(+)-Austrocorticin and Austrocorticinic Acid, the First Anthraquinones derived from a Propionate-triggered Octaketide

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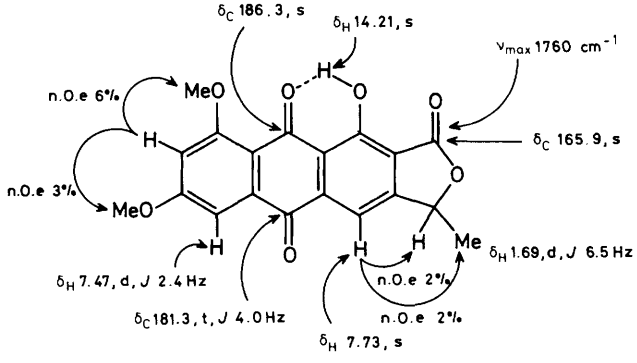
The toadstool pigments (**1**) and (**2**) bearing a unique C_2 side chain at C-3 in the anthraquinone nucleus have been isolated and characterised and their biogenesis studied by feeding ^{13}C labelled precursors to young fruit bodies; high specific incorporation of label from sodium [3-^{13}C]propionate into the C-3' methyl group of (**1**) indicates a clear propionate 'starter' effect is operating.

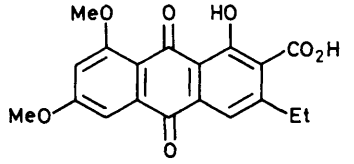
Anthraquinones form a large and important group of naturally occurring colouring matters distinguished by their wide distribution and structural diversity.[1] The majority of anthraquinone pigments isolated from plant and particularly from fungal sources have their origins in the polyketide pathway of secondary metabolism and these, in the main, are derived from octaketide progenitors.[2,3] Without exception[4] these octaketide-derived anthraquinones utilise acetate (as acetyl CoA) as the primer in their biosynthesis.[5] We report here the first naturally occurring anthraquinones, (**1**) and (**2**), which are shown to be derived *via* propionate-triggered octaketide assembly.



(**1**)

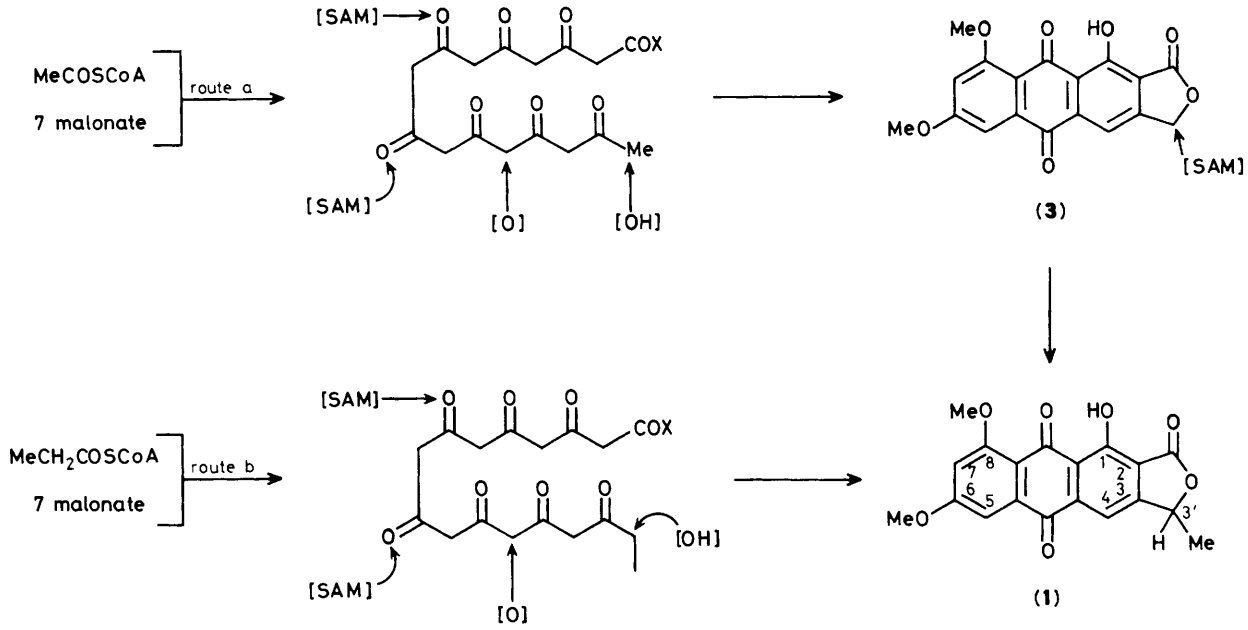
Figure 1. Selected spectroscopic data for austrocorticin (**1**).

The pigments (**1**) and (**2**), to which we give the names austrocorticin and austrocorticinic acid, respectively, have been isolated by us from the orange-red fruit bodies of an Australian toadstool belonging to the genus *Cortinarius*.† The



(**2**)

† This fungus is placed as a new taxon in the sub-genus *Dermocybe* of *Cortinarius* by Watling (personal communication). Photographs of the fruit bodies have been published.[6] Voucher specimens of the material described here are held in the herbarium of the Royal Botanic Garden, Edinburgh, under accession number Wat. 19352.



Scheme 1. Possible pathways for the biogenesis of austrocorticin (**1**).

principal pigment, austrocorticin, was obtained after extensive chromatography as orange needles (from $CHCl_3$–MeOH), $C_{19}H_{14}O_7$ (mass spectral and combustion analysis), m.p. 246–250 °C, $[\alpha]_D^{25}$ + 45° (*c* 1.2, $CHCl_3$), and could be unequivocally assigned the structure (**1**) on spectroscopic grounds. Selected spectroscopic data for (**1**) are presented in Figure 1. The absolute configuration at C-3′ in (**1**) is not yet known.

Similarly, the structure (**2**) for a minor yellow pigment, austrocorticinic acid, followed detailed analysis of mass, ^1H and ^{13}C n.m.r., i.r., and electronic spectra.‡

Being the first endocrocin-type anthraquinones bearing a C_2 side chain rather than a methyl substituent at C-3 we have studied the biosynthetic origin of the C-3′ methyl group in (**1**) and in (**2**). Two possible modes of biogenesis for the major pigment (**1**) are depicted in Scheme 1. 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 (**3**). *C*-Methylation of (**3**), for example by *S*-adenosylmethionine, would lead to austrocorticin (**1**). We were initially encouraged along these lines by our isolation of the anthraquinone (**3**) as a minor co-metabolite of austrocorticin (**1**) in this fungus. Alternatively, propionate-triggered octaketide assembly (Scheme 1, route b) and subsequent hydroxylation at C-2 of the propionate starter unit would also lead eventually to austrocorticin.

In order to differentiate these pathways [*Me*-^{13}C]methionine and sodium [3-^{13}C]propionate were administered individually to groups of toadstools growing in their natural habitat. While efficient assimilation of methionine was evident from high incorporation of label into the OMe groups at C-6 and C-8 in (**1**)§ no significant enrichment of the C-3′ methyl group in (**1**) 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 (**1**) which by ^{13}C and ^1H n.m.r. spectroscopy could clearly be seen to be considerably enriched exclusively at the C-3′ methyl group.¶

Thus, route b in Scheme 1 in which propionate initiates octaketide formation is established for the biosynthesis of austrocorticin (**1**). Analogous incorporation of the ^{13}C label into the ethyl side chain of austrocorticinic acid has also been observed but has not been quantified, to date, owing to lack of material.

Pigments (**1**) and (**2**) represent the first naturally occurring anthraquinones to be derived *via* a propionate-triggered octaketide. Propionate is known to initiate *deca*ketide formation during anthracyclinone biosynthesis in various *Streptomyces* species[8] and several anthraquinones have been isolated from blocked mutants of anthracyclinone-producing strains of *S. galilaeus*[9] and *S. coeruleorubidus*[10] to which propionate-triggered nona- and deca-ketide origins may be presumed, although this has not yet been verified experimentally. Nevertheless, a propionate start unit has, hitherto, never been observed in the octaketide series.

Our work on the pigments of other Australian *Cortinarius* toadstools is continuing. It may prove that the pigments (**1**) and (**2**) are the first examples of a whole family of ethyl homologues of anthraquinones of the emodin and endocrocin types.

We are grateful to Dr. R. Watling (Edinburgh) for expert advice and for lodging herbarium specimens, Mr. T. May and

‡ *Selected physical and spectroscopic data* for (**2**): yellow needles (from EtOAc–petrol), $C_{19}H_{16}O_7$ (mass spec. and combustion analysis), m.p. 250 °C (subl.), 280 °C (decomp.); ^1H n.m.r. ($CDCl_3$) δ 1.31 (t, *J* 7.3 Hz, CH_2CH_3), 2.75 (q, *J* 7.3 Hz, CH_2CH_3), 3.98 and 4.01 (each s, OMe), 6.77 (d, *J* 2.2 Hz, 7-H), 7.43 (d, *J* 2.2 Hz, 5-H), 7.63 (s, 4-H), 13.17 (s, chelated OH); mass spectrum *m/z* 356 (M^+, 18%), 338 ($M^+ - H_2O$, 52), 312 ($M^+ - CO_2$, 27), 310 (45).

§ A total enrichment of 33% followed 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 and 4.05, respectively, in the ^1H n.m.r. spectrum of (**1**).[7]

¶ 14% Enrichment was determined by integration of the ^{13}C satellites (J_{CH} 130.0 Hz) flanking the C-3′ Me resonance at δ 1.69 in the ^1H n.m.r. spectrum of (**1**).

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