New sesquiterpenes from *Marasmius cladophyllus*

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**Graphical abstract**

Four new sesquiterpenes, including a tremulane sesquiterpene (1), a drimane sesquiterpene (2), and two dimeric drimane sesquiterpenes (3 and 4), plus previously known dimeric drimane sesquiterpenes, cryptoporic acid D (5), cryptoporic acid E (6) and dimeric 15-hydroxyl cryptoporic acid H (7), were isolated from a fermentation extract of *Marasmius cladophyllus* F070624009. This strain was isolated from a spore suspension of a *Phylloporus* sp. collected from the Wuyi Mountain Conservation Area in Fujian Province, China. The chemical structures of 1–7 were elucidated by spectroscopic analyses, including 1D- and 2D-NMR, and on the basis of HR Q-TOF MS data.

**Keywords:** tricholomataceae; novel compounds; cladophyllol; drimane-3,8,11,12-tetraol; cryptoporic acid

1. Introduction

Mushrooms are known to be a rich source of natural products (Bose 1953; Monro 2003; Li and Oberlies 2005; Zaidman et al. 2005; Zheng and Shen 2009). *Marasmius cladophyllus* belongs to the family Tricholontataceae, and closely related genera have long been known to produce novel and active secondary metabolites (Kavanagh et al. 1949; Dugan et al. 1966; Hoeftle et al. 1976; King et al. 1977). Previously, two sesquiterpenes, namely alliacolide (King et al. 1977) and marasmic acid (Dugan et al. 1966), have been isolated from the genus *Marasmius*. Due to limitations in obtaining wild mushrooms, which usually replicate in small quantity and have a very short season annually, we employed the sustainable mycelia fermentation approach to mine for macrofungal products, especially sesquiterpenes, from the genus *Marasmius*. In
this paper, we report the isolation and structural elucidation of a new tremulane sesquiterpene (1) and six drimane sesquiterpenes (2–7), including three new ones (2–4), from *Marasmius cladophyllus* F070624009.

2. Methods

2.1. General

Column chromatography (CC): silica gel (SiO₂, 200–300 and 80–100 mesh; Qingdao Marine Chemical Factory, Qingdao, China), SiO₂ GF₂₅₄ (Merck, Darmstadt, Germany), RP-18 (Merck), and Sephadex LH-20 (Amersham Biosciences, Little Chalfont, UK) were used. TLC; precoated SiO₂ GF₂₅₄ plates (0.20–0.25 mm; Qingdao Marine Chemical Factory). Optical rotations: Perkin-Elmer 341 polarimeter with MeOH as solvent. IR spectra: Nicolet FT-IR 360 in KBr. HR-Q-TOF-MS: Bruker Daltonios BioTOF-Q mass spectrometers; in CD₃OD. FT -IR 360 in KBr. 1H- and 13C-NMR: Bruker DRX-600 spectrometer, at 600 (1H), and 150 (13C) MHz; in MeOD; Me₄Si, resp., 100 ml each) to obtain Fr.1.3.1–Fr1.3.2. Fr1.3.2 (30 g); gradient aq. MeOH (15%, 20%, 25% and 100% resp., 1 l each) to obtain 10 fractions. Fr.1.3 (56.7 mg) was further purified by MPLC [(RP-18 (30 g); gradient aq. MeOH (15%, 20%, 25% and 100% resp., 100 ml each)] to obtain Fr.1.3.1–Fr1.3.2. Fr1.3.2 (8.4 mg) was further purified by CC (SiO₂; petroleum ether (PE)/ethyl acetate (EA), 5:1, 3:1 and 1:1, v/v) to obtain 1 (1.2 mg) and 2 (1.2 mg).

Fr.7 (970 mg) was subjected to MPLC (RP-18 (80 g); gradient aq. MeOH (80%, 85%, 90% and 100% resp., 1 l each) to obtain six fractions. Fr.7.5 (31.4 mg) was further purified by CC (SiO₂; PE/EA, 10:1, 5:1 and 3:1, v/v) to obtain 6 (8.6 mg); Fr.7.6 (23.2 mg) was purified by CC (SiO₂; PE/EA, 10:1, 3:1 and 2:1, v/v) to obtain 3 (22 mg). Fr.7.2 (72.1 mg) was subjected to MPLC (PR-18, 30 g; 82% MeOH) to obtain three fractions Fr.7.2.1–Fr.7.2.3. Fr.7.2.2 (40 mg) was further purified by CC (SiO₂; PE/EA, 5:1, 3:1, 2:1 and 1:1, v/v) to obtain 7 (3 mg) and 4 (10.1 mg). Fr.7.3 (506.2 mg) was subjected to MPLC (PR-18 (80 g); 82% MeOH) to obtain five fractions Fr.7.3.1–Fr.7.3.5. Fr.7.3.1 (142.4 mg) was purified by CC (Sephadex LH-20 (80 g); MeOH) to obtain 5 (3.7 mg).

Cladophyllol

(=3R*,3aR*,4R*,6S*,6aS*,7S*)-6,8,8-trimethyl-1,3,3a,4,5,6,6a,7,8,9-decahydroazulenol[4,5-c]furan-3,4,7-triol; 1). White powder; UV (MeOH): vmax (lg ε) = 224 nm (2.17); IR (KBr) λmax: 3285, 1650 cm⁻¹; HR Q-TOF-MS m/z: 291.1575 [(M + Na)⁺, C₁₅H₂₃O₄; calcd. 291.1567]; 1H- and 13C-NMR: see Table 1.

Drimane-3,8,11,12-tetraol

(=25S*,4aS*,5S*,6R*,8aR*)-5,6-bis(hydroxymethyl)-1,1,4a-trimethyldecahydroazaphthalene-2,6-diol; 2). White powder. UV (MeOH): vmax (lg ε) = 216 nm (1.42); [α]D²⁰ = +15 (c = 0.05, MeOH); IR (KBr) λmax: 3285 cm⁻¹; HR Q-TOF-MS m/z: 295.1881 [(M + Na)⁺, C₁₅H₂₃O₄; calcd. 295.1880]; 1H- and 13C-NMR: see Table 2.

Table 1. 1H- and 13C-NMR data (600 and 150 MHz, resp.; in CD₃OD) of 1 (δ in ppm, J in Hz).

| Position | δ(H) (ppm) | δ(C) (ppm) |
|----------|------------|------------|
| 1        | 3.02 (br. s) | 48.0 (d) |
| 2        | 3.64 (d, J = 5.0) | 84.2 (d) |
| 3        | 41.6 (s) | 60.6 (s) |
| 4        | 2.18 (d, J = 12.0) | 42.9 (t) |
| 5        | 1.97 (d, J = 12.0) | 133.7 (s) |
| 6        | 127.3 (s) | 127.3 (s) |
| 7        | 2.75–2.78 (m) | 54.7 (d) |
| 8        | 4.13 (dd, J = 3.6, 6.9, 10.7) | 69.3 (d) |
| 9        | 2.47 (dd, J = 4.9, 10.7) | 44.9 (t) |
|          | 1.32–1.36 (m) | 72.9 (s) |
| 10       | 2.32 (quartet, J = 7.3) | 28.8 (d) |
| 11       | 0.96 (s) | 26.6 (q) |
| 12       | 1.05 (s) | 21.8 (q) |
| 13       | 4.52 (dd, J = 2.6, 12.4) | 68.6 (t) |
|          | 4.24–4.29 (m) | 72.9 (s) |
| 14       | 5.45 (d, J = 4.6) | 98.5 (d) |
| 15       | 1.16 (d, J = 7.3) | 20.0 (q) |
The molecular formula of 2 (δ in ppm, J in Hz).

| Position | δ(H) | δ(C) |
|----------|------|------|
| 1 1.82 (dt, J = 3.5, 13.0) 1.25 (dd, J = 3.5, 13.0) | 38.1 (t) 26.6 (t) |
| 2 1.64–1.66 (m) | 3.22 (dd, J = 5.9, 10.4) | 38.6 (s) 77.8 (d) |
| 4 5 1.02–1.05 (m) 1.67–1.69 (m) | 6 2.23 (dt, J = 3.0, 6.2) 1.24 (d, J = 3.0) |
| 7 8 1.55 (dd, J = 4.6, 7.9) | 9 3.99 (dd, J = 7.9, 11.1) 3.84 (dd, J = 4.6, 11.1) |
| 12 3.67 (q, J = 4.4) | 13 1.00 (s) 27.3 (q) |
| 14 0.80 (s) 14.7 (q) | 15 0.90 (s) 15.9 (q) |

Cryptoporic acid J

(=2(R,2S,5,8a)-trans-15S,4aR*,5R*,8aS*)-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenedecahydronaphthalen-1-yl)methoxy)-3-(methoxy carbonyl)pentanedioic acid; 3). White powder, [α]D20 = −6.6 (c = 0.033, CHCl3); UV (MeOH); vmax (lge): 207 nm (1.57); IR (KBr) λmax: 3415 cm⁻¹; HR Q-TOF-MS m/z: 848.4812 ([M + Na]⁺, C₄₅H₆₆O₁₄: calcd. 848.4791); ¹H- and ¹³C-NMR: see Table 3.

3. Results and discussion
3.1. Structural elucidation

The morphological properties of isolate F070624009 were examined after incubation for 14 days at 25°C on PDA plates. This organism was identified as *Marasmius cladophyllus* according to the ITS rDNA sequence (ITS1-5.8S-ITS2). The fermentation culture was extracted with EtOAc/MeOH/AcOH (80:15:5, v/v). The extract was separated by repeated column chromatography (RP-18, Sephadex LH-20 and SiO₂) to afford compounds 1–7 (Scheme 1).

Compound 1 was isolated as a white powder. The molecular formula of 1 was determined to be C₁₇H₂₃O₄ according to the HR Q-TOF-MS and NMR data. The IR absorptions at 3285, 1650 and 1300 cm⁻¹ indicated the presence of OH, C = C and ether groups, respectively. Inspection of the ¹H- and ¹³C-NMR spectra (Table 1) revealed the presence of three methyl groups (two singlets at δ 1.05 and 0.96, one doublet at δ 1.06), three methylenes, six methines and three quaternary carbons, including one oxymethylene and three oxymethine groups that were attributed to four oxygen substitutions in I. The HMBC correlations from the protons of the two methyl groups at δ 1.05 (s, H-C(11)) and 0.96 (s, H-C(12)) to corresponding carbons determined one 5-carbon moiety (including C(2), C(3), C(4), C(11) and C(12)) (Figure 1). This 5-carbon moiety was composed of two methyls, one methylenes and one oxymethylene, indicating the presence of gem-dimethyl groups. The ¹H-¹H COSY correlations determined the structure of one more 8-carbon moiety including C(2), C(1), C(10), C(15), C(9), C(8), C(7) and C(14) (Figure 1). The connection between C(1) and C(5) was assigned based on the HMBC correlations between H-C(4) and C(1) and C(5), and between H-C(10) and C(5), and H-C(2) and C(5). The connection between C(6) and C(7) was assigned according to the ¹H-¹³C long-range correlations between H-C(14) to C(6), C(7) and C(13). The four oxygen substitutions was assigned at C(2), C(8), C(14) and C(13) based on their downshift of chemical shifts. The presence of three free hydroxyl groups was determined by the ESI–MS m/z 417.2 [M + Na]⁺ of the fully acetylated 1, which further supported the elucidation of an ether bond between C(13) and C(14). Based on the above, a tetrahydrofuran, cycloheptane and cyclopentane ring system was assigned to 1, which presents a tremulane sesquiterpene (Ayer and Cruz 1993; Wu et al. 2010). The relative configurations of 1 were determined by analysis of the ROESY spectrum.

The presence of NOE correlations between H-C(1) and H-C(2), and H-C(1) and H-C(8), H-C(1) and H-C(10), and H-C(1) and H-C(11) indicated the α-orientation of these protons and Me(11) (Figure 2). Further, the NOESY cross-peaks between H-C(7) and H-C(14), and H-C(7) and Me(15) established the β-orientation of H-C(7), H-C(14) and Me(15) (Figure 2). Thus, the structure of 1 was established to be (3R*,3aR*,4R*,6S*,6aS*,7S*)-6,8-trimethyl-1,3,3a,4,5,6a,7,8,9-decahydroazuleno[4,5-c]furan-3,4,7-triol, named as cladophyllol.

Compound 2 was obtained as a white powder. The molecular formula of 2 was determined to be C₁₅H₂₅O₄ according to the HR Q-TOF-MS and NMR data. [α]D²⁰ = +15 (c = 0.05, MeOH). The IR absorption at 3285 cm⁻¹ indicated the presence of hydroxyl groups. The ¹³C-NMR and DEPT spectra of 2 (Table 2) revealed the presence of 15 resonance signals attributed to 3 Me, 6 CH₂ and three quaternary carbons (one oxygenated), indicating a two-ring sesquiterpene backbone. The HMBC correlations from the protons of the three methyl groups at δ 1.00 (s, Me(13)), 0.80 (s, Me(14)) and 0.90 (s, Me(15)) to corresponding carbons determined a 9-carbon moiety (including C(3), C(4), C(5), Me(13) and Me(14), and C(1), C(10), C(9), C(15) and C(5)) (Figure 1), which determined a six-membered ring together with the ¹H-¹H COSY correlations between H-C(1)/H-C(2)/H-C(3). The ¹H-¹H COSY correlations (H-C(6)/H-C(7), H-C(9)/H-C(11)) and the HMBC correlations from H-C(7), H-C(11) and H-C(12) to corresponding carbons determined the substructure of another six-membered ring (Figure 1). The NOEs between H-C(3)
Table 3. $^1$H- and $^{13}$C-NMR data (600 and 150 MHz, resp.; in CD$_3$OD) of 3 and 4 (δ in ppm, J in Hz).

| H-Atom | 3        | 4        |
|--------|----------|----------|
| 1      | 39.9 (t) | 38.3 (t) |
| 2      | 18.1 (t) | 18.1 (t) |
| 3      | 35.4 (t) | 35.4 (t) |
| 4      | 36.8 (s) | 36.8 (s) |
| 5      | 46.4 (d) | 45.3 (d) |
| 6      | 23.1 (t) | 23.1 (t) |
| 7      | 37.2 (t) | 37.2 (t) |
| 8      | 148.0 (s)| 148.0 (s)|
| 9      | 55.2 (d) | 55.1 (d) |
| 10     | 39.3 (s) | 39.3 (s) |
| 11     | 70.5 (t) | 70.5 (t) |
| 12     | 106.6 (t)| 106.5 (t)|
| 13     | 13.9 (q) | 13.9 (q) |
| 14     | 16.8 (q) | 16.8 (q) |
| 15     | 70.8 (t) | 70.6 (t) |
| 1'     | 79.2 (d) | 79.2 (d) |
| 2'     | 44.3 (d) | 44.3 (d) |
| 3'     | 31.9 (t) | 31.9 (t) |
| 4'     | 171.6 (s)| 171.0 (s)|
| 5'     | 170.9 (s)| 171.3 (s)|
| 6'     | 172.3 (s)| 173.8 (s)|
| 1''    | 39.9 (t) | 38.7 (t) |
| 2''    | 18.1 (t) | 18.1 (t) |
| 3''    | 35.6 (t) | 35.6 (t) |
| 4''    | 36.8 (s) | 36.8 (s) |
| 5''    | 46.5 (d) | 45.4 (d) |
| 6''    | 23.1 (t) | 23.1 (t) |
| 7''    | 37.2 (t) | 37.2 (t) |
| 8''    | 148.0 (s)| 148.0 (s)|
| 9''    | 55.3 (d) | 55.7 (d) |
| 10''   | 39.3 (s) | 39.3 (s) |
| 11''   | 70.6 (t) | 70.5 (t) |
| 12''   | 106.6 (t)| 106.5 (t)|
| 13''   | 13.9 (q) | 13.9 (q) |
| 14''   | 16.8 (q) | 16.8 (q) |
| 15''   | 70.9 (t) | 72.2 (t) |
| 1'''   | 79.4 (d) | 79.3 (d) |
| 2'''   | 44.5 (d) | 44.4 (d) |
| 3'''   | 31.9 (t) | 31.9 (t) |
| 4'''   | 171.8 (s)| 171.0 (s)|
| 5'''   | 170.9 (s)| 171.3 (s)|
| 6'''   | 172.3 (s)| 173.8 (s)|
| MeO    | 51.1 (q) | 51.1 (q) |

Note: Assignments may be interchangeable in each vertical column.

and Me(13), H-C(9) and Me(13), H-C(5) and H-C(9) indicated the α-orientation of these protons and Me(13) (Figure 2). Furthermore, the NOESY cross peaks between H-C(12) and Me(15), and Me(14) and Me(15) indicated the β-orientation of C(12), Me(14) and Me(15) (Figure 2). Therefore, a drimane-type backbone was assigned to 2 (Corté et al. 1998), and the relative configuration of 2 was established. Thus, the structure of 2 was established to be (2S*,4aS*,5S*,6R*,8aR*)-5,6-bis(hydroxymethyl)-1,1,4α-trimethyldecahydroginaphthalene-2,6-diol, named as drimane-3,8,11,12-tetraol.

Compound 3 was obtained as a white powder. The molecular formula of 3 was determined to be C$_{45}$H$_{66}$O$_{14}$ according to the HR Q-TOF-MS and NMR data (Table 3). [α]$_{D}^{20}$ = −6.6 (c = 0.033, CHCl$_3$). The IR absorptions at 3450 and 1740, and 1725 cm$^{-1}$ were in accordance
with the presence of carboxylic and ester groups, respectively. The $^{13}$C-NMR and DEPT spectra of 3 revealed the presence of 45 resonance signals attributed to seven methyl, eighteen methylene, eight methine and twelve quaternary carbons including six carboxylic carbon atoms. The $^1$H-NMR data of 3 showed the presence of four tertiary methyls and three methoxyl groups. The $^1$H- and $^{13}$C-NMR spectra of 3 resembled with those of cryptoporic acid D (5), except for the presence of a methoxyl group at $\delta_C$ 51.1 (OMe) and $\delta_H$ 3.68 (OMe), and the upfield shift of one of the carbonyl groups (Asakawa et al. 1992). The upfield shifted carbonyl was determined to be C (6') by comparing with the $^{13}$C-NMR spectrum of cryptoporic acid D, determining the position of the extra methoxyl group at C(6'), which was further supported by the HMBC correlation from $\delta_H$ 3.68 (OMe) to C(6') (Figure 1). The NOEs between H-C(11) and H-C(13), and H-C(13) and H-C(14), indicated the $\beta$-orientation of those protons (Figure 2). Furthermore, the NOESY cross peaks between H-C(5) and H-C(9), H-C(9), and H-C(15) indicated the $\alpha$-orientation of
the protons (Figure 2). Therefore, the chemical structure of 3 was established and named as cryptoporric acid J.

The molecular formula of compound 4 was deduced as C_{43}H_{62}O_{14} according to NMR data and comparison with 3. The structure of 4 was elucidated on the basis of 1D- and 2D-NMR, and by comparing with those of cryptoporric acid D (5). The NMR spectra of 4 (Table 3) were similar to those of 5. The only difference was the methoxyl group at C(4') in 5 was replaced by a hydroxyl group in 4. Therefore, the structure of 4 was elucidated as cryptoporric acid K.

### 3.2. Biological study
The antibacterial activities of compounds 1–3 were tested against bacteria *Escherichia coli*, *Bacillus subtilis* and *Mycobacterial smegmatis* via the filter paper disc diffusion method. Each compound was performed at a concentration of 1.0 mg/ml with the loading volume 30 μl. Compound 3 had an inhibitory zone ca. 8 mm against *Bacillus subtilis*, while other compounds had no effects on the growth of tested bacteria at 30 μg/disc.

### 4. Conclusions
In this paper, we report the isolation and structure elucidation of a new tremulane sesquiterpene (1) and six drimane
sesquiterpenes (2–7). Cladophyllol (1) was elucidated as a new tremulane-type sesquiterpenoid. The tremulanes, a class of unusual sesquiterpenes, first isolated in 1993 from the aspen tree rotting fungus *Phellinus tremulae*, were isolated recently from cultures of the basidiomycete *Conocybe siliginea* and cultures of *Phellinusig niarius* (Ayer and Cruz 1993; Zhou et al. 2008; Wu et al. 2010). Tremulane sesquiterpenoids have been assessed for their significant vascular-relaxing activities against PE-induced vasoconstriction (Wu et al. 2010). Compounds 2–7 were elucidated as drimane-type sesquiterpenoids. Cryptoporic acid J (3) and cryptoporic acid K (4) are new dimmers of drimane-type sesquiterpenes, belonging to the subfamily of cryptoporic acids (CAs) as a type of unusual esters of citric/isocitric acid. Our work was the first isolation of CAs from the cultures of higher fungi, rather than from fruiting bodies. CAs showed inhibitory effect on the release of superoxide anion radicals and displayed diverse bioactivities, including anticarcinogenic and the prevention human diseases caused by ischemia and inflammation (Asakawa et al. 1992). Thus, the biological activities of novel compounds 1–4 need to be further explored.

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