A New Dihydroanthracenone Glycoside from *Dermocybe sanguinea*[1]

Melvyn Gill* and Simon Saubern

School of Chemistry, University of Melbourne, Parkville, Victoria 3052, Australia

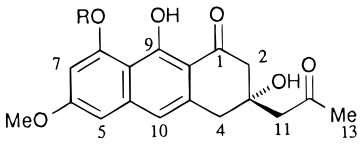
Received May 1, 1996[⊗]

The 8-*O*-*β*-D-gentiobioside (**1**) of the dihydroanthracenone dermochrysone (**3**) has been isolated from the H_2O-soluble extracts of the Australian toadstool *Dermocybe sanguinea* (*sensu* Cleland) and its structure deduced from the spectroscopic properties of its hepta-*O*-acetyl derivative (**2**).

In an earlier part of this series we described the isolation and structure elucidation of several new anthraquinone and dihydroanthracenone pigments from the organic soluble extracts of the Australian toadstool *Dermocybe sanguinea* (Wulf. ex Fr.) Wünsche *sensu* Cleland.[2] In a further investigation of this fungus, we describe herein the isolation from the H_2O-soluble extracts of *D. sanguinea* (*sensu* Cleland) of the novel *β*-D-gentiobioside (**1**), which is characterized spectroscopically as its heptaacetyl derivative (**2**).

The gentiobioside (**1**) was isolated from the lyophilized fruit bodies of *D. sanguinea* (*sensu* Cleland) after extraction with EtOH, partition of the extractives between EtOAc and H_2O, and gel permeation of the aqueous phase, as a chromatographically homogenous, hygroscopic, mustard-yellow powder that exhibited intense blue fluorescence when viewed under UV light ($\lambda = 365$ nm). The ^{1}H-NMR spectrum of the gentiobioside (**1**) in DMSO-d_6 was uniformative due to considerable line broadening, and this, together with our earlier experience involving dihydroanthracenone[3] and tetrahydroanthraquinone gentiobiosides,[4] prompted us to acetylate the natural product **1** prior to further structural analysis. Accordingly, exposure of **1** to Ac_2O and pyridine gave the heptaacetate **2** as a stable, optically active yellow powder, mp 50 °C (dec). FABMS, together with ^{13}C- and ^{1}H-NMR data, permitted assignment of the molecular formula of **2** as $C_{44}H_{52}O_{23}$. Identification of the aglycon portion of **2** was deduced from the presence in the ^{1}H-NMR spectrum of three methylene proton resonances (*δ* 2.86, ABq; 3.11, ABq; 2.74 br s), signals from C- and *O*-methyl groups (*δ* 2.16 and 3.96, respectively), two *meta*-coupled and one isolated aromatic proton (*δ* 6.74, 6.90, and 6.88, respectively) and a characteristic H-bonded phenolic OH signal (*δ* 14.57), all of which are close to the corresponding data for (*S*)-dermochrysone (**3**), a constituent of the organic soluble extractives of *D. sanguinea*.

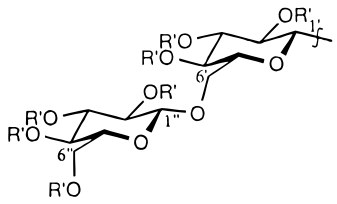
The nature of the sugars and their *β*-configurations in **2** was deduced from the results of DEPT, COSY, and HETCOR NMR experiments, while their connectivity to each other (1″→6′) was clear from the chemical shift of the C-6′ methylene protons (*δ* 3.78), which reveals that there is a glycosidic bond rather than an acetoxyl group at C-6′. Location of the gentiobiosyl moiety at C-8 in the aglycon is apparent from the observation of three-bond coupling between C-8 (*δ* 157.6) and the anomeric proton H-1′ (*δ* 5.22) in a COLOC NMR experiment. The identity of the disaccharide portion of **2** as the hepta-*O*-acetyl derivative of *β*-D-gentiobiose was confirmed by comparison of the spectroscopic data with those of several anthraquinone, tetrahydroanthraquinone, and dihydroanthracenone gentiobiosides described in the literature.[3,4]



(1) R = **A**

(2) R = **B**

(3) R = **H**



A R' = H

B R' = Ac

Based on the above results, the blue fluorescent material isolated from the fruit bodies of *D. sanguinea* (*sensu* Cleland) is 8-*O*-*β*-D-gentiobiosyl dermochrysone (**1**). The gentiobioside **1** joins a small but increasing number of diglycosidic anthraquinonoid pigments that have been isolated, exclusively to date, from Australian toadstools belonging to the genera *Dermocybe* and *Cortinarus*.[3,4] Significantly, the present case represents the first example of a disaccharide derivative of a nonaketide-derived pigment to be discovered in Basidiomycotina.

Experimental Section

General Experimental Procedures. Mps were taken on a Reichart-Jung micro-hot stage. Specific rotations were measured at 589 and 546 nm at 20 °C using a Perkin-Elmer 141 polarimeter for solutions in a 1-dm quartz cell. IR spectra were obtained using a Perkin-Elmer 983G spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX400 instrument; chemical shifts are shown as *δ* values with TMS as standard. FABMS were run on a JEOL JMS-AX505HF spectrometer. Preparative TLC used Merck Kieselgel 60 GF_{254} on glass plates (20 × 20 cm).

* To whom correspondence should be addressed. Phone: (3) 9344 6485. FAX: (3) 9347 5180. E-mail: m.gill@chemistry.unimelb.edu.au.

⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1996.

S0163-3864(96)00429-6 CCC: $12.00

Fungal Material. *Dermocybe sanguinea* (Wulf. ex Fr.) Wünsche *sensu* Cleland[5] was collected from mixed *Eucalyptus* forest in the Marysville State Park, Victoria, Australia, during June 1990, and stored at −20 °C prior to use. Voucher specimens have been deposited in the Herbarium of the Royal Botanic Garden, Edinburgh, U.K., under accession no. WAT 20931.

Extraction and Isolation. Frozen fruit bodies (91g) were freeze-dried, and the dried material (4.9g) was macerated in EtOH (200 mL) for 3 h. The extract was concentrated under reduced pressure, and the residual red paste was partitioned between EtOAc (3 × 50 mL) and H_2O (100 mL). The orange-red aqueous phase was lyophilized, and the residue (215 mg) in H_2O (10 mL) was applied to a column (2 × 60 cm) of Sephadex LH-20 gel, and a pale-orange zone exhibiting strong blue fluorescence under UV light (λ = 365 nm) that eluted with H_2O was collected and lyophilized. The hygroscopic orange residue (**1**) (2.0 mg, 2.2×10^{-3}% of the fresh wt, 4.1×10^{-2}% of the dry wt) was not characterized as such but was immediately acetylated as described below.

8-*O*-*β*-D-Gentiobiosyldermochrysone 2′,2″,3′,3″,4′,4″,6″-Hepta-*O*-acetate (2). The gentiobioside (**1**) (2.0 mg) in pyridine (2 mL) containing Ac_2O (0.5 mL) was stirred at room temperature for 20 min. The mixture was diluted with EtOAc (30 mL), and the solution was washed successively with aqueous HCl (1 M, 10 mL) and H_2O (10 mL). The organic phase was dried ($MgSO_4$) and concentrated to a yellow solid that was purified by preparative TLC using EtOAc as eluent to afford compound **2** (1.8 mg), as a yellow powder, mp 50 °C (dec), $[\alpha]_D$ −43°, $[\alpha]_{546}$ −54° (*c* 0.1, $CHCl_3$); IR (KBr) ν max 1751 br (C═O, Ac), 1633 br (C═O, chelated ketone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.57 (1H, s, OH-9), 6.90 (1H, d, J = 2.4 Hz, H-7), 6.88 (1H, s, OH-10), 6.74 (1H, d, J = 2.2 Hz, H-5), 5.41 (1H, dd, J = 9.4, 7.6 Hz, H-2′), 5.30 (1H, t, J = 9.4 Hz, H-3′), 5.22 (d, J = 7.6 Hz, H-1′), 5.02−5.10 (2H, m, H-3″ and H-4″), 4.95−5.02 (2H, m, H-2″ and H-4′), 4.52 (1H, d, J = 7.8 Hz, H-1″), 4.07−4.21 (2H, m, H_2-6″), 3.99 (1H, ddd, J = 9.8, 8.6, 2.4, Hz, H-5′), 3.96 (3H, s, OMe-6), 3.84 (1H, dd, J = 12.2, 8.6 Hz, Ha-6′), 3.73 (1H, dd, J = 12.2, 2.4 Hz, Hb-6′), 3.56 (1H, ddd, J = 10.0, 5.4, 2.2 Hz, H-5″), 3.16 (1H, d, J = 15.6 Hz, H-4β), 3.06 (1H, d, J = 15.6 Hz, H-4α), 2.93 (1H, d, J = 17.2, H-2β), 2.80 (1H, J = 17.2 Hz, H-2α), 2.74 (2H, s, H_2-11), 2.16 (3H, s, Me-13), 2.06 (9H, br s, Ac), 2.04 (6H, br s, Ac), 1.98 (3H, s, Ac), 1.83 (3H, s, Ac); ^{13}C NMR ($CDCl_3$) δ 209.8 (s, C-12), 201.3 (s, C-1), 170.6, 170.3, 170.2, 169.6, 169.5, 169.3, 169.2 (all s, Ac), 165.4 (s, C-9), 161.8 (s, C-6), 157.6 (s, C-8), 141.5 (s, C-10a), 136.0 (s, C-4a), 117.0 (d, C-10), 111.1 (s, C-8a), 109.6 (s, C-9a), 105.2 (d, C-7), 101.7 (d, C-5), 101.2 (d, C-1″), 99.6 (d, C-1′), 74.0 (d, C-5′), 73.0 (d, C-3″), 72.7 (d, C-3′), 71.8 (d, C-5″), 71.3 (s, C-3), 71.3 (d, C-2″), 71.1 (d, C-2′), 69.3 (d, C-4′), 69.1 (t, C-6′), 68.3 (d, C-4″), 61.9 (t, C-6″), 55.5 (q, OMe-6), 50.5 (t, C-11), 50.3 (t, C-2), 41.8 (t, C-4), 31.6 (q, C-13), 20.7 (q, 3 × Ac), 20.6 (q, 3 × Ac), 20.3 (q, Ac); FABMS (thioglycerol) m/z $[M + Na]^+$ 971, $[M + 1]^+$ 949, $[M -$ aglycon$]^+$ 619, [aglycon + 1]$^+$ 331.

Acknowledgment. The authors thank Dr. R. Watling, Royal Botanic Garden, Edinburgh, U.K., for the identification of the fungus and for lodging herbarium material. The work was supported by grants from the Australian Research Council; S.S. is the recipient of a Commonwealth Postgraduate Research Award.

References and Notes

(1) Pigments of Fungi. 42. Part 41: Cotterill, A. S.; Gill, M.; Milanovic, N. M. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1215−1223.
(2) Gill, M.; Gimenez, A. G. *J. Chem. Soc., Perkin Trans. 1*, **1990**, 2585−2591.
(3) Eagle, S. N.; Gill, M.; Saubern, S.; Yu, J. *Nat. Prod. Lett.* **1993**, *2*, 151−157.
(4) Gill, M.; Gimenez, A.; Saubern, S.; Smrdel, A. F. *Nat. Prod. Lett.* **1992**, *1*, 187−192.
(5) Cleland, J. B. *Toadstools and Mushrooms and Other Larger Fungi of South Australia*; Government Printer: South Australia, 1976; p 113. Depicted as "*Cortinarius* aff. *sanguineus*" in: Fuhrer, B. *A Field Companion to Australian Fungi*; The Five Mile Press: Hawthorne, Victoria, 1985; p 39.

NP960429X