Pigments of Fungi. Part 11.[1,2] (+)-Austrocorticin, Austrocorticinic Acid, Austrocorticone, and Related Pigments; the First Naturally Occurring Anthraquinones Derived from a Propionate-triggered Octaketide

Melvyn Gill * and Alberto Gimenez
Department of Organic Chemistry, The University of Melbourne, Parkville, Victoria, Australia, 3052

The anthraquinones (**7**), (**9**), (**11**), (**13**), (**15**), and (**17**) have been isolated from the orange fruit bodies of an Australian toadstool belonging to the genus *Dermocybe*. Five of these six pigments bear a unique C_2 side chain at C-3 in the anthraquinone nucleus, the biogenetic origin of which has been studied by feeding ^{13}C labelled precursors to young toadstools. High specific incorporation of isotope from sodium [3-^{13}C]propionate into the C-3' methyl group of pigments (**7**), (**11**), (**13**), (**15**), and (**17**) indicates that a propionate 'starter' effect is operating. The sixth pigment (**9**) is derived in the same organism by 'normal' acetate-triggered octaketide assembly.

Anthraquinones form a large and important group of naturally occurring colouring matters distinguished by their wide distribution and structural diversity.[3] The majority of anthraquinone pigments isolated from plant and particularly from fungal sources have their origins in the polyketide pathway and these, in the main, are derived from octaketide progenitors.[4,5] Without exception† these octaketide-derived anthraquinones utilise acetate (as acetyl CoA) as the primer in their biosynthesis.[8] Thus, it is well known that the pigments emodin (**1**) and endocrocin (**2**) are both derived from an octaketide which is itself assembled from an acetate starter and seven malonate units as depicted in Scheme 1.[9] In the higher fungi, particularly those toadstools belonging to the genus *Cortinarius* and its allies, emodin and endocrocin give rise in turn to a wide variety of hydroxylated anthraquinones which are responsible for the yellow, orange, and red colours of the fruit bodies.[5] Feeding experiments have established that radiolabelled emodin is effectively incorporated into the anthraquinones dermoglaucin (**3**) and dermocybin (**4**) by *Dermocybe semisanguinea*, while endocrocin acts as a precursor to the anthraquinone carboxylic acids dermolutein (**5**) and dermorubin (**6**) (but not to the neutral pigments) in *Dermocybe sanguinea* (Scheme 1).[10] In the same vein, all other anthraquinones isolated so far from Basidiomycetes,[5] with the exception of a small group found in *Cortinarius* subgenus *Leprocybe*,[11] possess structures which betray a similar, intimate biogenetic relationship either to emodin (**1**) or to endocrocin (**2**), and consequently an origin in acetate-triggered octaketide assembly.

We report here full details of the characterisation from an Australian toadstool of six new anthraquinones of which five are distinguished by being the first naturally occurring endocrocin-type anthraquinones bearing a C_2 side chain (rather than a methyl group) at C-3 in the anthraquinone nucleus. The origin of this unique C_2 side chain has been established by ^{13}C-labelling experiments and the results suggest a hitherto unprecedented pathway by way of which propionate rather than acetate initiates octaketide assembly.

The toadstool in question represents a new, and as yet unnamed, taxon within the genus *Dermocybe*.‡ Fruit bodies grow in mixed *Eucalyptus* forest and are distinguished by their brilliant orange tones. The cap is vermillion, particularly in young robust sporophores, and the stipe is orange-yellow with bright orange ring zones and orange fibrils. Photographs of the fruit bodies have been published[13] but unfortunately there and elsewhere[14] the fungus has been identified erroneously with the European *Cortinarius cinnabarinus* Fr. [= *Dermocybe cinnabarina* (Fr.) Wünsche].

Ethanol extraction of the fresh fruit bodies gave a rich orange solution which was concentrated and partitioned between ethyl acetate and water. Little, if any, pigment remained in the aqueous phase. TLC revealed the presence in the organic phase of at least ten pigments, six of which were subsequently purified and characterised. Physical and chromatographic properties for these pigments are collected in Table 1.

The neutral pigments (**7**) and (**9**) were separated from the carboxylic acids (**11**), (**13**), (**15**), and (**17**) by distribution of the total extract between ethyl acetate and aqueous buffer solution (pH 7.4). The major pigment fraction remained in the organic phase and the constituents (**7**) and (**9**) were purified further by preparative TLC on silica gel and by permeation through Sephadex LH-20.

The principal pigment in the toadstool, austrocorticin (**7**), was assigned the molecular formula $C_{19}H_{14}O_7$ from the mass spectrum and combustion analysis data. With acetic anhydride and a trace of concentrated sulphuric acid austrocorticin forms the monoacetyl derivative (**8**), $C_{21}H_{16}O_8$. The presence of a hydroxyanthraquinone chromophore in (**7**) was indicated by long wavelength UV absorption at 430 nm (log ε 3.78), and was confirmed when addition of sodium hydroxide caused a bathochromic shift (to λ_{max} 510 nm) typical of a 1-hydroxyanthraquinone.[3] The IR spectrum of the pigment shows absorption due to a chelated hydroxy group at 3 435 cm^{-1} and contains, in addition to quinonoid carbonyl bands at 1 673 (free) and 1 634 cm^{-1} (chelated), a third carbonyl absorption at 1 760 cm^{-1} consistent with the presence of a γ-lactone ring.

The ^1H NMR spectrum of the anthraquinone (**7**) reveals methoxy proton singlets at δ 4.02 and 4.05, and three aromatic protons appearing as a pair of *meta* coupled doublets (δ 6.84 and 7.47, *J* 2.2 Hz) and a singlet (δ 7.73). The remainder of the ^1H NMR spectrum consists of a singlet at δ 14.21, characteristic

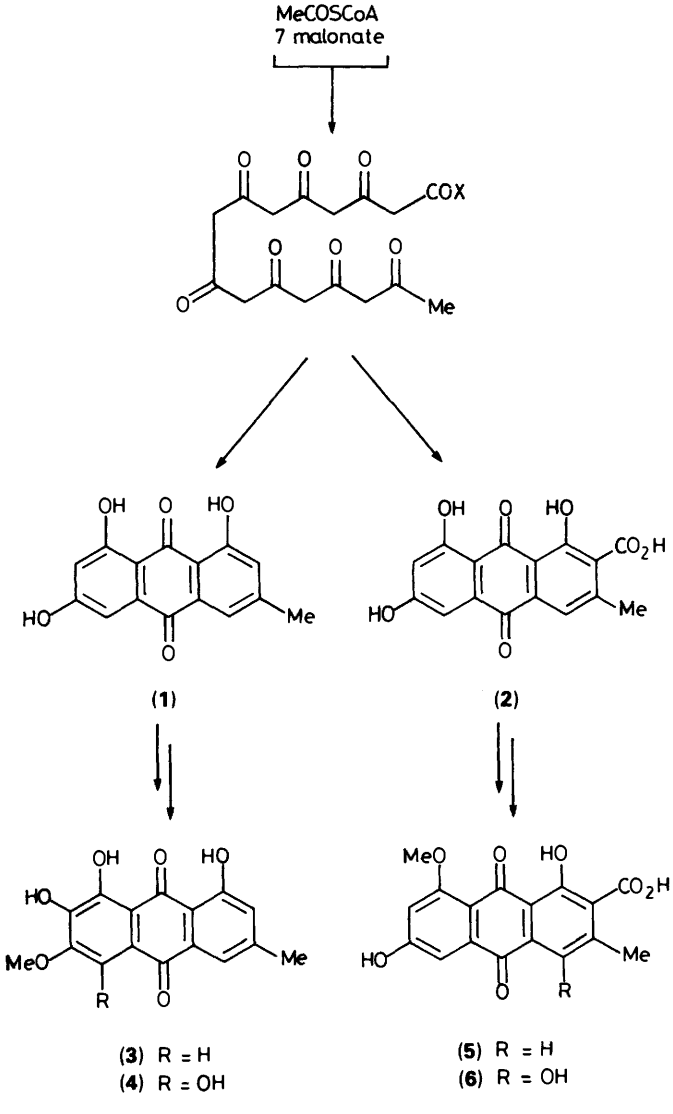
† It has been suggested[6] that the anthraquinone norsolorinic acid, the first enzyme-free intermediate involved in aflatoxin-B_1 biosynthesis, is derived from a hexanoate starter and seven malonates; this suggestion has been challenged.[7]

‡ Some mycologists regard *Dermocybe* as a genus on chemotaxonomic grounds,[12] others still treat *Dermocybe* as a subgenus of *Cortinarius*. In line with most recent chemical publications[5] we have elected here to refer to *Dermocybe* as a genus.

Table 1. Physical and chromatographic properties of toadstool pigments.

Pigment	Molecular formula	Form M.p./(°C)	Yield (%)[a]	TLC[b] R_F	Colour of pigment on TLC		
					Daylight	UV (366 nm)	$+NH_3$
(7)	$C_{19}H_{14}O_7$	Orange needles ($CHCl_3$–MeOH) 246–250	2.2×10^{-2}	0.28	Yellow	Bright red	Orange-brown
(9)	$C_{18}H_{12}O_7$	Orange needles ($CHCl_3$–MeOH) 250 (decomp.)	7.4×10^{-3}	0.28	Yellow	Bright red	Orange-brown
(11)	$C_{19}H_{16}O_7$	Yellow needles (EtOAc–light petroleum) 250 (soft), 280 (decomp.)	1.5×10^{-2}	0.35	Yellow	Bright red	Orange-brown
(13)	$C_{19}H_{16}O_8$	Red needles (EtOAc–HCO_2H) 254 (soft), 280 (decomp.)	1.3×10^{-2}	0.37	Red	Dark red	Violet
(15)	$C_{19}H_{14}O_8$	—	5.4×10^{-4}[c]	0.12	Yellow	Bright red	Orange-brown
(17)	$C_{19}H_{14}O_9$	—	1.4×10^{-3}[c]	0.18	Red	Dark red	Violet

[a] Based on fresh weight of fungus. [b] Merck Kieselgel 60 F254 precoated plates; solvent mixture A. [c] Yield based on isolated weight of the corresponding methyl ester.



Scheme 1. Biosynthesis of anthraquinones in *Cortinarius*.

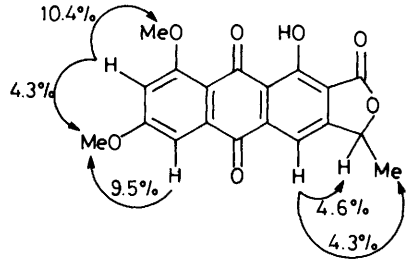
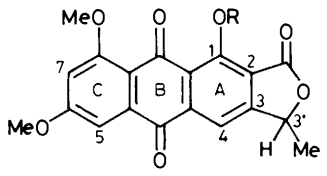


Figure 1. Nuclear Overhauser enhancement in the ^1H NMR spectrum of austrocorticin (7).

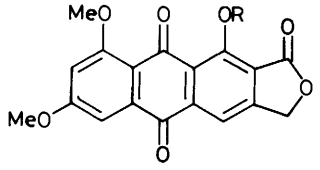
of a chelated hydroxy group, and a couplet (δ 5.55, 1 H, q, *J* 6.5 Hz; δ 1.69, 3 H, d, *J* 6.5 Hz) which identifies a Me-CH-O moiety. The data discussed so far are fully accommodated by, but do not uniquely define, the structure (7) for austrocorticin.

The relative disposition of substituents around the anthraquinone nucleus in (7) was deduced along the following lines. Nuclear Overhauser enhancement (NOE) experiments, the results of which are summarised in Figure 1, place both methoxyls together with the *meta* disposed aromatic protons in the same peripheral ring (ring C). Consequently, the isolated aromatic proton (δ 7.73) and the hydroxy group (δ 14.21) must be located in ring A and, furthermore, the hydroxy group by virtue of chelation must occupy a position *peri* to one of the quinonoid carbonyl groups. To complete the molecular formula ring A must abut the γ-lactone moiety. The connectivity between rings A–C in (7) is evident from the fully proton coupled ^{13}C NMR spectrum of austrocorticin (Table 2) in which one of the quinonoid carbonyl carbons (C-10) appears as a triplet (δ 181.3, *J* 4.0 Hz) due to three bond coupling to two *peri* hydrogens (4-H and 5-H). This interpretation is in full accord with the results of specific proton decoupling experiments and two dimensional heteronuclear correlation spectroscopy.

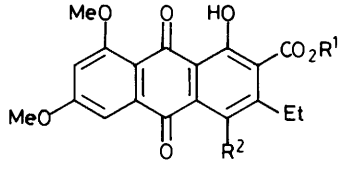
The *para* relationship between the free phenolic hydroxy group and the isolated aromatic proton (4-H) in ring A is consistent with a significant increase in the chemical shift of 4-H ($\Delta\delta$ + 0.44) on acetylation of austrocorticin (7).[15] Finally, the orientation of the γ-lactone with respect to ring A is evident from the appearance of the lactone carbonyl carbon atom as a singlet (δ 165.9) in the fully coupled ^{13}C NMR spectrum; the alternative ring fusion would render this same carbon atom prone to three bond coupling with 4-H which is not observed.



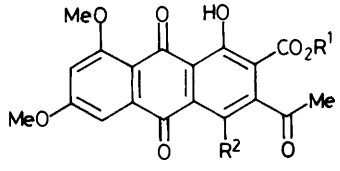
(7) R = H
(8) R = Ac



(9) R = H
(10) R = Ac



(11) R^1 = H, R^2 = H
(12) R^1 = Me, R^2 = H
(13) R^1 = H, R^2 = OH
(14) R^1 = Me, R^2 = OH



(15) R^1 = H, R^2 = H
(16) R^1 = Me, R^2 = H
(17) R^1 = H, R^2 = OH
(18) R^1 = Me, R^2 = OH

Conclusive proof that the Me-CH-O group and not the lactone carbonyl is adjacent to 4-H was obtained from the results of NOE enhancement (Figure 1) which clearly show that both the C-3′ methine proton (δ 5.55) and the protons of the C-3′ methyl group (δ 1.69) are in close spacial proximity to the aromatic proton at C-4 (δ 7.73).

Austrocorticin (**7**) and its acetate (**8**) are optically active although, as yet, the absolute configuration is not known.

Noraustrocorticin (**9**), the second yellow pigment from the neutral extractives from the fungus, is obtained free from austrocorticin (**7**) only with difficulty. The two anthraquinones are indistinguishable by TLC on silica gel in a number of solvent systems but have been separated by gel permeation through a long, narrow column (2 × 0.03 m) of Sephadex-LH20 using methanol. An alternative and more convenient procedure involves conversion to the respective acetyl derivatives (**8**) and (**10**) which are more soluble and sharply separated from each other on Sephadex gel. Brief exposure of the individual acetates (**8**) and (**10**) to dilute aqueous pyridine smoothly regenerated the corresponding natural products (**7**) and (**9**). With the exception of those resonances in the ^{1}H and ^{13}C NMR spectra of austrocorticin (**7**) and noraustrocorticin (**9**) which reflect the presence in the former of the C-3′ methyl group and its replacement in the latter with a hydrogen atom, the spectroscopic properties of (**7**) and (**9**) are very similar (Tables 2 and 3, and Experimental), and complementary arguments for the pattern of substitution around the anthraquinone nucleus in the second neutral pigment (**9**) as in the first are applicable.

The presence of a γ-lactone moiety linearly fused to an anthraquinone nucleus as is found in the pigments (**7**) and (**9**) is without precedent in nature.

Four anthraquinones, two yellow and two red, have been characterised from the 'acidic' fraction of the toadstool extractives (Table 1). The pigments (**11**) and (**13**) were separated from their more polar counterparts (**15**) and (**17**) by preparative TLC on silica gel. In turn, the pigments (**11**) and (**13**) were separated one from the other by column chromatography on acetylated polyamide resin. A faster moving yellow band gave austrocorticinic acid (**11**) which possesses the same anthraquinone chromophore as austrocorticin (**7**) and a molecular formula, $C_{19}H_{16}O_7$, which reveals an increase in the number of hydrogens by two. The IR spectrum shows (non-quinonoid) carbonyl absorption at 1 715 cm^{-1} suggesting that the γ-lactone moiety in (**7**) is replaced in (**11**) by a free carboxylic acid group. This is supported by the formation of the methyl ester (**12**) (ν_{max} 1 716 cm^{-1}) on brief exposure of austrocorticinic acid to an excess of ethereal diazomethane. The location of the carboxyl group at C-2 in ring A and the nature and positions of the other substituents in the anthraquinone nucleus of (**11**) followed from NMR experiments including NOE studies similar to those performed with austrocorticin (**7**). These studies place two methoxyl (δ 3.98 and 4.01) and two *meta* coupled aromatic protons (δ 6.77 and 7.43, *J* 2.2 Hz) in the same peripheral ring (ring C) in austrocorticinic acid. The ^{1}H NMR spectrum further identified a chelated hydroxyl (δ 13.17), an isolated aromatic proton (δ 7.63), and an ethyl group (δ 2.75, 2 H, q, *J* 7.3 Hz; δ 1.31, 3 H, t, *J* 7.3 Hz) as the remaining substituents in ring A of (**11**). The location of the aromatic proton at C-4 and hence the establishment of the connectivity between the rings A and C through the quinonoid ring B followed from the appearance of the C-10 carbonyl resonance as a triplet (δ 182.4, *J* 4.4 Hz) in the ^{13}C NMR spectrum. Since the hydroxy group is chelated with a quinonoid carbonyl group it must necessarily flank C-9. Finally, mutual NOE between 4-H and the methylene protons of the ethyl group place the C_2 side chain at C-3, and hence the carboxy group at C-2, in (**11**). The ^{13}C NMR spectrum of austrocorticinic acid (Table 2) and its ester (**12**) (Experimental) are in full accord with structure (**11**) for the natural product.

A slower moving red band gave 4-hydroxyaustrocorticinic acid (**13**) which from its molecular formula, $C_{19}H_{16}O_8$, contains one oxygen atom more than austrocorticinic acid. The incorporation of this atom into a hydroxy group and the location of this group at C-4 in (**13**) was deduced from the ^{1}H NMR and electronic spectra. Thus, whereas signals due to the presence of methoxy groups at C-6 and C-8, and to chelated hydroxy, carboxy, and ethyl groups at C-1, C-2, and C-3, respectively, correspond to signals in the spectrum of (**11**) (Table 3), no resonance attributable to 4-H is observed in the ^{1}H NMR spectrum of the red pigment (**13**). Instead it is replaced by a low field signal (δ 13.66) which may be assigned to a second chelated hydroxyl. Consequently, the ^{13}C NMR spectrum of (**13**) now reveals the C-10 carbonyl as a doublet (δ 186.7, *J* 4.4 Hz), while the long wavelength region of the UV spectrum (λ_{max} 473, 498, and 534 nm) is in accord with a quinizarin chromophore.[3] With diazomethane (**13**) yields the methyl ester (**14**). All aspects of the ^{13}C NMR spectra of (**13**) (Table 2) and the ester (**14**) (Experimental) are fully consistent with the assigned structures.

Table 2. ^{13}C NMR chemical shifts and coupling constants (δ/ppm, relative to Me_4Si in $CDCl_3$).[a]

	(7)	(9)	(11)[b]	(13)[b]	(18)[c]
C-1	161.9 (d, 5.9)	162.2 (d, 5.9)	159.8 (br s)	153.6 (br s)	153.5
C-2	117.4 (t, 5.9)	117.5 (t, 5.9)	127.3 (m)	132.3 (t, 5.1)	131.8
C-3	158.8 (br s)	154.5 (dd, 5.2, 4.4)	150.8 (sextet, 5.2)	141.9 (septet, 5.4)	136.6
C-3′	77.0 (dm, 154.3)	69.0 (td, 154.1, 2.9)	27.4 (tp, 129.0, 4.4)	21.7 (tq, 130.6, 4.4)	199.4
3′-Me	19.9 (q, 130.0)	—	14.8 (qt, 127.6, 5.1)	13.5 (qt, 129.0, 5.9)	31.5
C-4	110.5 (d, 168.7)	111.0 (d, 168.5)	119.1 (dt, 165.8, 5.9)	155.9 (q, 4.9)	153.9
C-4a	137.4 (br s)	137.5 (br s)	133.0 (br s)	112.6 (d, 4.4)	115.0
C-5	104.7 (dd, 159.2, 4.4)	104.7 (dd, 159.0, 4.4)	104.7 (dd, 167.2, 4.4)	104.4 (dd, 167.3, 4.4)	104.1
C-6	165.9 (m)	166.0 (m)	165.8 (m)	165.9 (m)	165.1
C-7	105.0 (dd, 167.3, 4.1)	105.2 (dd, 167.5, 4.1)	104.8 (dd, 161.3, 4.4)	104.9 (dd, 160.0, 5.1)	105.3
C-8	163.5 (m)	163.6 (m)	163.2 (pentuplet, 2.9)	163.3 (qd, 4.4, 2.9)	163.6
C-8a	114.5 (t, 5.9)	114.7 (t, 5.9)	114.5 (t, 5.1)	114.8 (t, 5.1)	115.5
C-9	186.3 (s)	187.3 (s)	187.1 (s)	185.6 (s)	185.5
C-9a	117.8 (t, 5.9)	117.9 (t, 5.9)	114.9 (d, 5.9)	111.9 (br s)	114.4
C-10	181.3 (t, 4.0)	182.3 (t, 4.0)	182.4 (t, 4.4)	186.7 (d, 4.4)	187.1
C-10a	136.9 (br s)	136.2 (br s)	137.2 (br s)	137.1 (br s)	136.9
6-OMe	56.0 (q, 145.2)	56.2 (q, 145.2)	56.1 (q, 145.3)	56.0 (q, 145.3)	56.2
8-OMe	56.3 (q, 145.2)	56.8 (q, 145.2)	56.5 (q, 145.3)	56.0 (q, 145.3)	56.5
CO_2R	165.9 (lactone) (s)	167.2 (lactone) (s)	171.4 (R = H) (s)	170.1 (R = H) (s)	165.8 (R = Me)
CO_2Me	—	—	—	—	53.0

[a] Chemical shifts are from proton noise decoupled spectra. Coupling constants were measured in fully proton coupled spectra. Assignments have been corroborated by two dimensional heteronuclear correlation spectroscopy: direct correlation (140 Hz) 2 000 data points × 64 and 340 scans; long range correlation (10 Hz) 8 000 data points × 128 and 680 scans; both with pulse delay plus acquisition time 2.0 s. [b] Solvent $CDCl_3$ + 1 drop HCO_2H. [c] Insufficient material to measure the fully proton coupled spectrum; assignments made by analogy with spectra of closely related compounds.

Table 3. ^1H NMR chemical shifts and coupling constants (δ/ppm, relative to Me_4Si in $CDCl_3$).

	(7)	(9)	(11)	(13)[a]	(16)	(18)
3′-H	5.55 (q, 6.5)	5.39 (s)	2.75 (q, 7.3)	2.76 (q, 7.5)	—	—
3′-Me	1.69 (d, 6.5)	—	1.31 (t, 7.3)	1.25 (t, 7.5)	2.69 (s)	2.69 (s)
4-H	7.73 (s)	7.73 (s)	7.63 (s)	—	8.13 (s)	—
5-H	7.47 (d, 2.2)	7.47 (d, 2.5)	7.43 (d, 2.2)	7.45 (d, 2.5)	7.50 (d, 2.2)	7.54 (d, 2.5)
7-H	6.84 (d, 2.2)	6.83 (d, 2.5)	6.77 (d, 2.2)	7.02 (d, 2.5)	6.83 (d, 2.2)	6.85 (d, 2.5)
6-OMe	4.02 (s)	4.02 (s)	3.98 (s)	4.03 (s)	4.03 (s)	4.02 (s)
8-OMe	4.05 (s)	4.05 (s)	4.01 (s)	4.05 (s)	4.05 (s)	4.05 (s)
1-OH	14.21 (s)	14.23 (s)	13.17 (s)	13.20 (s)	13.39 (s)	13.14 (s)
4-OH	—	—	—	13.66 (s)	—	13.61 (s)
CO_2Me	—	—	—	—	4.00 (s)	3.94 (s)

[a] Solvent [$^2H_6$]acetone.

Austrocorticone (**15**) and its 4-hydroxy analogue (**17**) are the most polar pigments to be characterised from the toadstool extractives. They were separated collectively from the pigments (**11**) and (**13**) by preparative TLC but proved extremely difficult to separate from each other principally due to their acute insolubility in common organic solvents. However, exposure of a mixture of (**15**) and (**17**) to an excess of ethereal diazomethane gave the methyl esters (**16**) and (**18**) which proved readily soluble and hence conveniently separable by silica gel chromatography. Consequently, all subsequent work was carried out on the methyl ester derivatives of these natural products.

The methyl ester (**16**) of austrocorticone is the more mobile and was ascribed the formula $C_{20}H_{16}O_8$ from high resolution mass spectroscopic data alone since insufficient material was available for combustion analysis.

A close structural relationship between (**16**) and austrocorticinic acid (**11**) was evident from the similarities of their respective molecular formulae, their near superimposable electronic spectra, and the observation of many signals common to the ^1H NMR spectra of both yellow pigments. Thus, the ^1H NMR spectrum of (**16**) reveals the presence of two methoxyls (δ 4.03 and 4.05) and two *meta* coupled aromatic protons (δ 6.83 and 7.50, *J* 2.2 Hz), a chelated hydroxy group (δ 13.39), and an isolated aromatic proton (δ 8.13). The remainder of the ^1H NMR spectrum comprises two, three proton singlets, one at δ 4.00 which confirms the presence of a methyl ester, and a second at δ 2.69 which strongly suggested replacement of the ethyl side chain in (**11**) by an acetyl group in austrocorticone (**15**).

The less mobile, red pigment may be assigned the structure (**18**) corresponding to the methyl ester of 4-hydroxyaustrocorticone (**17**), principally by comparison of the molecular formula, $C_{20}H_{16}O_9$ (high resolution mass spectrometry), and spectroscopic data with those of the esters (**14**) and (**16**) of 4-hydroxyaustrocorticinic acid and austrocorticone, respectively. Thus, in common with the ^1H NMR spectrum of (**14**) this second red pigment exhibits two methoxy signals (δ 4.02 and 4.05), (only) two aromatic proton resonances (δ 6.85 and 7.54, *J* 2.5 Hz), and two low field, chelated hydroxyl signals (δ 13.14 and 13.61). The remaining signals may be compared with the data for (**16**) in that they reveal the presence of a methyl ester (three proton singlet at δ 3.94) and an acetyl group (three proton singlet at δ 2.69) in (**18**). The presence of a quinizarin chromophore is consistent with the electronic spectrum (λ_{max} 458, 485, and 520 nm).

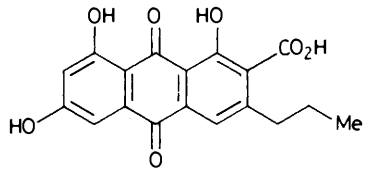
The ^{13}C NMR spectrum of the ester (**18**) exhibits four signals

which may be assigned to carbonyl groups. Thus, quinonoid carbons appear at δ 187.1 and 185.5, a carbonyl at δ 165.8 is in line with the chemical shift of the corresponding carbons in the esters (**12**) (δ 167.1) and (**14**) (δ 166.2), and a fourth resonance at δ 199.4 confirms the presence of the acetyl group. The remainder of the ^{13}C NMR spectrum of ester (**18**) (Table 2) is fully consistent with the assigned structure in which the C_2 side chain is at the oxidation level of an acetyl group.

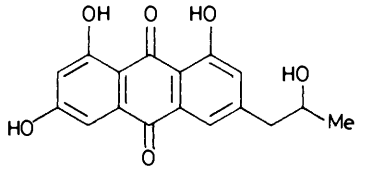
The toadstool pigments (**7**), (**11**), (**13**), (**15**), and (**17**) are the first endocrocin-type anthraquinones to be reported which contain a C_2 side chain at C-3 in the nucleus. Only one other analogue of endocrocin (**2**) is known in which the methyl group at C-3 is replaced by a longer side chain. This pigment, ptilometric acid (**19**), occurs in echinoderms[16] as do several homologues of emodin such as isorhodoptilometrin (**20**)[17] and the quinone (**21**)[18] which presumably arise by way of a similar biosynthesis to that giving rise to ptilometric acid. While details of this biosynthesis are not known it is significant that each of these pigments falls rationally into the polyketide pattern and, furthermore, may be assumed to originate, like endocrocin itself, from precursors assembled from an acetate starter and an appropriate number of malonate units.

In contrast, the structures deduced here for this new group of toadstool pigments each incorporating a two carbon side chain raise immediate questions concerning their biosynthesis. Of particular moment is the source of the C-3′ methyl group. Two possible modes of biogenesis for the major pigment austrocorticin (**7**) are depicted in Scheme 2. Of these, route *a* would involve 'normal' acetate-triggered octaketide assembly followed at some stage by hydroxylation of the methyl group of the starter-acetate unit to provide a phthalide of the type (**9**). According to this route C-methylation of (**9**), for example by *S*-adenosylmethionine, would subsequently lead to austrocorticin (**7**). Following route *a* austrocorticinic acid (**11**) and austrocorticone (**15**) would presumably arise by reductive and by oxidative cleavage, respectively, of the γ-lactone ring in (**7**). We were initially encouraged to support this pathway by our isolation of noraustrocorticin (**9**) as a cometabolite of the C_2 side chain quinones in this fungus. Alternatively, propionate-triggered octaketide assembly (Scheme 2, route *b*) and subsequent hydroxylation at C-2 of the propionate starter unit would explain the origin of the C-3′ methyl group in that it too would lead eventually to austrocorticin. Whereas the latter route held appeal in that the putative sequence austrocorticinic acid ⟶ austrocorticin ⟶ austrocorticone would necessitate a familiar series of sequential oxidations at the benzylic (C-3′) position, it presented in the nature of the starter unit a pathway without precedent in octaketide biosynthesis.

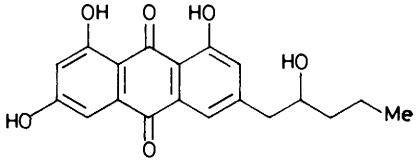
In order to differentiate these pathways [Me-^{13}C]methionine



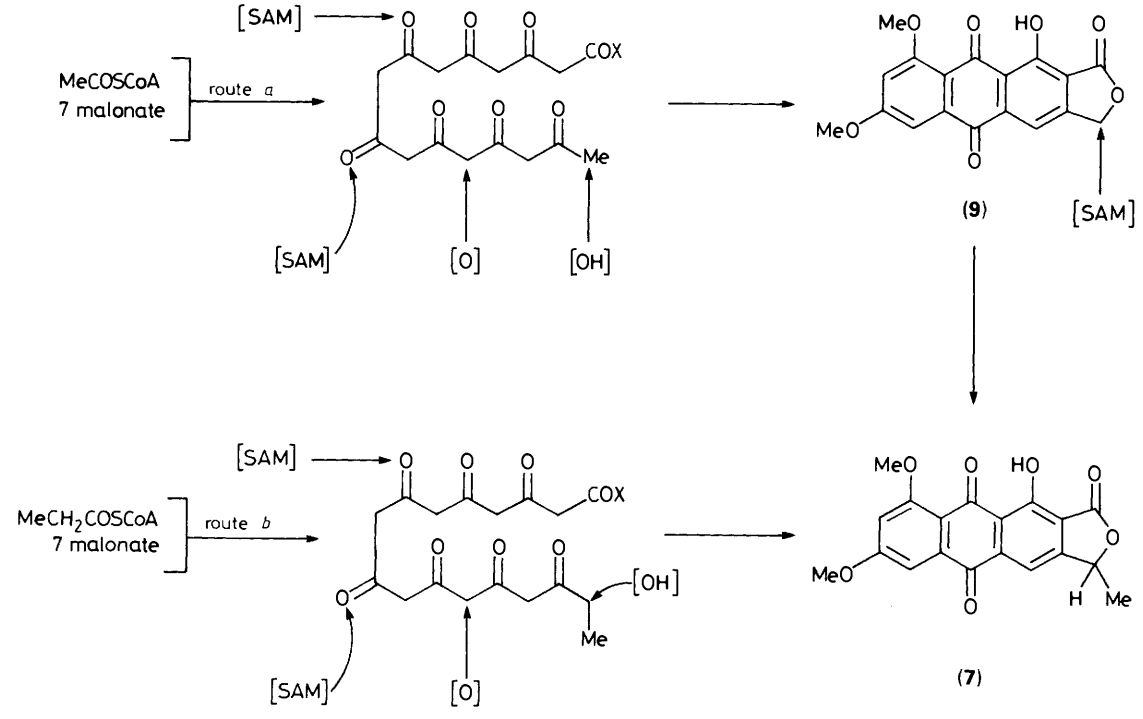
(**19**)



(**20**)



(**21**)



Scheme 2. Possible pathways for the biogenesis of austrocorticin (**7**).

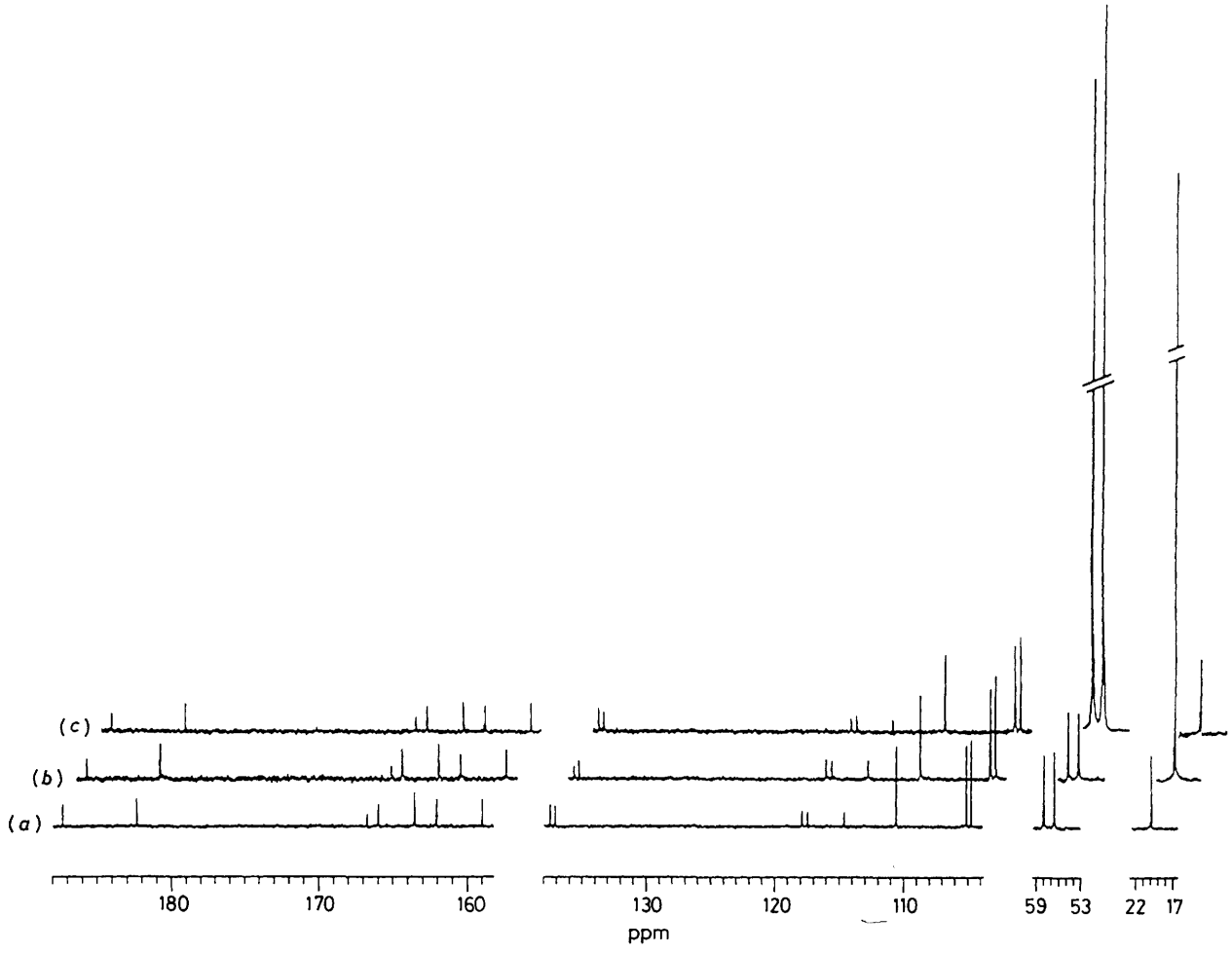


Figure 2. ^{13}C NMR spectrum of austrocorticin (**7**): (*a*) natural abundance; (*b*) enhanced by incorporation of sodium [3-^{13}C]propionate; and (*c*) enhanced by incorporation of [Me-^{13}C]methionine.

Table 4. Enrichment in ^{13}C content as a result of feeding [Me-^{13}C]-methionine.

Pigment	Atom% enrichment[a]	
	6-OMe	8-OMe
(**7**)	31	30
(**9**)	32	34
(**11**)	29	29
(**13**)	29	29
(**16**)	24	24
(**18**)	24	24

[a] Refers to atom% enrichment over and above natural abundance (1.1%). Measured by integration of the ^{13}C satellite bands (J_{CH} *ca.* 145 Hz) flanking the near coincident C-6 and C-8 OMe resonances close to δ 4.0 in the ^1H NMR spectra.

and sodium [3-^{13}C]propionate were administered individually by syringe to groups of toadstools growing in their natural habitat. After several days the fruit bodies were harvested and the pigments were isolated and purified as described above.

Efficient assimilation of methionine during the feeding experiment was immediately apparent from the ^{13}C NMR spectrum of enriched austrocorticin when this was compared with the natural abundance spectrum (Figure 2). The incorporation of label into the methoxy groups at C-6 and C-8 in (**7**) was quantified by integration of the ^{13}C satellites (J_{CH} 145.2 Hz) flanking the near coincident C-6 and C-8 OMe resonances at δ 4.02 (31 atom% enrichment over and above natural abundance) and 4.05 (30%) respectively, in the ^1H NMR spectrum of (**7**).[19] Similar levels of incorporation of methionine took place into the OMe groups of pigments (**9**), (**11**), (**13**), (**15**),* and (**17**)* (Table 4) thereby firmly establishing the involvement of *S*-adenosylmethionine in the biosynthesis of these anthraquinones. Significantly, no enrichment of the C-3′ methyl group in (**7**), or in the pigments (**11**), (**13**), (**15**), and (**17**) took place during the same experiment. In marked contrast, those fruit bodies which had been fed sodium [3-^{13}C]propionate afforded, after extraction and purification, austrocorticin (**7**) which by ^{13}C NMR spectroscopy (Figure 2) could clearly be seen to be enriched exclusively at the C-3′ methyl group. An enrichment of 14 atom% above natural abundance was determined by integration of the ^{13}C satellites (J_{CH} 130.0 Hz) flanking the C-3′ methyl resonance at δ 1.69 in the ^1H NMR spectrum of (**7**). Correspondingly high incorporation was also observed into the terminal methyl group of the C_2 side chain of pigments (**11**) and (**13**), and into the acetyl group of pigments (**15**)* and (**17**)* (Table 5). Thus, route *b* in Scheme 2 in which propionate initiates octaketide formation is established for the biosynthesis of the anthraquinones (**7**), (**11**), (**13**), (**15**), and (**17**).

* Isolated as the corresponding methyl ester.

Table 5. Enrichment in ^{13}C content as a result of feeding sodium [3-^{13}C]propionate.

Pigment	Atom% enrichment at C-3′ methyl group[a]	
	From ^1H NMR[b]	From ^{13}C NMR[c]
(**7**)	14	13
(**11**)	12	15
(**13**)	10	11
(**16**)	16	—
(**18**)	13	—

[a] Refers to atom% enrichment over and above natural abundance (1.1%). [b] Measured by integration of the ^{13}C satellite bands (J_{CH} *ca.* 130 Hz) flanking the C-3′ methyl resonance. [c] Measured from the proton noise decoupled ^{13}C NMR spectrum by comparing peak heights in the enriched and natural abundance spectra after normalisation.

These pigments represent the first naturally occurring anthraquinones to be derived *via* a propionate-triggered octaketide. Propionate is well known to initiate decaketide formation during anthracyclinone biosynthesis in various *Streptomycetes* species[20] and several anthraquinones have been isolated from blocked mutants of anthracyclinone producing strains of *S. galilaeus*[21] and *S. coerulcorubidus*[22] to which propionate-triggered nona- and decaketide origins may be presumed, although this has not yet been verified experimentally. Nevertheless, a propionate initiator has, hitherto, never been observed in the octaketide series.

A final feeding experiment involving the administration of sodium [2-^{13}C]acetate was carried out in order to elucidate the relationship between noraustrocorticin (**9**) and its homologous cometabolites. Owing to a dearth of healthy fruit bodies at the time that this experiment was executed the amounts of pigments available after extraction and purification was strictly limited, in the case of the minor pigments (**11**), (**13**), (**15**), and (**17**) to trace amounts. However, a sufficient quantity of a mixture of the major neutral pigments (**7**) and (**9**) was obtained to record the ^{13}C NMR spectrum. From this it could be ascertained that whereas label was not incorporated to any discernible extent into the C-3′ carbon atom in (**7**) (in full accord with the results of propionate feeding experiments discussed above), a significant enrichment in label (9 atom%; from comparison of the ^{13}C spectra of enriched and natural abundance material after normalisation) was observed at C-3′ in noraustrocorticin (**9**). The pigment (**9**) may therefore be assumed to arise by way of a 'regular' acetate-triggered octaketide pathway which operates in this organism in tandem with the predominant propionate-triggered pathway.

Incorporation of label from sodium [2-^{13}C]acetate into the anthraquinone nucleus of austrocorticin (**7**) and noraustrocorticin (**9**) is clearly discernible in the ^{13}C NMR spectrum of the mixture of these metabolites but has, to date, proved difficult to quantify accurately. Nevertheless, levels of enrichment ranging from 0.2–1.5 atom% occur specifically at C-2, C-4, C-5, C-7, C-8a, C-9a, and C-10 in (**7**) and (**9**) and this distribution is entirely consistent with the formation of these pigments, after initiation, by head-to-tail linkage of acetate units.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, IR spectra as potassium bromide discs on a Perkin-Elmer 983G spectrophotometer, UV spectra on a Varian SuperScan 3 spectrophotometer, ^1H and ^{13}C NMR spectra on a JEOL-JNM-GX 400 spectrometer operating at 399.65 MHz (^1H) and 100.40 MHz (^{13}C) for solutions in deuteriochloroform unless stated otherwise, mass spectra on a V.G. Micromass 7070F spectrometer (e.i. probe, 70 eV), and optical rotations on a Perkin-Elmer 241MC polarimeter. All TLC and preparative TLC (PLC) was performed on Merck Kieselgel 60 GF254, using either toluene–ethyl formate–formic acid (10:5:3) (solvent mixture A) or toluene–ethyl formate–formic acid (50:49:1) (solvent mixture B) as eluant. Column chromatography used Macherey Nagel Polyamide 6Ac resin in acetone. Gel permeation chromatography employed Pharmacia Sephadex LH-20 in methanol. Solutions were routinely dried over Na_2SO_4 prior to concentration under reduced pressure (water bath, 35 °C).

Voucher specimens are lodged in the herbariums of the Royal Botanic Garden, Edinburgh, UK, and the New South Wales Department of Agriculture Chemical and Biological Research Station, Rydalmere, NSW, Australia under accession numbers WAT 19352 and DAR 61417, respectively.

Isolation of Anthraquinones (**7**), (**9**), (**11**), (**13**), (**15**), and (**17**).—Fresh toadstools (95 g) collected in the Kinglake National Park, Victoria, during June 1987 were homogenised and macerated in ethanol (750 ml) at room temperature for 16 h. The orange extract was evaporated under reduced pressure and the residue partitioned between ethyl acetate (3 × 100 ml) and aqueous buffer solution [200 ml, pH 7.4, $(NH_4)_2SO_4$ (4 g), K_2HPO_4 (7.6 g)]. The organic and aqueous phases were separated and purified individually.

The Organic Phase.—This was evaporated to dryness under reduced pressure and the orange residue (0.24 g) was chromatographed (PLC solvent mixture A) to afford a predominant yellow fraction (28 mg, R_F 0.28, solvent mixture A). This fraction was dissolved in methanol, applied to a column (0.03 × 2 m) of Sephadex LH-20 gel and eluted with the same solvent to afford two yellow zones which were collected separately and evaporated to dryness. The faster moving zone gave *austrocorticin* (**7**) (21 mg) (Found: C, 64.7; H, 4.0. $C_{19}H_{14}O_7$ requires C, 64.45; H, 4.0%); $[\alpha]_D^{22}$ +59° (*c* 0.25 in $CHCl_3$); ν_{max} 3 435 (OH), 1 760 (γ-lactone), 1 673 (conj. CO), 1 634 (conj. chelated CO), 1 594, 1 330, and 1 275 cm^{-1}; λ_{max}(EtOH) 227 (log ε 4.40), 232sh (4.32), 282 (4.19), 350sh (3.20), and 430 nm (3.78); λ_{max}(EtOH + OH^-) 510 nm (log ε 3.66); *m/z* 354 (M^+, 81%), 336 (100), 308 (40), and 280 (14).

The slower moving zone gave *noraustrocorticin* (**9**) (7 mg) (Found: M^+, 340.0583. $C_{18}H_{12}O_7$ requires *M*, 340.0583); ν_{max} 3 446, 1 776, 1 666, 1 643, 1 593, 1 330, and 1 277 cm^{-1}; λ_{max} (EtOH) 214 (log ε 4.56), 228sh (4.30), 383 (3.97), 350sh (3.23), 430 (3.55), and 448sh nm (3.50); λ_{max}(EtOH + OH^-) 500 nm (log ε 3.35); *m/z* 340 (M^+, 34%), 322 (100), 296 (26), 266 (17), and 15 (71).

The Aqueous Phase.—This was acidified using aqueous phosphoric acid (8 ml; 85%, v/v) and the constituents extracted into ethyl acetate (2 × 100 ml). Evaporation of the solvent gave a red solid (0.15 g) which was chromatographed (PLC, solvent mixture A) to afford two fractions.

(*a*) The first was a red powder (35 mg, R_F 0.35–0.37, solvent mixture A) which was dissolved in acetone and applied to a column (2.8 × 50 cm) of acetylated polyamide resin. Elution with the same solvent gave *austrocorticinic acid* (**11**) (14 mg) (Found: C, 64.3; H, 4.6. $C_{19}H_{16}O_7$ requires C, 64.05; H, 4.5%); ν_{max} 3 441, 1 715, 1 666, 1 625, 1 593, 1 360, and 1 246 cm^{-1}; λ_{max}(EtOH) 210 (log ε 4.76), 225 (4.57), 274 (4.43), 307sh (3.97), 423 (3.71), and 493sh nm (3.68); λ_{max}(EtOH + OH^-) 520 nm (log ε 3.78); *m/z* 356 (M^+, 20%), 338 (52), 310 (42), and 15 (100). After austrocorticinic acid (**11**) had completely eluted from the column the solvent was modified by addition of formic acid (1%, v/v). Continued elution gave 4-*hydroxyaustrocorticinic acid* (**13**) (12 mg) (Found: C, 61.3; H, 4.6. $C_{19}H_{16}O_8$ requires C, 61.3;

H, 4.3%); ν_{max} 3 443, 1 730, 1 681, 1 588, 1 398, 1 319, and 1 228 cm^{-1}; λ_{max}(EtOH) 228 (log ε 4.28), 277 (4.25), 315 (3.79), 473sh (3.88), 498 (3.93), and 534 nm (3.73); λ_{max}(EtOH + OH^-) 520sh (log ε 3.74), 553 (4.00), and 591 nm (3.92); *m/z* 372 (M^+, 56%), 354 (100), 326 (49), and 15 (41).

(*b*) The second fraction was a red powder (5.2 mg, R_F 0.12–0.18, solvent mixture A) which was insoluble in common organic solvents. The powder was suspended in chloroform (2 ml) and treated with an excess of ethereal diazomethane at 0 °C for 1 min whereupon the excess reagent was destroyed by dropwise addition of acetic acid. Removal of the solvent gave a red solid which was chromatographed (PLC, solvent mixture B) to afford a more mobile yellow zone (R_F 0.70, solvent mixture B) and a less mobile red zone (R_F 0.60, solvent mixture B). The yellow zone gave *austrocorticone methyl ester* (**16**) as orange granular microcrystals (0.5 mg), m.p. 235–238 °C from ethyl acetate–light petroleum (b.p. 40–60 °C) (Found: M^+, 384.0844. $C_{20}H_{16}O_8$ requires *M*, 384.0845); ν_{max} 3 435, 1 715, 1 679, 1 630, 1 590, 1 360, and 1 238 cm^{-1}; λ_{max}(EtOH) 215 (log ε 4.62), 280 (3.85), and 490 nm (3.61); λ_{max}(EtOH + OH^-) 510 nm (log ε 3.30); *m/z* 384 (M^+, 17%), 352 (36), 296 (30), 32 (20), and 28 (100). The red zone yielded *4-hydroxyaustrocorticone methyl ester* (**18**) as red needles (1.3 mg), m.p. 225–228 °C from ethyl acetate–light petroleum (b.p. 40–60 °C) (Found: M^+, 400.0793. $C_{20}H_{16}O_9$ requires *M*, 400.0794); ν_{max} 3 452, 1 720, 1 655, 1 592, 1 396, 1 310, and 1 225 cm^{-1}; λ_{max}(EtOH) 216 (log ε 4.93), 288sh (4.31), 279 (4.00), 458sh (3.66), 485 (3.76), and 520sh nm (3.62); λ_{max}(EtOH + OH^-) 560sh (log ε 3.66) and 604 nm (3.73); *m/z* 400 (M^+, 72%), 368 (83), 340 (45), 302 (41), 32 (49), and 28 (100).

O-*Acetylaustrocorticin*.—A solution of austrocorticin (12 mg) in acetic anhydride (2 ml) containing a trace of concentrated sulphuric acid was stirred at room temperature for 3 h. The mixture was diluted with iced water (25 ml) and extracted with chloroform (3 × 25 ml). The combined extracts were dried and evaporated to afford a pale yellow solid which was purified by PLC using solvent mixture B, to afford O-*acetylaustrocorticin* (**8**) as yellow needles (13 mg), m.p. 163–165 °C from acetone–light petroleum (b.p. 40–60 °C) (Found: C, 64.0; H, 4.3. $C_{21}H_{16}O_8$ requires C, 63.6; H, 4.0%); $[\alpha]_D^{22}$ +33° (*c* 0.52 in $CHCl_3$); ν_{max} 1 767, 1 713, 1 676, 1 617, 1 594, 1 332, and 1 191 cm^{-1}; λ_{max}(EtOH) 214 (log ε 4.67), 250 (4.43), 273 (4.31), and 402 nm (3.58); *m/z* 396 (M^+, 9%), 354 (62), 336 (100), and 43 (62); δ_H 1.71 (3 H, d, *J* 6.6 Hz, 3′-Me), 2.56 (3 H, s, CH_3CO), 3.98 (3 H, s, 6-OMe), 3.99 (3 H, s, 8-OMe), 5.60 (1 H, q, *J* 6.6 Hz, 3′-H), 6.81 (1 H, d, *J* 2.5 Hz, 7-H), 7.37 (1 H, d, *J* 2.5 Hz, 5-H), and 8.17 (1 H, s, 4-H); δ_C 19.9 (q, *J* 129.3 Hz, 3′-Me), 20.9 (q, *J* 130.6 Hz, *Me*CO_2), 56.0 (q, *J* 145.2 Hz, 6-OMe), 56.7 (q, *J* 145.2 Hz, 8-OMe), 77.2 (dm, *J* 154.0 Hz, C-3′), 103.1 (dd, *J* 160.0 and 5.9 Hz, C-5), 105.6 (dd, *J* 167.3 and 4.4 Hz, C-7), 116.9 (t, *J* 8.8 Hz, C-8a), 117.8 (d, *J* 170.2 Hz, C-4), 123.9 (d, *J* 7.3 Hz, C-2), 127.6 (d, *J* 5.9 Hz, C-9a), 136.2 (br s, C-4a), 138.7 (br s, C-10a), 149.4 (s, C-1), 155.3 (br s, C-3), 162.5 (m, C-8), 164.7 (m, C-6), 165.7 (s, lactone-CO), 168.8 (q, *J* 7.3 Hz, MeCO_2), 179.1 (s, C-9), and 182.4 (t, *J* 4.4 Hz, C-10).

O-*Acetylnoraustrocorticin*.—Noraustrocorticin (3 mg) was treated with acetic anhydride and purified as described above to yield O-*acetylnoraustrocorticin* (**10**) as yellow needles (3 mg), m.p. 275–280 °C from acetone–light petroleum (b.p. 40–60 °C) (Found: M^+, 382.0691. $C_{20}H_{14}O_8$ requires *M*, 382.0689); ν_{max} 1 776, 1 675, 1 660, 1 597, 1 324, 1 262, and 1 191 cm^{-1}; λ_{max}(EtOH) 215 (log ε 4.64), 249 (4.30), 275 (4.0), and 400 nm (3.30); *m/z* 382 (M^+, 5%), 340 (45), 322 (100), and 43 (22); δ_H 2.57 (3 H, s, CH_3CO_2), 3.99 (3 H, s, 6-OMe), 4.00 (3 H, s, 8-OMe), 5.39 (2 H, s, 3′-H_2), 6.83 (1 H, d, *J* 2.2 Hz, 7-H), 7.39 (1 H, d, *J* 2.2 Hz, 5-H), and 8.24 (1 H, s, 4-H); δ_C 20.8 (q, *J* 130.6 Hz, *Me*CO_2), 56.0 (q, *J* 145.2 Hz, 6-OMe), 56.7 (q, *J* 145.2 Hz, 8-OMe), 68.7 (td, *J* 154.1 and 2.9 Hz, C-3′), 103.1 (dd, *J* 160.0 and 5.9 Hz, C-5), 105.7 (dd, *J* 167.5 and 4.4 Hz, C-7), 117.1 (t, *J* 8.8 Hz, C-8a), 118.3 (d, *J* 168.9 Hz, C-4), 123.9 (d, *J* 5.9 Hz, C-2), 127.6 (d, *J* 5.9 Hz, C-9a), 136.2 (br s, C-4a), 138.7 (br s, C-10a), 149.6 (s, C-1), 150.5 (dd, *J* 5.2 and 4.4 Hz, C-3), 162.5 (m, C-8), 164.8 (m, C-6), 166.3 (s, lactone CO), 168.8 (q, *J* 7.3 Hz, MeCO_2), 179.0 (s, C-9), and 182.4 (t, *J* 4.4 Hz, C-10).

Alternatively, a mixture of (**7**) and (**9**) (25 mg, 4:1 respectively by ^1H NMR spectroscopy) obtained directly from the fungus extracts was treated with acetic anhydride (5 ml) containing a trace of concentrated sulphuric acid. Work up and purification by PLC (solvent mixture B) gave a yellow fraction (27.5 mg) which was dissolved in methanol and applied to a column (0.03 × 2 m) of Sephadex LH-20. Elution with the same solvent and evaporation gave two fractions: a faster moving band containing *O*-acetylaustrocorticin (**8**) (21.5 mg), and a slower moving band containing *O*-acetylnoraustrocorticin (**10**) (5.3 mg). The acetates (**8**) and (**10**) were identical in all respects with the acetyl derivatives of the natural products (**7**) and (**9**), respectively.

Hydrolysis of O-*Acetylaustrocorticin*.—*O*-Acetylaustrocorticin (5 mg) was stirred at 0 °C in a mixture of water (6 ml) and pyridine (12 drops) until the solution became bright red. The homogeneous solution was stirred at room temperature for 45 min, cooled on ice, acidified with cold phosphoric acid (5 ml; 1M), and extracted with chloroform (3 × 20 ml). Concentration of the organic layer gave austrocorticin (**7**) (4.4 mg) identical in all respects with material obtained directly from the toadstool extract. Similarly, and using the same procedure, *O*-acetylnoraustrocorticin (**10**) (3.5 mg) was transformed into noraustrocorticin (**9**) (3.1 mg).

Methyl Austrocorticinate.—A solution of austrocorticinic acid (9 mg) in chloroform (2 ml) was stirred at 0 °C for 1 min with an excess of ethereal diazomethane. Evaporation of the solvent and purification of the residue by PLC (solvent mixture B) gave *methyl austrocorticinate* (**12**) as orange needles (8 mg, 86%), m.p. 210–212 °C from ethyl acetate–light petroleum (b.p. 40–60 °C) (Found: C, 64.7; H, 4.9. $C_{20}H_{18}O_7$ requires C, 64.9; H, 4.9%); ν_{max} 3 432, 1 716, 1 674, 1 629, 1 592, 1 358, 1 334, and 1 240 cm^{-1}; λ_{max}(EtOH) 216 (log ε 4.66), 268sh (3.94), 281 (4.02), 423 (3.67), and 493sh nm (3.66); λ_{max}(EtOH + OH^-) 510 nm (log ε 3.49); *m/z* 370 (M^+, 44%), 338 (93), and 310 (100); δ_H 1.28 (3 H, t, *J* 7.3 Hz, 3′-Me), 2.69 (2 H, q, *J* 7.3 Hz, 3′-H_2), 3.99 (3 H, s, 6-OMe), 3.98 (3 H, s, CO_2Me), 4.02 (3 H, s, 8-OMe), 6.77 (1 H, d, *J* 2.2 Hz, 7-H), 7.44 (1 H, d, *J* 2.2 Hz, 5-H), 7.63 (1 H, s, 4-H), and 13.43 (1 H, s, 1-OH); δ_C* 14.7 (3′-Me), 27.3 (C-3′), 52.6 (CO_2Me), 56.1 (6-OMe), 56.5 (8-OMe), 104.2 (C-5), 104.8 (C-7), 114.9 (C-8a), 115.0 (C-9a), 118.6 (C-4), 128.9 (C-2), 132.7 (C-4a), 137.4 (C-10a), 149.6 (C-3), 159.6 (C-1), 163.1 (C-8), 165.5 (C-6), 167.1 (CO_2Me), 182.4 (C-10), and 186.9 (C-9).

Methyl 4-*Hydroxyaustrocorticinate*.—4-Hydroxyaustrocorticinic acid (8 mg) was esterified using ethereal diazomethane and purified as described above to yield *methyl* 4-*hydroxyaustrocorticinate* (**14**) as red needles (6.8 mg, 82%), m.p. 219–223 °C from ethyl acetate–formic acid (Found: C, 62.2; H, 4.7. $C_{20}H_{18}O_8$ requires C, 62.5; H, 4.7%); ν_{max} 3 456, 1 719, 1 620, 1 590, 1 402, 1 309, and 1 223 cm^{-1}; λ_{max}(EtOH) 215 (log ε 4.79), 275 (3.89), 305sh (3.61), and 482 nm (3.66); λ_{max}(EtOH + OH^-) 520sh (log ε 3.50), 556 (3.70), and 593 nm (3.68); *m/z* 386 (M^+,

* Assignments made by analogy with the spectrum of austrocorticinic acid, and corroborated by 2D ^1H-^{13}C heteronuclear correlation spectroscopy.

94%), 354 (100), and 326 (83); δ_H 1.24 (3 H, t, *J* 7.5 Hz, 3'-Me), 2.68 (2 H, q, *J* 7.5 Hz, 3'-H_2), 3.98 (3 H, s, CO_2Me), 3.99 (3 H, s, 6-OMe), 4.02 (3 H, s, 8-OMe), 6.78 (1 H, d, *J* 2.5 Hz, 7-H), 7.49 (1 H, d, *J* 2.5 Hz, 5-H), 13.21 (1 H, s, 1-OH), and 13.66 (1 H, s, 4-OH); δ_C* 13.7 (3'-Me), 21.9 (C-3'), 52.6 (CO_2*Me*), 56.0 (6-OMe), 56.6 (8-OMe), 103.4 (C-5), 105.0 (C-7), 111.8 (C-9a), 112.4 (C-4a), 115.3 (C-8a), 133.6 (C-2), 137.2 (C-10a), 140.9 (C-3), 153.8 (C-1), 155.7 (C-4), 163.1 (C-8), 165.3 (C-6), 166.2 (CO_2Me), 185.5 (C-9), and 186.9 (C-10).

Feeding Experiments.—[Me-^{13}C]*Methionine.* Young fruit bodies growing in their natural habitat were each injected using a syringe three times (days 1, 4, and 8) over an eleven day growing period with an aqueous solution of [Me-^{13}C]methionine (250 μl, 0.33M, 99.6 atom% ^{13}C, Sigma-Aldrich). After being harvested (day 11) the toadstools were extracted with ethanol and the pigments isolated in the usual way. The enhancement in ^{13}C content over and above natural abundance (1.1%) for each of the quinones (**7**), (**9**), (**11**), (**13**), (**16**), and (**18**) is listed in Table 4. The natural abundance and methionine-enriched ^{13}C NMR spectra of austrocorticin (**7**) form part of Figure 2.

Sodium [3-^{13}C]*propionate.* Young fruit bodies growing in their natural habitat were each injected using a syringe four times (days 1, 4, 8, and 11) over a fifteen day growing period with an aqueous solution of sodium [3-^{13}C]propionate (250 μl, 0.33M, 97.8% atom% ^{13}C, Amersham). After being harvested (day 15) the toadstools were extracted with ethanol and the pigments isolated in the usual way. The enhancement in ^{13}C content over and above natural abundance (1.1%) for each of the quinones (**7**), (**11**), (**13**), (**16**), and (**18**) is listed in Table 5. The natural abundance and propionate-enriched ^{13}C NMR spectra of austrocorticin (**7**) form part of Figure 2.

Sodium [2-^{13}C]*acetate.* An aqueous solution of sodium [2-^{13}C]acetate (250 μl, 2.44M, 99.5 atom% ^{13}C, Sigma–Aldrich) was injected using a syringe four times (days 1, 4, 8, and 11) over a period of 15 days into each member of a group of toadstools growing in their natural habitat. After being harvested (day 15) the toadstools were extracted with ethanol and the pigments isolated in the usual way. The enhancement in ^{13}C content over and above natural abundance (1.1%) is discussed in the text.

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