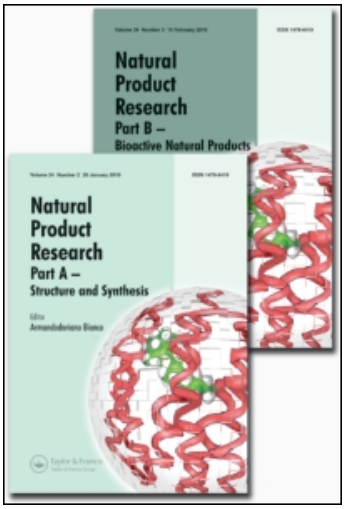

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Chemical constituents of *Antrodia camphorata* submerged whole broth

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One new compound, 10-hydroxy-γ-dodecalactone (**1**) and three natural new compounds, 11-hydroxy-γ-dodecalactone (**2**), 2-(2-hydroxyethyl)phenol (**3**) and 12-hydroxydodecanoic acid methyl ester (**4**), together with eight known compounds, ergostatrien-3β-ol, ergosterol peroxide, methyl (4-hydroxyphenyl)acetate, vanillin, 4-hydroxybenzaldehyde, hexadecanoic acid, 5-methoxymethylfuran-2-carbaldehyde and 5-hydroxymethylfuran-2-carbaldehyde, all were isolated from the submerged whole broth of *Antrodia camphorata*. The structures of **1** and **2** were principally elucidated by spectral evidence and the absolute configuration was elucidated by the modified Mosher's method.

Keywords: Antrodia camphorata; Submerged; 10-hydroxy-γ-dodecalactone; 11-hydroxy-γ-dodecalactone; 2-(2-hydroxyethyl)phenol; 12-hydroxydodecanoic acid methyl ester

1. Introduction

The fruiting body of *Antrodia camphorata* (Polyporaceae, Aphyllophorales) is well known in Taiwan as a traditional Chinese medicine. It has been used for the treatment of food and drug intoxication, diarrhoea, abdominal pain, hypertension, skin itching, and cancer [1]. It is rare and expensive. The submerged fermentation of *A. camphorata* has been developed and commercialised by local companies. In biological studies, the fruiting bodies exhibited immunomodulating [2], antioxidative and hepatoprotective effects [3]. The fermented culture broth had cytotoxic activity against several tumour cell lines [4], and the cultured mycelia showed anti-inflammatory [5], vasorelaxation [6] and cytotoxic activity against several tumour cell lines [7–9], protection from oxidative damage in normal human erythrocytes [7] and anti-hepatitis B virus activity [10].

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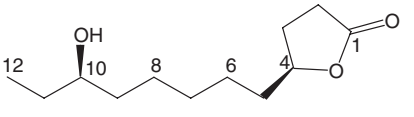
The filtrate in submerged culture also had protective effects against CCl_4-induced hepatic toxicity [11] and high antioxidant properties [12].

By previous chemical studies on its fruiting body, components including steroids, triterpenoids, sesquiterpenes, lignoids, fatty acids, phenyl methanoids and its dimers were identified by several investigators [13–19].

Recently, Nakamura et al. [9] discovered five new maleic and succinic acid derivatives together with ergosterol peroxide from the mycelium of *A. camphorata* and two of the maleimide derivatives showed appreciable cytotoxic activity against LLC cells. This encouraged us to study the chemical principles from the submerged whole broth. The methanol extract of freeze-dried powder of *A. camphorata* of the submerged whole broth was partitioned with EtOAc and H_2O. The EtOAc soluble fraction was repeatedly open column chromatographed and HPLC chromatographed on normal phase to afford one new compound, 10-hydroxy-γ-dodecalactone (**1**) and three first isolated from natural, 11-hydroxy-γ-dodecalactone (**2**), 2-(2-hydroxyethyl)phenol (**3**) and 12-hydroxydodecanoic acid methyl ester (**4**) along with eight known compounds, ergostatrien-3β-ol [20], ergosterol peroxide [21], methyl (4-hydroxyphenyl)acetate [22], vanillin [23], 4-hydroxybenzaldehyde [24], hexadecanoic acid [25], 5-methoxymethylfuran-2-carbaldehyde [26] and 5-hydroxymethylfuran-2-carbaldehyde [27]. In our studies, only ergosterol peroxide was present, and the maleic and succinic acid derivatives were not observed. All isolated components expressed no significant cytotoxic activity. In this report, we deal with the structural elucidation of one new compound **1**.



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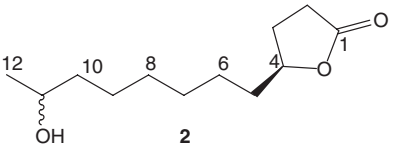

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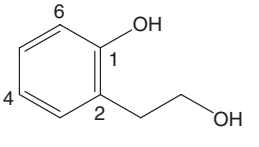

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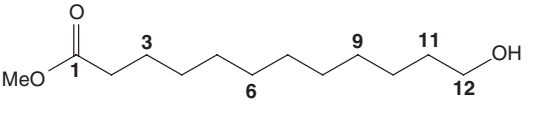


4

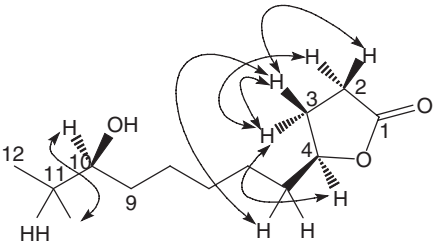
2. Results and discussion

10-Hydroxy-γ-dodecalactone (**1**) was isolated from the fraction eluted with ethyl acetate/*n*-hexane of 30–40%; its molecular formula of $C_{12}H_{22}O_3$ was established through ^{13}C-NMR and high-resolution electron impact mass spectral (HR-EI-MS) data. The index of hydrogen deficiency (IHD) of **1** is 2. The IR spectrum of **1** confirmed the presence of a γ-lactone group (1772 cm^{-1}) and a hydroxyl group (3449 cm^{-1}). The ^{1}H-NMR spectrum (table 1) exhibited signals for one primary methyl group [δ_H 0.91 (3H, t, $J = 7.6$ Hz)], a carbinol proton neighbouring with two methylene groups [δ_H 3.46 (quin, $J = 6.4$ Hz)], a methine proton linked to a γ-lactone group (δ_H 4.46, m). The consecutive protons δ_H 2.50 (2H, dd, $J = 8.8$, 9.6 Hz, H_2-2), 1.87 and 2.29 (1H each, m, H_β-3 and H_α-3), 4.46(1H, m, H-4) and 1.73 and 1.56 (each 1H, m, H_2-5) were revealed from correlation spectroscopy (COSY). The signals at δ_H 2.50 (2H), 1.87 and 2.29 were assigned as H_2-2 and H_2-3 due to having heteronuclear multiple bond correlation (HMBC) with lactone carbonyl (δ_C 176.9) (table 1). The ^{13}C-NMR data (table 1) and the distortionless enhancement by polarisation transfer (DEPT) spectroscopy analysis showed 12 signals including one CH_3 [δ_C 9.9 (C-12)], eight CH_2 [δ_C 28.9 (C-2), 28.1 (C-3), 35.5 (C-5), 25.5 (C-6), 30.2 (C-7), 25.3 (C-8), 29.4 (C-9), 36.7 (C-11)], two CH [δ_C 80.9 (C-4), 73.1 (C-10)] and one lactone carbon [δ_C 176.9 (C-1)].

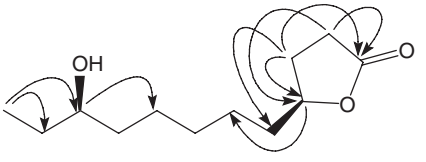
Table 1. ^{1}H-and ^{13}C-NMR spectral data of compounds **1**–**4** (400 and 100 MHz in $CDCl_3$).

	1		**2**		**3**		**4**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	176.9		177.1		155.0		174.1	
2	28.9	2.50 dd (8.8, 9.6)	29.5	2.50 dd (9.6, 10.0)	126.7		34.2	2.27 t (7.2)
3α	28.1	2.29 m	29.4	2.29 m	131.0	7.06 dd (7.8, 1.6)	25.0	1.59 quin (7.2)
3β		1.87 m		1.81 m				
4	80.9	4.46 m	81.0	4.45 m	120.7	6.85 td (7.8, 1.6)	29.2	
5	35.5	1.73 m, 1.56 m	35.6	1.72 m, 1.62 m	128.3	7.15 td (7.8, 1.6)		
6	25.5	1.33 m, 1.40 m	25.2	1.37 m, 1.42 m	116.8	6.92 dd (7.8, 1.6)		1.24 br s
7	30.2	1.44 m	29.3	1.30 m	34.3	2.90 t (5.2)		
8	25.3	1.31 m	29.5	1.24 m	63.8	4.00 t (5.2)		
9	29.4	1.38 m, 1.44 m	25.6	1.35 m			29.9	
10	73.1	3.46 quin (6.4)	39.2	1.40 m			25.7	
11	36.7	1.48 m, 1.52 m	68.1	3.75 sex (6.0)			32.8	1.52 quin (6.4)
12	9.9	0.91 t (7.6)	25.7	1.15 d (6.0)			63.0	3.61 t (6.4)
$COOCH_3$								3.64 s
$COO\underline{C}H_3$							51.5	

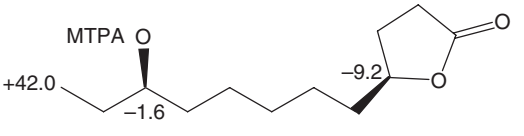
The nuclear Overhause enhancement exchange spectroscopy (NOESY) (structure **5**) and COSY spectra clarified the relative configuration and locations of H-4/H-3, H-4/H-5, H-9/H-10, H-3/H-5. Key HMBC spectrum (structure **6**) confirmed the correlation of C-10/H-12, H-11, H-9; C-4/H-6, H-5, H-3, H-2, and also verified the hydroxyl group on C-10. The fragment peak in MS spectrum at m/z 85 (100%) is the proof of the presence of γ-lactone moiety. Based on the above evidence, compound **1** was proposed as 10-hydroxy-γ-dodecalactone. The absolute configuration of **1** was determined by the modified Mosher's method [28]. Treatment of **1** with (*R*)- and (*S*)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) afforded the (*S*)- and (*R*)-MTPA esters, respectively. $\Delta\delta$ values (in Hz) (δ_S–δ_R) of H-12 (+42.0 Hz) showed positive values, while H-10 (−1.6 Hz) and H-4 (−9.2 Hz) were negative (structure **7**), thus indicating a (10*R*)-configuration. Therefore, the absolute configuration C-10 of **1** was determined.



5 (key NOESY of **1**)



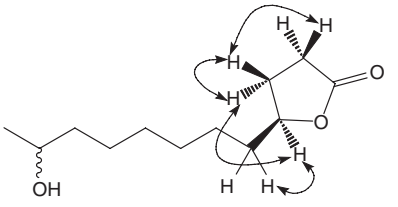
6 (key HMBC of **1**)



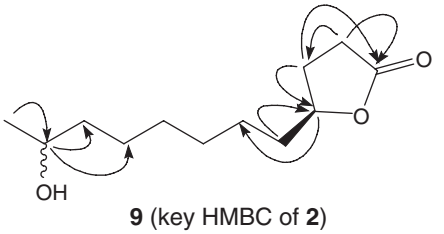
7 ($\Delta\delta$values [$\Delta\delta$(in Hz)=δ_S–δ_R] obtained for the (*S*)-and (*R*)-MTPA esters)

Based on the HR-EI-MS and ^{13}C-NMR data (table 1), compound **2** has the molecular formula of $C_{12}H_{22}O_3$ with IHD of 2. The IR spectrum of **2** confirmed to the presence of a lactone carbonyl group (1772 cm^{-1}) and a hydroxyl group (3422 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral data (table 1) of compound **2** is similar to compound **1**, they are positional isomers. The only difference is the position of the hydroxyl group (justified by the NOESY and HMBC spectra, see structures **8** and **9**). The hydroxyl group located at C-11 in compound **2** instead of located at C-10 in compound **1**. This compound has been prepared by oxidising dodecanoic acid or its esters with aerobic or

facultative aerobic micro-organisms or extracts of the microbes [29]. Treatment of **2** with one of two (*R* and *S*) MTPACl afforded the two MTPA esters with equivalent amount (counted by ^{1}H-NMR integration). The evidence indicated that the C-11 chiral centre is a racemic.



8 (key NOESY of **2**)



9 (key HMBC of **2**)

Compound **3**, a viscid oil, was formulated as $C_8H_{10}O_2$ on the base of HR-EI-MS. From its physical data (table 1) [ν_{max} 3350, 1600 and 1493; ^{1}H-NMR δ_H 2.90, 4.00 (each 2H, d, $J = 5.2$ Hz), 6.92, 7.06 (each 1H, dd, $J = 7.8$, 1.6 Hz), 6.85 and 7.15 (each 1H, td, $J = 7.8$, 1.6 Hz) and ^{13}C-NMR], the structure of **3** was confirmed as 2-(2-hydroxyethyl)phenol. It is a synthetic intermediate and first isolated from nature [30].

Based on the HR-EI-MS and ^{13}C-NMR data (table 1), compound **4** was assigned as 12-hydroxydodecanoic acid methyl ester from its physical data assignment and 2D spectra. It was first isolated from a natural source and a synthetic product from the methylation of 12-hydroxydodecanoic acid [31].

3. Experimental

3.1. *General*

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Specific rotations were recorded on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 983 G spectrometer. ^{1}H- and ^{13}C- NMR spectra were, respectively, recorded on a Bruker DMX-400 spectrometer. EIMS and HR-EIMS were measured with a JEOL Finnigan TSQ-46C and JEOL SX-102A mass spectrometers, respectively. Extracts were chromatographed on silica gel (Merck 70–230 mesh, 230–400 mesh) and purified on a semi-preparative normal-phase HPLC column [250 × 10 mm, Licrosorb Si 60 (7 μm)] carried out with a LCD Rafracto Monitor III.

3.2. *Fungus material*

Freeze-dried powder of *A. camphorata* of the submerged whole broth (Batch No. MZ-247) was provided by the Biotechnology Center of Grape King Inc., Chung-Li City, Taiwan, Republic of China.

3.3. *Extraction and isolation*

Freeze-dried powder of *A. camphorata* of the submerged whole broth (1.6 kg) was extracted three times with methanol (16 L) at room temperature (1 day each). The methanol extract was evaporated *in vacuo* to give a brown residue, which was suspended in H_2O (1 L), and then partitioned (3 times) with 1 L of ethyl acetate. The EtOAc fraction (95 g) was chromatographed on silica gel using mixtures of hexane and EtOAc of increasing polarity as eluents and further purified with HPLC. Twelve components were identified. Ergostatrien-3β-ol (5.4 g) was eluted with 10% EtOAc in hexane. Eight components, 2-(2-hydroxyethyl)phenol (**3**) (6.2 mg), 12-hydroxydodecanoic acid methyl ester (**4**) (9.5 mg), ergosterol peroxide (3.0 mg), methyl (4-hydroxyphenyl)acetate (11.2 mg), vanillin (1.9 mg), 4-hydroxybenzaldehyde (2.3 mg), hexadecanoic acid (8.3 mg) and 5-methoxymethylfuran-2-carbaldehyde (3.7 mg) were eluted with 15–20% EtOAc in hexane. Three components, 10-hydroxy-γ-dodecalactone (**1**) (12.8 mg), 11-hydroxy-γ-dodecalactone (**2**) (12.3 mg) and 5-hydroxymethylfuran-2-carbaldehyde (142.6 mg) were eluted with 30–40% EtOAc in hexane.

3.3.1. 10-Hydroxy-γ-dodecalactone (1). Viscid liquid; $[\alpha]_D^{24} = -10.1^\circ$ ($c = 2.33$, $CHCl_3$). IR (KBr) ν_{max} 3449, 1772, 1465, 1355, 1183, 1017, 918 cm^{-1}, ^{1}H- and ^{13}C-NMR ($CDCl_3$, 400 and 100 MHz) data: see table 1. EI-MS (70 eV) (rel. int.%) *m/z* 214 [M^+] (3), 152 (61), 85 (100); HR-EIMS *m/z* 214.1566 (M^+, Calcd for $C_{12}H_{22}O_3$, 214.1563). (*S*)-MTPA ester of **1**:^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, t, $J = 7.6$ Hz, H-12), 2.29 (1H, m, H-3α), 2.51 (2H, dd, $J = 8.8$, 9.6 Hz, H-2), 3.54 (3H, s, OMe), 4.43 (1H, m, H-4), 5.01 (1H, quin, $J = 6.4$ Hz, H-10), 7.39 (3H, m), 7.53 (2H, m). (*R*)-MTPA ester of **1**:^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, t, $J = 7.6$ Hz, H-12), 2.29 (1H, m, H-3α), 2.51 (2H, dd, $J = 8.8$, 9.6 Hz, H-2), 3.56 (3H, s, OMe), 4.45 (1H, m, H-4), 5.02 (1H, quin, $J = 6.4$ Hz, H-10), 7.39 (3H, m), 7.53 (2H, m).

3.3.2. 11-Hydroxy-γ-dodecalactone (2). Viscid liquid; $[\alpha]_D^{24} = -10.0^\circ$ ($c = 0.81$, $CHCl_3$). IR (KBr) ν_{max} 3422, 2933, 1772, 1464, 1423, 1183, 1127, 916 cm^{-1}, ^{1}H- and ^{13}C-NMR ($CDCl_3$, 400 and 100 MHz) data: see table 1. EI-MS (70 eV) (rel. int.%) *m/z* 214 [M^+] (2), 152 (22), 85 (100); HR-EI-MS *m/z* 214.1562 (M^+, Calcd for $C_{12}H_{22}O_3$, 214.1563).

3.3.3. 2-(2-Hydroxyethyl)phenol (3). Viscid oil. IR (KBr) ν_{max} 3350, 2932, 1600, 1493, 1459, 1248, 1108, 1049, 758 cm^{-1}, ^{1}H- and ^{13}C-NMR ($CDCl_3$, 400 and 100 MHz) data: see table 1. EI-MS (70 eV) (rel. int.%) *m/z* 138 [M^+] (35), 120 [$M^+ - H_2O$] (44), 107 (100); HR-EI-MS *m/z* 138.0675 (M^+, Calcd for $C_8H_{10}O_2$, 138.0678).

3.3.4. 12-Hydroxypentanoic acid methyl ester (4). Needles, m.p. 86–87°C. IR (KBr) ν_{max} 3421, 2930, 2858, 1740, 1465, 1413, 1249, 976, 727 cm^{-1}, ^{1}H- and ^{13}C-NMR ($CDCl_3$, 400 and 100 MHz) data: see table 1. EI-MS (70 eV) (rel. int.%) *m/z* 230 [M^+] (4), 74 (100); HR-EIMS *m/z* 230.1877 (M^+, Calcd for $C_{13}H_{26}O_3$, 230.1875).

Acknowledgement

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