Victoria in June 1984. The fresh sporophores were chopped and extracted exhaustively with EtOH (6 × 500 ml) at room temp. and the combined extracts were concentrated under red. pres. The resulting aq. suspension was diluted with H_2O (150 ml) and continuously extracted overnight with petrol; the petrol extract, which contained a proportion of the anthraquinones, was retained (see below). The deep red aq. phase was continuously extracted overnight with EtOAc and the organic phase was dried ($MgSO_4$) and evaporated. The resulting deep red solid (1.1 g) was chromatographed on a column (3.5 × 25 cm) of silica gel using C_6H_6–EtOAc–HOAc–HCO_2H (12:6:1:1) and the eluate was collected in 10 ml fractions. The first coloured fractions (containing the anthraquinones) were combined and evaporated to dryness under red. pres. Later fractions contained **9** and **10** [10] and their purification will be described in detail elsewhere. The anthraquinone residues were combined with the petrol extractives described above and the whole (200 mg) was chromatographed on a column (5.0 × 20 cm) of silica gel using petrol–EtOAc–HOAc (40:10:1) as eluant. Early fractions contained chromatographically and spectroscopically homogeneous triolein (80 mg). The slower moving anthraquinones were eluted together and subsequently separated by prep. TLC with CH_2Cl_2–petrol–HOAc (60:40:1) (four developments). This gave, in order of decreasing R_f: 6-methylxanthopurpurin-3-methyl ether (**7**), yellow needles (from $CHCl_3$–petrol), mp 186–187° (lit. [12] 184–185°) (21 mg) (Found: C, 71.9; H, 4.55. Calc. for $C_{16}H_{12}O_4$: C, 71.65; H, 4.5%); MS *m/z* (rel. int.): 268 (100), 239 (10), 225 (8), 210 (8), 120 (7), 115 (7), identical with authentic material [12]; acetate (Ac_2O–H_2SO_4) pale yellow needles (from EtOH), mp 173–177° (lit. [19] 171–173°) (Found: C, 69.6; H, 4.75. Calc. for $C_{18}H_{14}O_5$; C, 69.65; H, 4.55%); MS *m/z* (rel. int.): 310 (2) 284 (1), 270 (2), 269 (18), 268 (100), 239 (6); xanthorin (**2**), red needles (from $CHCl_3$–petrol), mp 245–250° (lit. [20] 250–251°) (2 mg); MS *m/z* (rel. int.): 301 (17.5), 300 (100), 284 (12), 282 (10), identical with a synthetic sample [14]; and *austrocortinin* (**8**), red needles (from $CHCl_3$–petrol), mp 237–240° (lit. [13] 237–239°) (3 mg) (Found: C, 67.65; H, 4.25. $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.25%); UV λ_{max}^{EtOH} nm (log ε): 210 (4.51), 262.5 (4.50), 302 (3.91), 458 sh (3.85), 482 (3.93), 514 (3.76); IR ν_{max}^{KBr} cm^{-1}: 1593, 1620 (w); ^{1}H NMR: δ2.55 (3H, *s*, CMe), 4.01 (3H, *s*, OMe), 6.70 (1H, *s*, H-3), 7.63 (1H, *dd*, *J* = 8.1, 1.9 Hz, H-6), 8.13 (1H, *s* (*br*), H-8), 8.24 (1H, *d*, *J* = 8.1 Hz, H-5), 13.46 and 13.58 (each 1H, *s*, *peri*-OH); MS *m/z* (rel. int.): 284 (100), 266 (9), 255 (8), 254 (9), 241 (12), 115 (14); diacetate (Ac_2O–H_2SO_4) yellow needles (from $CHCl_3$–petrol), mp 245–252° (lit. [13] 242–248°) (Found: C, 65.35; H, 4.4, $C_{20}H_{16}O_7$ requires C, 65.2; H, 4.4%); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 270 (4.40), 286 sh (4.10), 345 (3.48), 366 (3.54); IR ν_{max}^{KBr} cm^{-1}: 1592, 1672, 1770; ^{1}H NMR: δ2.49 (9H, *s*, CMe, 2 × OAc), 3.94 (3H, *s*, OMe), 6.94 (1H, *s*, H-3), 7.53 (1H, *d* (*br*), *J* = 8.1 Hz, H-5), 7.94 (1H, *s* (*br*), H-7), 8.06 (1H, *d*, *J* = 8.1 Hz, H-4); MS (10 eV) *m/z* (rel. int.): 368 (3), 327 (19), 326 (98), 286 (14), 285 (92), 284 (100).

Acknowledgements—We thank Dr. R. Watling, Royal Botanic Gardens, Edinburgh, U.K. and Dr. J. Walker, NSW Department of Agriculture Biological and Chemical Research Institute, Rydalmere, NSW, for lodging voucher specimens and for their help in the difficult area of identification and nomenclature. We are grateful to Dr. A. Stoessl for authentic samples of 6-methylxanthopurpurin-3-methyl ether and its acetate derivative, and to Professor T. Ross Kelly for synthetic austrocortinin. The Australian Research Grants Scheme provided financial support.

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Phytochemistry, Vol. 24, No. 11, pp. 2758–2760, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

PYROGALLOL DERIVATIVES FROM *LEUCANTHEMOPSIS PALLIDA* SUBSP. *FLAVEOLA*

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(*Received* 7 *February* 1985)

Key Word Index—*Leucanthemopsis pallida* subsp. *flaveola*; Compositae; pyrogallol derivatives.

Abstract—From *Leucanthemopsis pallida* subsp. *flaveola*, three new pyrogallol derivatives were isolated: 4-hydroxy-5-propionyl-1,3-di-*O*-methylpyrogallol, 4-hydroxy-5-propionyl-1,3-di-*O*-methyl-2-*O*-isopentenylpyrogallol and 5-(1′-isovalerianoyloxy)-ethyl-1,3-di-*O*-methyl-2-*O*-isopentenylpyrogallol.

INTRODUCTION

In previous papers [1–4], we described the components of *Leucanthemopsis pulverulenta* (Lag.) Heywood. Now, following the study of the genus *Leucanthemopsis*, we report the first results from *L. pallida* (Miller) Heywood, subsp. *flaveola* (Hoff. & Link.) Ladero & Velasco. From the aerial parts of the plant, we isolated three new pyrogallol derivatives, **1**–**3**, two phloroglucinols, **4** and **5**, previously isolated from roots of *L. pulverulenta* [1], (+)-sesamin and cumambrin A [5].

RESULTS AND DISCUSSION

The UV spectrum of **1** showed characteristic absorptions due to a conjugated C=O group, with a polyoxygenated aromatic ring. The bathochromic shift (28 nm) induced by addition of $AlCl_3$–HCl, was indicative of the presence of one *ortho*-hydroxyl group associated with the C=O group [6].

The ^{1}H NMR spectrum of **1** (Table 1) was in agreement with a 1,2,3,4,5-pentasubstituted aromatic ring, with COEt, two hydroxyl and two methoxy groups as substituents. The intramolecular association of the C=O group and the chemical shift of the unique aromatic proton in the ^{1}H NMR (δ6.91), allowed us to conclude that the COEt group was flanked by OH and H [7]. The chemical shifts of the methoxyl groups in the ^{13}C NMR spectrum (Table 2), showed that one of them was *ortho*-disubstituted and the other *ortho*-monosubstituted [8].

Acetylation of **1**, gave a diacetate, **1a**, whose mass spectrum showed an $[M]^+$ at m/z 310, in agreement with the formula $C_{15}H_{18}O_7$. The ^{1}H NMR spectrum of **1a**, showed additional signals due to two acetoxyl groups. All these data allowed us to identify **1** as 4-hydroxy-5-propionyl-1,3-di-*O*-methylpyrogallol. The ^{13}C NMR spectra of **1** and **1a** (Table 2) confirmed the proposed structure.

The spectral data of compound **2** were all very similar to those of **1**. The only significant differences were in the ^{1}H and ^{13}C NMR spectra which exhibited additional signals due to an *O*-isopentenyl group (Tables 1 and 2). The intramolecular association of the C=O group through hydrogen bonding and the chemical shift of the unique aromatic proton (δ6.92), suggested that the *O*-isopentenyl group was at C-2, identifying **2** as 4-hydroxy-5-propionyl-1,3-di-*O*-methyl-2-*O*-isopentenylpyrogallol.

Compound **3** was isolated as an optically active oil, whose UV spectrum was typical of a polyoxy-