New Xanthorin Glycosides from a *Dermocybe* Species[1]

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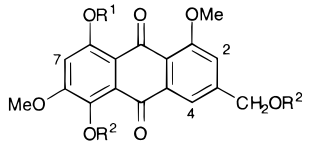
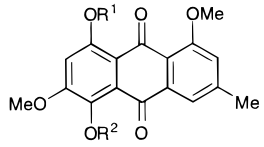
A mixture of the 8-*O*-*β*-D-glucopyranoside of *ω*-hydroxyxanthorin 1-*O*-methyl ether (**3**) and the 8-*O*-*β*-D-gentiobioside of xanthorin 1-*O*-methyl ether (**4**) was isolated from the water-soluble extractives of the Australian toadstool *Dermocybe* sp. WAT 22963. Compounds **3** and **4** were identified by characterization of their respective peracetyl derivatives **5** and **6**.

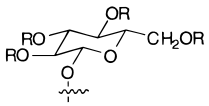
Earlier we described the isolation and structure elucidation of *ω*-hydroxyxanthorin 1-*O*-methyl ether (**1**) and xanthorin 1-*O*-methyl ether (**2**) from the organic-soluble extractives of the indigenous Australian toadstool *Dermocybe* sp. WAT 22963 (Agaricales).[2] Drawn by the intensity of the pigmentation that remained in the aqueous phase when the total EtOH extract of *Dermocybe* sp. WAT 22963 was partitioned between EtOAc and H_2O, the constituents of this fungus were reexamined. We report here isolation of a mixture of the 8-O-*β*-D-glucopyranoside of *ω*-hydroxyxanthorin 1-*O*-methyl ether (**3**) and the 8-*O*-*β*-D-gentiobioside of xanthorin 1-*O*-methyl ether (**4**). Compounds **3** and **4** were acetylated in admixture, and the acetylated derivatives were separated and identified as the new compounds **5** and **6**.



1 $R^1 = R^2 = H$
3 R^1 = glu, $R^2 = H$
5 R^1 = glu-*O*-Ac, R^2 = Ac



2 $R^1 = R^2 = H$
4 R^1 = gent, $R^2 = H$
6 R^1 = gent-*O*-Ac, R^2 = Ac



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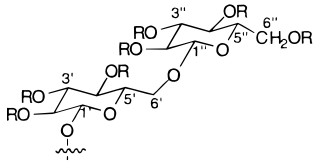
glu-*O*-Ac: R = Ac



gent: R = H
gent-*O*-Ac: R = Ac

Fruiting bodies of *Dermocybe* sp. WAT 22963 were collected during June 1993, and April 1995, from native forest comprising parts of the Kinglake and Otway Ranges National Parks, Victoria, Australia. Fresh fruiting bodies were macerated in EtOH overnight, the extracts were evaporated, and the residual slurry was partitioned between EtOAc and H_2O. The deep red organic phase afforded, among other things the xanthorin derivatives **1** and **2**, which were identified by direct comparison with authentic materials.[2] A small aliquot of the orange aqueous phase was stirred at room temperature with dilute HCl, and the resulting hydrolysate was extracted with EtOAc. All of the pigments then passed into the organic phase, and the principal components of the extract were separated by preparative silica TLC. Two red zones (R_f 0.20 and 0.55) were isolated and identified as *ω*-hydroxyxanthorin 1-*O*-methyl ether (**1**) and xanthorin 1-*O*-methyl ether (**2**) by comparison with authentic materials.[2] It seemed probable, then, that the pigments present in the H_2O extractives were glycosidic derivatives of **1** and **2**. Consequently, the remainder of the orange H_2O phase was passed through a column of Sephadex LH-20 using EtOH−H_2O (1:1) as eluent, and the single orange band that eluted was evaporated to dryness. The ^{1}H NMR spectrum of the residue in DMSO-d_6 was complex and relatively uninformative due to considerable line broadening. Nevertheless, it served not only to suggest that the residue consisted of at least two compounds but also to show that there were no acetoxy groups in the mixture of natural products as it was isolated. To purify the individual components of this mixture prior to further structure analysis it was first necessary to acetylate the mixture by using Ac_2O and pyridine and then to separate the resulting peracetyl derivatives by preparative silica TLC with $CHCl_3$−HCO_2-Et (1: 1) as eluent.

The more polar fraction (R_f 0.35) obtained in this way gave a yellow powder, mp 124−126 °C, that exhibited pseudomolecular ions at *m*/*z* 767 {[M + Na]$^+$} and 745 {[M + H]$^+$} in the FABMS. This corresponds to the molecular formula $C_{35}H_{36}O_{18}$. The ^{1}H NMR spectrum consisted of signals from two methoxy groups (δ 3.92 and 3.93), one aromatic methylene group (δ 5.16), and three aromatic protons (δ 7.201, 7.204, and 7.68), among others, which identified this compound as a derivative of *ω*-hydroxyxanthorin 1-*O*-methyl ether (**1**).[2] Furthermore, the appearance of methyl singlets at δ 2.02, 2.06, 2.07, 2.16, 2.31, and 2.46 established the presence of six acetoxy groups, the first four being appended to aliphatic hydroxy groups and the last two being attached to phenolic and benzylic hydroxy groups.

Because an *ω*-hydroxyxanthorin nucleus together with six acetoxy groups accounts for the elements $C_{29}H_{29}O_{17}$, the glycoside is most likely a hexose derivative. It was identified as a *β*-D-tetra-*O*-acetylglucopyranoside from the ^{1}H and ^{13}C NMR data (Table 1) and by comparison of the proton chemical shifts and couplings with the corresponding data for some other fungal *β*-D-tetra-*O*-acetylglucopyranosides.[3−5] Thus, in the ^{1}H NMR spectrum, five methine and two methylene protons resonate between δ 3.70 and 5.40. Because all of the methine protons, including the anomeric proton (δ 5.25), exhibit vicinal coupling constants of at least 7 Hz, each one must be trans-diaxially disposed to each of its neighbors, and the anomeric linkage must be *β*. Furthermore, the ^{13}C NMR spectrum (Table 1) and the results

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Table 1. ^{1}H NMR Data [Chemical Shift (δ), Multiplicity, and Coupling Constants (J/Hz)] ($CDCl_3$; 400 MHz) for the Peracetyl Derivatives **5** and **6**, and ^{13}C Data (δ) ($CDCl_3$; 100 MHz) for **5**

	5		**6**[a]
position	^{1}H	^{13}C	^{1}H
1		159.1	
1-OMe	3.93 (s)	56.3	3.92 (s)
2	7.20(1) (br s)	116.3	7.03 (br s)
3		142.5	
3-CH	5.16 (s)	65.2	2.45 (s)
4	7.68 (br s)	117.9	7.52 (br s)
4a		135.8	
5		136.1	
6		155.7	
6-OMe	3.93 (s)	56.6	3.90 (s)
7	7.20(4) (br s)	114.9	7.18 (br s)
8		153.8	
8a		120.9	
9		180.4	
9a		122.7	
10		182.4	
10a		125.9	
1′	5.25 (d, 7.3)	101.2	5.28 (d, 9.35)
2′	5.40 (dd, 7.3, 9.2)	72.2	5.36 (t, 9.35)
3′	5.35 (t, 9.2)	70.9	5.38 (t, 9.35)
4′	5.17 (t, 9.2)	68.6	5.16 (t, 9.35)
5′	3.70 (m)	72.4	3.71 (m)
6′	4.25 (2H, m)	61.5	4.24 (m)
1″			5.05 (d, 10.26)
2″			5.49 (dd, 9.50, 10.26)
3″			5.40 (t, 9.50)
4″			5.05 (t, 9.50)
5″			4.05 (ddd, 2.00, 5.50, 9.50)
6″			4.00 (dd, 2.00, 12.42; 4.27, dd, 5.50, 12.42)
ω-OAc	2.46 (s)	167.6	
5-OAc	2.31 (s)	170.5	2.32 (s)
gly-OAc	2.02, 2.06, 2.07, 2.16 (all s)	20.7 (3C), 20.8 (2C), 20.9; 169.6, 170.1 (2C), 170.5 (2C), 170.6	2.02, 2.04, 2.05(2), 2.05(4), 2.06, 2.08, 2.09 (all s)

[a] Assignments are supported by the results of 2D ^{1}H−^{1}H COSY and NOESY experiments.

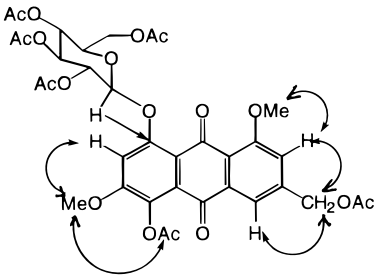

Figure 1. Selected correlations from NOESY and HMBC experiments involving the peracetyl derivative **5** of the 8-*O*-β-D-glucopyranoside of ω-hydroxyxanthorin 1-*O*-methyl ether (**3**).

of HMBC experiments are in complete accord with the β-D-tetra-*O*-acetylglucopyranoside formulation shown in **5**.

Placement of the glucopyranosyl residue at C-8 in the ω-hydroxyxanthorin nucleus was strongly suggested from the results of NOESY and HMBC experiments; some important correlations are summarized in Figure 1. However, an HMBC experiment was conclusive in this regard in that it shows clear correlation between C-8 (δ 153.8) and the anomeric proton H-1′ (δ 5.25).

The more polar yellow peracetate can, therefore, be assigned the structure **5**, and hence, the natural product, as it occurs in *Dermocybe* sp. WAT 22963, must be the 8-*O*-β-D-glucopyranoside **3** of ω-hydroxyxanthorin 1-*O*-methyl ether (**1**). The glucopyranoside **3** is a new natural product and is the only known glycoside of an ω-hydroxyanthraquinone. A small number of other anthraquinone β-D-glucosides are known from Basidiomycotina;[6] however, and with only one exception,[7] these are all 1-*O*-β-D-glucopyranosides.

The less polar yellow zone (R_f 0.46) obtained by gel permeation of the acetylation mixture gave compound **6** as an orange powder, mp 131−133 °C, which exhibits pseudomolecular ions at m/z 997 {[M + Na]$^+$} and at m/z 975 {[M + H]$^+$} in the FABMS, consistent with the molecular formula $C_{45}H_{50}O_{24}$. The ^{1}H NMR spectrum of **6** (Table 1) shows the presence of one aromatic methyl group (δ 2.45), three aromatic protons (δ 7.03, 7.18, and 7.52), and two methoxy groups (δ 3.90 and 3.92), confirming that **6** is a glycoside of xanthorin 1-*O*-methyl ether (**2**) (see above). Furthermore, because there are signals from seven aliphatic acetoxy groups between δ 2.02 and 2.09 and one phenolic acetoxy group (δ 2.32), the carbohydrate must be the peracetate of a disaccharide with the formula $C_{12}H_{22}O_{11}$. From our previous experience with naturally occurring dihydroanthracenones,[4,5] tetrahydroanthraquinones,[3] and anthraquinones[7] with an appended disaccharide unit from fungi, this strongly suggested to us that the carbohydrate residue in the present case is a β-D-gentiobioside (see formulas **4** and **6**). This suggestion was confirmed by comparison of the ^{1}H data (Table 1) with those of other peracetylated 8-*O*-β-D-gentiobiosides[3−5] and by the results of homonuclear COSY and NOESY experiments. Thus, beginning from the innermost anomeric proton, H-1′ (δ 5.28), it was possible to trace the COSY correlation first to H-2′ (δ 5.36) and thence, sequentially, to H-3′ (δ 5.38), H-4′ (δ 5.16), H-5′ (δ 3.71) and H_2-6′ (δ 4.24). The sequence of assignments from H-1″ (δ 5.05) through to H_2-6″ (δ 4.00 and 4.27) followed in the same way. All of the vicinal coupling constants around both rings are at least 9 Hz, and, therefore, all methine protons, including the two anomeric protons, must be axially disposed.

The peracetylated β-D-gentiobioside moiety was placed on the C-8 hydroxyl in **6** as determined from NOE correlations. Thus, irradiation at δ 3.90 (the C-6 methoxy group) caused significant enhancement to the signal due to H-7 and to the phenolic acetate group. Irradiation of the protons of the other methoxy group (δ 3.92) enhanced only H-2 (δ 7.03), proving that the second methoxy group must be located at C-1. This leaves only C-8 as the site for attachment of the gentiobioside residue.

The yellow octa-acetate must, therefore, have the structure **6**, and the second orange glycoside from *Dermocybe* sp. WAT 22963 is the 8-*O*-β-D-gentiobioside **4** of xanthorin 1-*O*-methyl ether (**2**). This is the first report of the quinone **4** as a natural product; it joins only a handful of anthraquinone β-D-gentiobiosides that have been isolated exclusively from Australian toadstools belonging to the genera *Cortinarius* and *Dermocybe*.[8]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM GX-400 spectrometer operating at 399.65 MHz (^{1}H) and 100.4 MHz (^{13}C) fitted with Varian Unity Plus version 5.1 software. Chemical shifts (δ) are quoted in parts per million from standard TMS in $CDCl_3$. UV spectra were recorded on a Varian SuperScan 3 spectrophotometer using 10 mm quartz cells and EtOH as the solvent. FABMS were obtained in a thioglycerol matrix using a JEOL JMS-AX505HF mass spectrometer. Melting points (mps) were determined on a Köfler micro hot stage apparatus and are uncorrected. Preparative TLC (silica) was performed on 20 × 20 cm glass plates coated with 1.0 mm of silica (Merck Kieselgel GF_{254} applied as a suspension in H_2O). Plates were activated at 110 °C for 1.5 h prior to use. Analytical TLC was performed on Macherey−Nagel precoated aluminum plates (0.25 mm, Macherey−Nagel SIL G-25 UV_{254}) and visualized both in daylight and under short (254 nm) and long (360 nm) wavelength UV light. All R_f values quoted refer to analytical TLC with toluene−HCO_2Et−HCO_2H (50:49:1) as eluent, unless stated otherwise. Gel permeation was carried out using columns packed with Sephadex LH-20 (Pharmacia) suspended in and eluted with MeOH, unless stated otherwise. Commercial $CDCl_3$ (Cambridge Isotope Laboratories) was washed with H_2O, dried (K_2CO_3), distilled, and stored in the dark. All other solvents and reagents were purified before use by published procedures.[9]

Fungal Material. Lyophilized fruiting bodies of *Dermocybe* sp. WAT 22963 are lodged in the herbarium of the Royal Botanic Garden, Edinburgh, UK, under accession number WAT 22963. The species was placed in *Dermocybe* by Dr. R. Watling. A description of the fungus has been published previously.[2]

Extraction, Isolation, and Derivatization of Natural Products 3 and 4. Fresh fruiting bodies of *Dermocybe* sp. WAT 22963 (6 g) from the Otway Ranges National Park were finely chopped and soaked overnight in EtOH (300 mL) at room temperature. The solvent was evaporated, and the green-brown residue was partitioned between EtOAc (150 mL) and H_2O (150 mL). A second collection of the same fungus (24 g), from the Kinglake National Park, was extracted in the same way.

The organic phase was dried (Na_2SO_4) and evaporated to dryness to afford a green-brown residue (40 mg) that was purified by preparative TLC on silica using toluene−HCO_2-Et−HCO_2H (50:49:1) as the eluent to give, among other things xanthorin 1-*O*-methyl ether (**2**) (R_f 0.55) (2.7 mg, 9.0×10^{-3}% fr wt) as red needles ($CHCl_3$), mp 204−205 °C (lit.[2] mp 205−209 °C) and ω-hydroxyxanthorin 1-*O*-methyl ether (**1**) (R_f 0.20) (1.5 mg, 5.0×10^{-3}% fr wt) as red needles ($CHCl_3$), mp 220−222 °C (lit.[2] mp 220−223 °C).

The H_2O phase was purified by gel permeation using, sequentially, EtOH−H_2O (1:1) and MeOH as eluent. The solvent was evaporated, and to a small quantity of the residue (10 mg) was added aqueous HCl (1M, 1 mL). The mixture was stirred at room temperature for 15 min, and the products were extracted into $CHCl_3$ (3 × 5 mL). Evaporation of the solvent and purification of the residue by preparative TLC using toluene−HCO_2Et−HCO_2H (50:49:1) as the eluent gave xanthorin 1-*O*-methyl ether (**2**) (R_f 0.55) (2 mg) and ω-hydroxyxanthorin 1-*O*-methyl ether (**1**) (R_f 0.20) (1.5 mg), both identical with material described earlier.

The rest of the residue obtained from evaporation of the H_2O phase was subjected to gel permeation using, sequentially, EtOH−H_2O (1:1) and MeOH as eluent. An orange zone that eluted was evaporated, and the residue (27 mg) was treated with pyridine (3.5 mL) and Ac_2O (3.5 mL) at room temperature for 90 min. Iced H_2O (20 mL) was added, and the products were extracted into EtOAc (3 × 50 mL). Removal of the solvent left a yellow powder that was purified by preparative TLC using $CHCl_3$−HCO_2Et (1:1) as the eluent. Two yellow zones that were obtained gave, in order of increasing R_f, **5** and **6**.

***ω*,5,2′,3′,4′,6′-Hexaacetyl-1-*O*-methylxanthorin 8-*O*-β-D-glucopyranoside (5):** R_f 0.35 {$CHCl_3$−HCO_2Et [1:1]} (6.2 mg) as a yellow powder ($CHCl_3$); mp 124−126 °C; UV λ_{max} (log ε) 219 (3.27), 266 (4.40), 328 (2.35) nm; ^{1}H and ^{13}C NMR spectra, see Table 1; FABMS *m*/*z* 767 ([M + Na]$^+$, 4), 745 ([M + 1]$^+$, 1), 331 (40), 169 (100) (calcd for $C_{35}H_{36}O_{18}$, 744).

5,2′,3′,4′,2″,3″,4″,6″-Octaacetyl-1-*O*-methylxanthorin 8-*O*-β-D-gentiobioside (6): R_f 0.46 {$CHCl_3$−HCO_2Et [1:1]} (3.5 mg) as a yellow powder ($CHCl_3$); mp 131−133 °C; UV λ_{max} (log ε) 221 (3.14), 267 (4.32), 325 (2.45) nm; ^{1}H and ^{13}C NMR spectra, see Table 1; FABMS *m*/*z* 997 ([M + Na]$^+$, 1), 975 ([M + 1]$^+$, 1), 331 (22), 169 (100) (calcd for $C_{45}H_{50}O_{24}$, 974).

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References and Notes

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