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PIGMENTS OF FUNGI, PART 8. BIANTHRAQUINONES FROM *DERMOCYBE AUSTROVENETA*[1]

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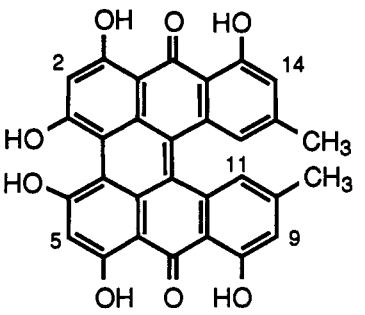
Fruiting bodies of the fungus *Dermocybe austroveneta* (Cleland) Moser (Agaricaceae) are common in *Eucalyptus* woodlands throughout the eastern states of Australia from May to August. The mushroom is easily recognized by its emerald green cap skin, yellow gills, and yellow-orange stipe and is now recognized as the sole Australian member of the genus *Dermocybe* possessing these unmistakable taxonomic characters. The closest known relatives of *D. austroveneta* are to be found in South America. Thus, *D. austroveneta* is placed in the section *Pauperae* of the subgenus *Icterinula* of *Dermocybe* by Moser in Singer (1) along with several South American species, e.g., *Dermocybe luteostriatula, Dermocybe olivipes, Dermocybe obscureolivea* var. *brunnea, Dermocybe hypoxantha*, and *Dermocybe alcalisensibilis*.

When sporophores of *D. austroveneta* are attacked by insects or suffer damage on aging, the flesh assumes a red-violet coloration. Similarly, on extraction of the mushrooms under normal laboratory conditions, deep red-violet solutions displaying fiery red fluorescence are obtained. It is worth noting that the South American species mentioned above are reported to possess a red fluorescent pigment (1).

We report here the isolation, after extraction of *D. austroveneta* in the dark, of a purple pigment which is identical with the rare natural product protohypericin [**1**]. This is the first record of protohypericin as a fungal metabolite. In laboratory daylight the protohypericin present in extracts of *D. austroveneta* is rapidly transformed to the photodynamic pigment hypericin [**2**], which is thereafter responsible for the red-violet color and fiery red fluorescence mentioned earlier. The principal orange pigment of *D. austroveneta* is identified as (+)-*S*-skyrin [**3**].



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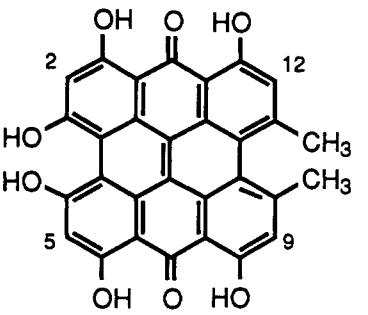

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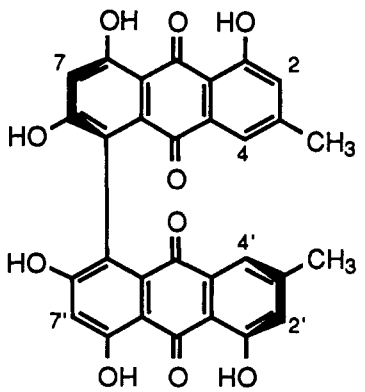

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[1]For Part 7, see M. Gill, A. Gimenez, and R.J. Strauch, *Phytochemistry*, **26**, 2815 (1987).

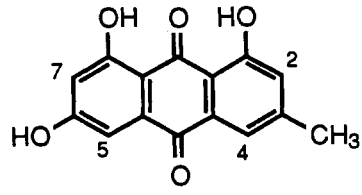
Fresh mushrooms were macerated in the dark and extracted with Me_2CO. Subsequent operations, all carried out in the dark, served to concentrate the extracts, distribute the pigments between EtOAc and H_2O, and concentrate the coloring matters in the organic phase. Preparative tlc and gel permeation then afforded the major purple and orange pigments, each in pure form.

The purple pigment (43 ppm fresh wt) was found to be identical in chromatographic behavior and electronic, ir, and nmr spectra with protohypericin [**1**], a synthetic sample of which was available for direct comparison. On exposure to light, protohypericin is rapidly converted to hypericin [**2**] (2), and we have for the first time monitored the progress of this transformation using ^{1}H-nmr spectroscopy. Accordingly, when a fresh solution of the *D. austroveneta* pigment prepared in the dark in Me_2CO-d_6 was examined at 400 MHz, a spectrum fully consistent with the structure **1** was observed (Figure 1a). Periodic exposure of this solution to light resulted in the gradual appearance of proton resonances consistent with the presence of hypericin [**2**] together with a concomitant diminution in intensity of resonances due to protohypericin itself (Figure 1b–e). After 8 min of cumulative exposure to sunlight the solution contained no protohypericin (Figure 1f). Evaporation of the solution and chromatographic purification of the residue gave hypericin [**2**], identified by its ^{1}H-nmr spectrum (Figure 1f) and by comparison with an authentic specimen.

Protohypericin [**1**] has previously been isolated from plants of the genus *Hypericum* (3,4) and from various insects (5), but this is the first report of its occurrence as a fungal metabolite.

As was expected from our observations, extraction of *D. austroveneta* under normal laboratory light conditions gave hypericin as the only red-violet pigment. Although the presence of hypericin in extracts obtained under these conditions is demonstrably artifactual, it must also be recognized that protohypericin too could be formed, at least in part, from an oxygen-sensitive precursor even in the absence of light.

The major pigment of *D. austroveneta*, orange-red plates (94 ppm fresh wt) was cleaved with alkaline dithionite to give emodin [**4**]. It was identified as the 5,5′-linked bianthraquinone skyrin [**3**] from this (6) and by comparison of its ^{1}H- and ^{13}C-nmr spectra with those of the anthraquinone **4**. The unequivocal assignment of resonances in the ^{13}C-nmr spectra of emodin and skyrin is reported here for the first time in Tables 1 and 2, respectively. Assignments are consistent with ^{1}H-^{13}C couplings in the proton-coupled ^{13}C-nmr spectra of pigments **3** and **4**.



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Skyrin is chiral by virtue of restricted rotation about the biaryl linkage: the absolute configuration of the dextrorotatory enantiomer isolated here is *S*, as depicted in formula **3**, according to Karl and Steglich.[2]

Skyrin, although widespread among the microfungi (7,8), has rarely been found in macromycetes. It has been isolated from *Cortinarius atrovirens*, *Cortinarius ionochlorus*, and *Cortinarius odoratus* (9), and from the hypochraceous ascomycetes *Hypomyces aurantius* (10), *Hypomyces lactifluorum* (11,12), and *Hypomyces trichothecoides* (13). Of particular significance in the present context, skyrin has been detected recently by Keller *et al.* (14) using tlc in extracts of dried specimens of *D. alcalisensibilis*, *Dermocybe alienata*, *D. luteostriatula*, *D.*

[2]U. Karl and W. Steglich, unpublished data.

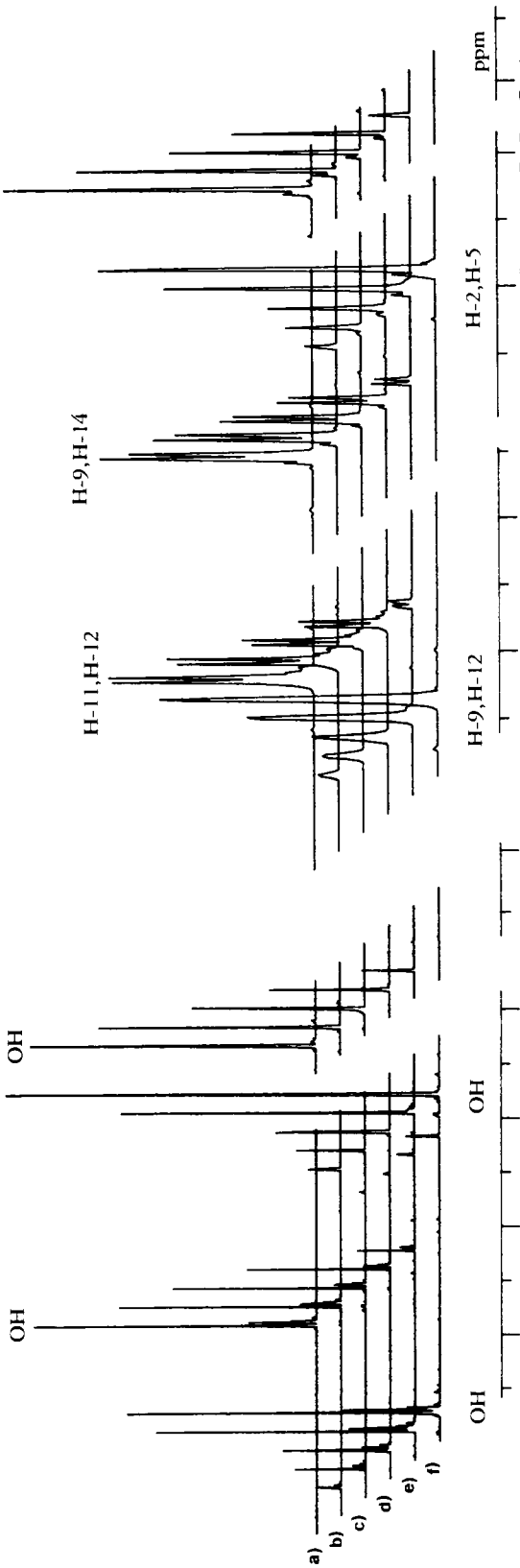

FIGURE 1. Hydroxy and aromatic proton region of nmr spectrum of (a) protohypericin [**1**] in Me_2CO-d_6; (b) the same sample after exposure to sunlight for 30 sec; (c) 60 sec; (d) 120 sec; (e) 240 sec; (f) 480 sec. Spectra recorded at 400 MHz while irradiating the protons of the methyl groups in **1**.

TABLE 1. ^{13}C Chemical Shifts and One-bond and Long Range Carbon-Hydrogen Coupling Constants (Hz) in Emodin [**4**].[a]

Carbon	Chemical shift	Multiplicity	Coupling constants
1	161.2	bs[b]	—
2	123.7	dtq	164.4, *7.3*, *5.0*
3	147.9	q	**5.9**
4	120.1	ddq	164.3, *7.3*, *5.0*
4a	132.4	s[c]	—
5	108.6	dd	166.5, *5.0*
6	165.4	bs	—
7	107.6	dm[d]	162.8
8	164.3	bs[e]	—
8a	108.6	bq[f]	—
9	189.3	s	—
9a	112.9	bq[c]	*4.4*
10	180.8	t	*4.4*
10a	134.7	s	—
Me	21.3	qt	127.6, *4.4*

[a]^1J values printed in roman type, ^2J values in **bold** type, ^3J values in *italics*; b is used to indicate broad and not clearly resolved.
[b]On irradiation of chelated OH: d, $^2J = \mathbf{4.4}$ Hz.
[c]Sharpened on irradiation of Me.
[d]On irradiation of chelated OH: dd, $^1J = 162.8$, $^3J = 4.4$ Hz.
[e]On irradiation of H-7: d, $^2J = \mathbf{5.9}$ Hz.
[f]On irradiation of chelated OH: t, $^3J = 5.9$ Hz.

obscureolivea var. *brunnea*, and *D. olivipes* from South America, species that have been placed close to *D. austroveneta* as noted earlier in this paper. The developing chemotaxonomic link between the Australian and South American species is made stronger by the fact that hypericin [**2**] has also been detected by tlc in these South American fungi (14). As no statement is made to the contrary, it is conceivable that the extraction and workup employed by Keller *et al.* (14) were carried out without protection from light and that much of their hy-

TABLE 2. ^{13}C Chemical Shifts and One-bond and Long Range Carbon-Hydrogen Coupling Constants (Hz) in Skyrin [**3**].[a]

Carbon	Chemical shift	Multiplicity	Coupling constants
1,1′	161.0	bs	—
2,2′	123.5	dm	162.9
3,3′	148.1	q	**5.9**
4,4′	120.4	dm	164.3
4a,4a′	131.2	s	—
5,5′	123.6	d	*5.9*
6,6′	164.6	bs	—
7,7′	107.2	dd	162.7, *6.6*
8,8′	164.3	t	**4.4**
8a,8a′	108.9	t	*5.0*
9,9′	189.5	s	—
9a,9a′	131.1	bm	—
10,10′	182.0	d	*4.4*
10a,10a′	133.2	s	—
Me	21.5	qt	127.6, *4.4*

[a]^1J values printed in roman type, ^2J values in **bold** type, ^3J values in *italics*; b is used to indicate broad and not clearly resolved.

pericin [**2**] arose as a consequence of photooxidation of protohypericin [**1**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were measured at 99.55 MHz with a JEOL JNM-FX 100 spectrometer or at 399.65 MHz on a JEOL JNM-GX 400 spectrometer for solutions in Me_2CO-d_6. ^{13}C-nmr spectra were measured at 100.40 MHz on a JEOL JNM-GX 400 spectrometer for solutions in DMSO-d_6. Electron impact (70 eV) mass spectra were obtained with a V.G. Micromass 7070F spectrometer. Ir spectra were obtained for samples as KBr discs using a Perkin-Elmer 983G spectrometer. Uv spectra were recorded with a Varian SuperScan 3 spectrometer for solutions in MeOH. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter.

COLLECTION, EXTRACTION, AND ISOLATION.—Mushrooms of *D. austroveneta* were collected from mixed *Eucalyptus* woodland near Gembrook, Victoria. Voucher specimens are lodged in the herbariums of the New South Wales Department of Agriculture, Chemical and Biological Research Station, Rydalmere, NSW, and the Royal Botanic Garden, Edinburgh, UK, under accession numbers DAR 55994 and WAT 19344, respectively. As far as possible, all subsequent operations were carried out in the absence of light or in subdued light. Fresh mushrooms (530 g) were homogenized with Me_2CO (2.5 liters) and extracted with a further quantity (2.5 liters) of the same solvent overnight. The combined Me_2CO extracts were evaporated to dryness under reduced pressure, and the residue (14 g) was partitioned between EtOAc and H_2O. The organic phase was dried ($MgSO_4$) and evaporated to leave a residue (5 g) that was dissolved in Me_2CO (500 ml) and filtered through a column of Celite (6 × 15 cm). Me_2CO was removed under reduced pressure, and the residue (1 g) was chromatographed on plates of Si gel (Merck Kieselgel 60 PF_{254}) using C_6H_6-EtOAc-HOAc-HCO_2H (12:6:1:1) as eluent. In this way the major orange pigment (R_f 0.80) was separated from a purple substance (R_f 0.54).

(+)-S-*Skyrin* [**3**].—The orange zone (R_f 0.80) (280 mg) was subjected to further preparative tlc on Si gel in C_6H_6-HCO_2Et-HCO_2H (10:5:3) and finally to gel filtration (Sephadex LH-20 suspended in MeOH). Crystallization from MeOH gave the pure pigment (50 mg) as red-orange plates, mp >340°; $[\alpha]^{25}D$ +100° (c = 0.117, dioxan); ^{13}C nmr see Table 2. Skyrin isolated from *D. austroveneta* proved chromatographically indistinguishable from an authentic sample.

Protohypericin [**1**].—The purple zone (R_f 0.54) was partitioned between EtOAc and a buffered aqueous solution (pH 7.4). The material so obtained was subjected first to preparative tlc in C_6H_6-HCO_2Et-HCO_2H (10:5:3) and then to gel filtration (Sephadex LH-20 suspended in MeOH) to afford protohypericin [**1**] (23 mg) as a deep purple powder (from MeOH), mp >340°, identical in chromatographic properties with an authentic sample.

REDUCTIVE CLEAVAGE OF SKYRIN [**3**].—To compound **3** (7 mg) in 2 M Na_2CO_3 solution (2 ml) was added dropwise an aqueous solution of sodium dithionite (1.5 g in 10 ml) until the color changed from red to orange. The mixture was acidified and extracted with Et_2O to afford emodin [**4**] identical in all respects with a commercial sample.

HYPERICIN [**2**].—*From protohypericin.*—Protohypericin (ca. 5 mg) was dissolved in Me_2CO-d_6 (0.5 ml) in the dark, and the solution was transferred to an nmr tube. After the ^{1}H-nmr spectrum (shown in part in Figure 1a) was recorded, the solution was exposed to sunlight for 30 sec, and the spectrum was again measured; after an accumulated exposure of 480 sec, the ^{1}H-nmr spectrum revealed the absence of protohypericin (Figure 1f). Evaporation of the solvent under reduced pressure and chromatographic purification of the residue on layers of Si gel using C_6H_6-HCO_2Et-HCO_2H (10:5:3) followed by gel filtration (Sephadex LH-20 suspended in MeOH) gave hypericin [**2**], mp >340°, identical in chromatographic behavior with an authentic sample.

From D. austroveneta.—A small quantity of *D. austroveneta* was homogenized with Me_2CO in the presence of light, and the extracts were worked up as described above but under normal laboratory light conditions. Si gel chromatography [C_6H_6-EtOAc-HCO_2H-HOAc (12:6:1:1)] gave skyrin [**3**] (R_f 0.80) and hypericin [**2**] (R_f 0.50). The hypericin so obtained was indistinguishable (uv, tlc, ^{1}H nmr) from an authentic specimen and from material obtained by photooxidation of protohypericin.

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