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PIGMENTS OF FUNGI, PART 26.[1] INCORPORATION OF SODIUM [1,2-$^{13}C_2$]ACETATE INTO TOROSACHRYSONE BY MUSHROOMS OF THE GENUS *DERMOCYBE*

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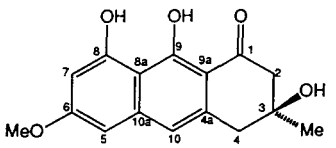
ABSTRACT.—The pattern of incorporation of sodium [1,2-$^{13}C_2$]acetate into torosachrysone [**1**] in intact mushrooms belonging to the genus *Dermocybe* defines unequivocally the folding pattern of the precursor octaketide.

Torosachrysone [**1**] is believed to occupy a pivotal position in the biogenesis of anthraquinone and dihydroanthracenone pigments in fungi (1,2). It was first isolated from the seeds and seedlings of the higher plant *Cassia torosa* (3,4). We demonstrated earlier (2) that sodium [2-^{13}C]acetate and [*Me*-^{13}C]methionine are efficiently incorporated into torosachrysone [**1**] by intact fruiting bodies of an Australian fungus growing in their natural habitat. These experiments confirmed the polyketide origin of the carbon backbone in the dihydroanthracenone **1**, with the carbon atom of the OMe group coming from *S*-adenosylmethionine. Furthermore, the specific incorporation of label from [2-^{13}C]acetate into C-2, C-4, C-5, C-7, C-8a, C-9a, C-10, and the *C*-Me group in **1**, when taken together with a considerable amount of circumstantial evidence drawn from previous biosynthetic studies of related anthraquinones such as emodin (5) and islandicin (6,7), led us to propose that torosachrysone arises from an octaketide precursor by the pathway delineated in Scheme 1, route a (2). While folding of the octaketide in the way shown in route a is entirely consistent with the observed distribution of ^{13}C in **1**, it is not possible to distinguish between the outcome of route a and the alternative folding pattern shown in route b of Scheme 1, since both pathways would lead ot torosachrysone [**1**] in which precisely the same carbons are enriched. Although folding as depicted in route b of Scheme 1 has not been previously documented in the octaketide series, it is well known that it occurs in analogous decaketides involved in the biosynthesis of pyrromycinones by *Streptomyces* (8). We describe here the results of feeding experiments employing sodium [1,2-$^{13}C_2$]acetate, which establish unequivocally that the pathway depicted in route a of Scheme 1 is operational in these Australian mushrooms.



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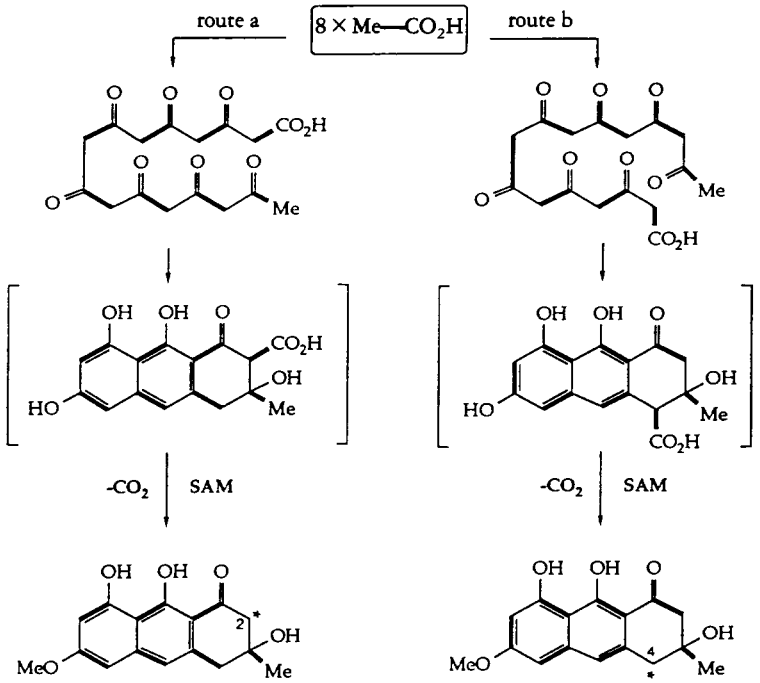
RESULTS AND DISCUSSION

WAT 20880 served admirably as the substrate in our earlier, single labelling studies (2). Now, in order to differentiate route a from route b in Scheme 1, it was necessary to deliver to the intact fruiting bodies doubly $^{13}C_2$ labeled acetate (7).

Accordingly, two fruiting bodies of WAT 20880 were periodically supplied by syringe over 8 days with a total of 150

[1]For Part 25, see M. Gill, A. Giménez, A.G. Jhingran, and A. Qureshi, *Phytochemistry*, in press.



SCHEME 1. Possible folding of an octaketide during the biosynthesis of torosachrysone [**1**].

mg of sodium [1,2-$^{13}C_2$]acetate. After a further 4 days growth, the mushrooms were harvested and torosachrysone [**1**] was isolated and purified in the usual way (2). From the ^{13}C-nmr spectrum of torosachrysone [**1**] obtained in this way it is clearly discernible that ^{13}C-^{13}C coupling occurs between seven pairs of adjacent nuclei, indicating that seven intact acetate units have been incorporated into the molecule. Furthermore, inspection of the chemical shifts of the mutually coupled nuclei (Table 1) reveals that incorporation of the double label has taken place with a distribution consistent only with the folding pattern depicted in route a in Scheme 1. By way of illustration, the carbonyl carbon C-1 (δ 204.4) in the spectrum of doubly-labeled **1** shows ^{13}C-^{13}C coupling ($J = 55.7$ Hz) with C-9a (δ 109.4) but no coupling with C-2 (compare route b). In complete accord with this conclusion, the resonance due to C-2 (δ 51.4) in the spectrum of enriched torosachrysone is measurably enhanced but is not discerni-

TABLE 1. ^{13}C-nmr Data (100.40 MHz, Me_2CO-d_6) for Torosachrysone [**1**] Enriched with Sodium [1,2-$^{13}C_2$]Acetate.

Carbon[a]	Chemical shift (δ)	J_{CC} (Hz)
C-1	204.4	55.7
C-2	51.4	—[b]
C-3	70.8	36.9
C-4	43.6	41.1
C-4a	138.2	41.1
C-5	100.2	69.0
C-6	164.3	69.0
C-7	101.3	74.8
C-8	160.4	74.8
C-8a	108.4	66.0
C-9	166.2	66.0
C-9a	109.4	55.7
C-10	118.0	55.8
C-10a	142.1	55.8
3-Me	29.3	36.9
6-OMe	55.8	—

[a]Assignments are consistent with the fully-proton-coupled spectrum obtained in $CDCl_3$ (2) and have been confirmed by 2D ^{1}H-^{13}C correlation experiments.

[b]Signal enriched by 0.6% above natural abundance.

bly coupled. Enrichment of the ^{13}C content at this site was quantified by comparison of the intensity of the resonance in both the natural abundance (1.1%) and enriched spectra after normalization (13).

In summary, the incorporation of label from sodium [2-^{13}C]acetate, [*Me*-^{13}C] methionine (2) and now sodium [1,2-$^{13}C_2$]-acetate into torosachrysone [**1**] by fruiting bodies of WAT 20880 supports a biosynthesis of this important dihydroanthracenone by folding of a single, intact octaketide chain, itself assembled (at least formally) by head-to-tail linkage of eight acetate units, according to route a of Scheme 1. It should be noted that the putative precursor relationship between torosachrysone [**1**] and fungal anthraquinones of the emodin type (1) has not yet been established experimentally; the possibility that the pigment **1** is derived from an anthraquinone by reductive hydration cannot be completely ruled out.

EXPERIMENTAL

TAXONOMIC DIAGNOSIS.—The taxonomic rank of *Dermocybe* remains a matter for debate. Consistent with most recent chemical publications (1) and in line with Moser (9), we have elected here to treat *Dermocybe* as a genus. We have found in southeast Victoria several species of *Dermocybe* mushrooms that contain torosachrysone [**1**] as the major coloring matter. One of these, WAT 20880, is placed as a new taxon in *Dermocybe* and is referred to here by the accession number under which a voucher specimen is held in the Herbarium of the Royal Botanic Garden, Edinburgh, U.K.

WAT 20880.—Material examined: In grassy area in mixed *Eucalyptus* forest, Kinglake National Park, Victoria, July 1988, legit. M. Gill. Basidia 4-spored, 25–27.5 × 6–6.5 μm excluding sterigmata (<4.5 μm long), clavate-elongate, tapered downwards, honey-colored. Basidiospores 8–9 (−9.5) × (4.5−) 4.8 (−5) μm, elongate sub-amygdaliform, elongate elliptic in face-view, verruculose with prominent but small apiculus, bright honey-yellow slightly darker in ammoniacal solutions. Pleurocystidia absent; cheliocystidia replaced by sterile cells hardly differentiated from basidia <30 μm long × 6–7 μm. Hymenophoral trama regular, composed of honey-colored to rich tawny honey, inflated cells, some even ellipsoid, 11–22 μm broad, intermixed with filamentous elements >9 μm broad, not differentiated into medio and lateral strata; subhymenium composed of compacted, filamentous, non-gelatinized hyphae >10 μm broad, many elements strongly colored, supporting hymenial cells full of bright tawny orange inclusions or cytoplasmic material in addition to basidia. Gill-edge ± fertile, basidia interspersed with poorly differentiated, elongate-clavate cells. Pileipellis a cutis of repent, flexuous, filamentous, loosely intertwined, clamp-connected, hyaline or pigmented, smooth or encrusted golden red-yellow hyphae, 4.5–6.5 μm broad or containing a honey-colored strand of cytoplasmic material, interspersed with similarly colored amorphous material and seated on compacted subpellis; end-cells not differentiated. Context composed of honey-colored inflated hyphae 20–25 μm broad compacted towards hymenium, and towards the subpellis resembling a loose pseudoparenchyma of rounded cells 9–30 μm broad; fluorescent under uv and with a pale yellow, soluble pigment in ammoniacal solutions.

This fungus differs from *Cortinarius* sp. 1 (WAT 16091) described by Høiland and Watling (10) from Queensland in the lack of sulfur-yellow colors below the cortinate zone and in the gills, and the almost smooth basidiospores of WAT 16091, the faint finely punctuated ornamentation being seen only with the aid of the scanning electron microscope. *Cortinarius alkalivirens* Høiland and Watling (10) differs in the broader, distinctly verrucose basidiospores and smaller stature. *Dermocybe canaria* Horak is a bulky agaric (80 mm) with ovoid, yellow brown (tinged with rust-color) basidiospores minutely asperulate at the apex [(7−) 7.5–8.5 (−a) × 4–5 μm] and more differentiated suprapellis. It is found with *Nothofagus* and associated conifers in New Zealand (11). WAT 20880 approaches *D. canaria* in many other ways. *Dermocybe aurantiella* Horak, also from New Zealand (11), differs markedly in its elongate, longer basidiospores, slight stature, and more differentiated pileipellis.

At the moment WAT 20880 cannot be satisfactorily placed. The fact that WAT 20880 contains torosachrysone [**1**] as the major pigment and the fact that torosachrysone is believed to be the precursor of several tetrahydroanthraquinone pigments in *Dermocybe splendida* (12) suggest that *D. splendida* and WAT 20880 are probably in the same taxonomic consortium. *D. canaria* and WAT 20880 hold an isolated position in *Dermocybe*.

GENERAL EXPERIMENTAL PROCEDURES.—^{13}C-nmr spectra were measured at 100.40 MHz on a JEOL JNM-GX 400 spectrometer for solutions in Me_2CO-d_6. Sodium [1,2-$^{13}C_2$]acetate (99.0 atom % ^{13}C) was used as purchased from Sigma-Aldrich. For details of the isolation and

purification of torosachrysone [**1**] from WAT 20880 see Gill *et al.* (2).

FEEDING EXPERIMENT.—Two young fruiting bodies of WAT 20880, growing in their natural habitat in *Eucalyptus* forest in the Kinglake National Park, Victoria, during late June 1988, were impregnated by using a syringe (ca. 10 a.m. on days 1, 4, and 8) with an H_2O solution of Na [1,2-$^{13}C_2$]OAc (250 μl, 2.44 M). On day 12 (ca. 4 p.m.) the mushrooms were collected, diced, and soaked in EtOH (250 ml). Work up and chromatography (2) gave torosachrysone [**1**] (5 mg). The ^{13}C-nmr chemical shifts and heteronuclear coupling constants of this material are collected in Table 1.

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