Prenylated Phenolics from *Ganoderma fornicatum*

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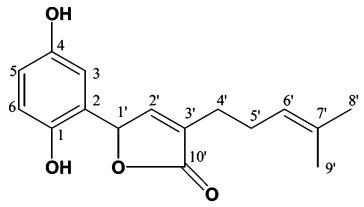
Received May 12, 2006

Three new prenylated phenolic compounds, fornicins A−C (**1**−**3**), were obtained from the fruiting bodies of *Ganoderma fornicatum*. The structure elucidation was achieved by interpretation of spectroscopic data. These compounds exhibited moderate cytotoxic activity with IC_{50} values of 15 to 30 μg/mL in Hep-2 cells.

Lingzhi (*Ganoderma* spp., Ganodermataceae) has been a well-known Chinese medicine since ancient times. Recorded under the superior rank of herbs, it is renowned for its health-promoting and tonic properties.[1] In the medicinal literature, over 10 *g*anoderma species, along with the well-known *G. lucidum* (Leyss. ex Fr.) Karst., are collectively known as Lingzhi. They are used as medicinal materials in various regions in China.[1−3] Most of the phytochemical and pharmacological investigations have been reported on *Ganoderma* triterpene acids or its polysaccharides.[4−8] In a previous phytochemical study on the fruiting bodies of *G. fornicatum* (Fr.) Pat., which is occasionally used as a substitute for *G. lucidum*, we obtained two novel nortriterpenoids, fornicatins A and B.[6] Further work has now led to the isolation of three prenylated phenolics, fornicins A−C (**1**−**3**), from the EtOAc-soluble fraction of an aqueous acetone extract. In this paper, we report the isolation and structural elucidation of **1**−**3**.



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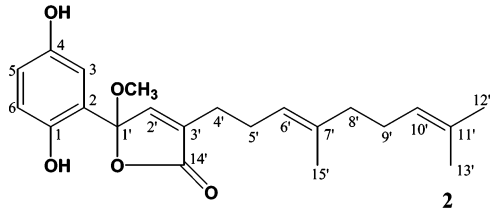

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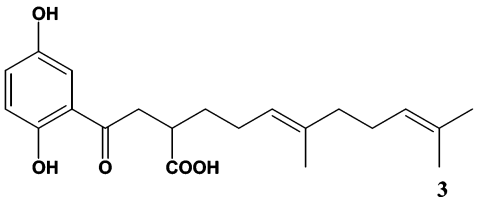

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Compound **1** displayed an $[M]^+$ ion at *m*/*z* 274 in the EIMS, consistent with a molecular formula of $C_{16}H_{18}O_4$. The ^{13}C and ^1H NMR spectra of **1** exhibited signals for two methyls, two methylenes, an oxymethine, five methines, five aromatic/olefinic quaternary carbons (two oxygenated), and an ester carbonyl carbon. The data suggested the presence of a 1,2,4-trisubstituted dihydroxybenzene structure connected to a 10-carbon side chain. The observation that the NMR data of **1** was similar to those of 2-geranyl-1,4-dihydroxybenzene[9,10] led to the establishment of a benzene oxygenation pattern as shown. It further revealed that C-1′ was oxygenated and the methyl group attached to C-3′ of the geranyl moiety was replaced by an ester carbonyl. Thus, the proton at δ_H 6.20 (d, $J = 1.4$ Hz, H-1′) exhibited HMBC long-range correlations with the ester carbonyl carbon (δ_C 174.6, C-10′) as well as the olefinic methine at δ_C 149.5 (C-2′), suggesting the formation of a γ-lactone between C-1′ and C-10′. Available data are consistent with the structure of 3-(4-methyl-3-pentenyl)-5-(2,5-dihydroxyphenyl)-2(5*H*)-furanone for fornicin A (**1**).

Compound **2** displayed a quasi-molecular ion peak at *m*/*z* 371 $[M - H]^-$ in the negative FABMS, in agreement with the molecular formula $C_{22}H_{28}O_5$. The NMR spectra of **2** suggested the presence of a 1,4-dihydroxybenzene connected to a 15-carbon side chain. Comparison of the NMR data with those of 2-farnesyl-1,4-dihydroxybenzene[10] disclosed that, in addition to the presence of a methoxy group in **2**, signals for a methylene and a methyl group of the farnesyl moiety were replaced by a downfield shifted ketacetal carbon at δ_C 107.6 (C-1′) and an ester carbonyl carbon (δ_C 171.6, C-14′), respectively. Further comparison of the NMR data of **2** with those of **1** suggested the presence of a γ-lactone in **2**. In the HMBC spectrum, long-range correlations were observed between C-1′ and H-3, H-2′, and OCH_3. The structure of fornicin B (**2**) was elucidated as 3-(4,8-dimethyl-3,7-nonadienyl)-5-(2,5-dihydroxyphenyl)-5-methoxy-2(5*H*)-furanone.

Compound **3** displayed a quasi-molecular ion at *m*/*z* 361 ($[M + H]^+$), consistent with the molecular formula $C_{21}H_{28}O_5$. It contained three methyls, five methylenes, six methines, five aromatic/olefinic quaternary carbons, a carboxyl group, and one conjugated ketone group, as shown by the ^{13}C and ^1H NMR data. The HMBC correlation between H-3 (δ_H 7.38, d, $J = 2.9$ Hz′) and the ketone carbon (δ_C 205.6, C-1′) revealed that the latter was attached to C-2 of the 1,4-dihydroxybenzene. Comparison of the NMR data of **3** with those of 2-farnesoyl-1,4-dihydroxybenzene[9] revealed the presence of a carboxylic acid at C-3′ and saturation between C-2′ and C-3′. The structure of fornicin C (**3**) was determined to be 2-(4,8-dimethyl-3,7-nonadienyl)-3-gentisoylpropanoic acid.

The biological effects of *G. lucidum* are generally attributed to triterpene ganoderic acids as the active principles. Thus far, we have not been able to find such triterpene acids from *G. fornicatum*. Instead, our findings indicate that the ingredients of this species belong to nortriterpenes and prenylated phenolics. From a chemical point of view, *G. fornicatum* should not be used as a substitute of *G. lucidum* due to the different chemical compositions in the two species.

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10.1021/np060218k CCC: $33.50
Published on Web 07/15/2006

Compounds **1**, **2**, and **3** were evaluated in vitro for cytotoxic activity against the Hep-2 (human larynx carcinoma) cell line as previously described.[11,12] They displayed moderate inhibitory activity, with IC_{50} values of 30, 15, and 23 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer, and IR spectra on a Bio-Rad FTS-135 infrared spectrophotometer. MS spectra were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR experiments were run on a DRX-500 instrument. Chemical shifts (δ) are expressed in ppm with reference to TMS. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10−40 μm, Qingdao Marine Chemical Inc., China), Lichroprep Rp_{18} gel (40−63 μm, Merck, Darmstadt, Germany), or MCI gel (70−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH.

Materials. The fruiting bodies of *Ganoderma fornicatum* (Fr.) Pat. (1.5 kg) were purchased from the Honghe Prefecture of Yunnan Province, People's Republic of China, and authenticated by Professor Zang Mu at the Kunming Institute of Botany. A voucher specimen (KIB 2003-02-10) has been deposited in the same institution.

Extraction and Isolation. The fruiting bodies (1.5 kg) were powdered and macerated in 70% aqueous acetone (3 × 10 L). The extract was filtered, concentrated under reduced pressure, and partitioned between water and EtOAc (3 × 2 L each). The EtOAc portion was brought to dryness to afford 95 g of residue, which was subjected to silica gel column chromatography using $CHCl_3$, $CHCl_3$/Me_2CO (9:1 → 8:2 → 7:3), and Me_2CO as eluents to obtain four fractions: fractions A (3 L, $CHCl_3$), B (4 L, $CHCl_3$/Me_2CO, 9:1), C (2 L, $CHCl_3$/Me_2CO, 4:1), and D (1.5 L, Me_2CO). Fraction B (45.2 g) was further separated over silica gel eluted by petroleum ether/Me_2CO (9:1 → 8:2 → 6:4 → 0:1) to obtain four fractions. Fraction B-2 (5.1 g, obtained from petroleum ether/Me_2CO, 8:2) was separated on a macroporous resin column (100 g) developed with aqueous CH_3OH (60% → 80% → 100) to obtain four fractions. Fraction B-2-2 (2.0 g, eluted from 60% CH_3OH) was rechromatographed over Rp_{18} gel (120 g) washing with aqueous CH_3OH (50% → 60% → 70%) to obtain three fractions. Compound **1** (36 mg) was purified from fraction B-2-2-1 (eluted from 50% CH_3OH) after silica gel column chromatography developed with $CHCl_3$/Me_2CO (15:1). Fraction B-2-2-2 (eluted from 60% CH_3OH) was repeatedly chromatographed over silica gel eluted by $CHCl_3$/Me_2CO (15:1) to afford compounds **2** (38 mg) and **3** (7 mg).

Fornicin A (1): gum; $[\alpha]^{23.0}_D$ +9.52 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 267.8 (4.1), 203.6 (4.6) nm; IR (KBr) ν_{max} 3426, 2923, 2854, 1728, 1641, 1507, 1456, 1377, 1297, 1194, 1083, 1054, 984, 869, 814, 742, 578 cm^{-1}; ^1H NMR (500 MHz, CD_3COCD_3), 7.35 (1H, d, J = 1.4 Hz, H-2′), 6.76 (1H, d, J = 8.6 Hz, H-6), 6.65 (1H, dd, J = 8.6 and 2.9 Hz, H-5), 6.53 (1H, d, J = 2.9 Hz, H-3), 6.20 (1H, d, J = 1.4 Hz, H-1′), 5.12 (1H, br t, J = 6.9 Hz, H-6′), 2.30 (2H, m, H-4′), 2.28 (2H, m, H-5′), 1.64 (3H, s, H-8′), 1.57 (3H, s, H-9′); ^{13}C NMR (125 MHz, CD_3COCD_3), 174.6 (C-10′), 151.3 (C-4), 149.5 (C-2′), 148.2 (C-1), 133.1 (C-7′), 132.7 (C-3′), 123.9 (C-6′), 123.6 (C-2), 117.0 (C-6), 116.8 (C-5), 113.2 (C-3), 78.3 (C-1′), 26.6 (C-4′), 25.9 (C-5′), 25.7 (C-8′), 17.7 (C-9′); EIMS *m*/*z* (rel int) 274 ($[M]^+$, 36), 256 (21), 241 (15), 229 (17), 218 (19), 213 (44), 204 (19), 161 (36), 149 (38), 137 (39), 123 (42), 111 (36), 97 (52), 85 (54), 69 (94), 57 (100); HRESIMS *m*/*z* 275.1281 $[M + H]^-$ (calcd for $C_{16}H_{19}O_4$, 275.1283).

Fornicin B (2): gum; $[\alpha]^{18.0}_D$ +7.69 (*c* 0.78, MeOH); UV (MeOH) λ_{max} (log ϵ) 268.6 (4.1), 226.2 (4.5) nm; IR (KBr) ν_{max} 3425, 2925, 2855, 1752, 1642, 1501, 1450, 1377, 1294, 1194, 1109, 1040, 970, 887, 823, 783, 566 cm^{-1}; ^1H NMR (500 MHz, CD_3COCD_3), 7.38 (1H, s, H-2′), 6.93 (1H, d, J = 2.8 Hz, H-3), 6.75 (1H, d, J = 8.6 Hz, H-6), 6.71 (1H, dd, J = 8.6 and 2.8 Hz, H-5), 5.11 (1H, br t, J = 7.1 Hz, H-6′), 5.06 (1H, br t, J = 6.8 Hz, H-10′), 3.52 (3H, s, OCH_3), 2.32 (2H, m, H-4′), 2.27 (2H, m, H-5′), 2.00 (2H, m, H-9′), 1.92 (2H, m, H-8′), 1.63 (3H, s, H-12′), 1.56 (3H, s, H-13′), 1.54 (3H, s, H-15′); ^{13}C NMR (125 MHz, CD_3COCD_3), 171.6 (C-14′), 151.1 (C-4), 148.7 (C-1), 146.9 (C-2′), 137.1 (C-7′), 135.8 (C-3′), 131.6 (C-11′), 125.0 (C-10′), 123.6 (C-6′), 123.8 (C-2), 118.3 (C-6), 118.0 (C-5), 114.1 (C-3), 107.6 (C-1′), 56.9 (OCH_3), 43.0 (C-8′), 27.2 (C-9′), 26.1 (C-4′), 25.8 (C-12′), 25.7 (C-5′), 17.7 (C-13′), 16.1 (C-15′); negative FABMS *m*/*z* (rel int) 371 ($[M - H]^-$, 47), 340 (100), 311 (18), 202 (52), 175 (40), 159 (43), 108 (22), 79 (12); HRESIMS *m*/*z* 371.1847 $[M - H]^-$ (calcd for $C_{22}H_{27}O_5$, 371.1858).

Fornicin C (3): gum; $[\alpha]^{24.0}_D$ +28.15 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 268.2 (4.2), 262.0 (4.2), 203.4 (4.6) nm; IR (KBr) ν_{max} 3440, 2924, 2854, 1708, 1629, 1484, 1457, 1377, 1273, 1176, 831, 792, 730, 577 cm^{-1}; ^1H NMR (500 MHz, CD_3COCD_3), 7.38 (1H, d, J = 2.9 Hz, H-3), 7.08 (1H, dd, J = 8.9 and 2.9 Hz, H-5), 6.79 (1H, d, J = 8.9 Hz, H-6), 5.18 (1H, br t, J = 7.1 Hz, H-6′), 5.10 (1H, br t, J = 6.9 Hz, H-10′), 3.50 (1H, dd, J = 17.9 and 9.2 Hz, H-2′a), 3.16 (1H, dd, J = 17.9 and 4.4 Hz, H-2′b), 3.03 (1H, m, H-3′), 2.14 (2H, m, H-5′), 2.05 (2H, m, H-9′), 1.99 (2H, m, H-8′), 1.87 (2H, m, H-4′a), 1.67 (2H, m, H-4′b), 1.64 (3H, s, H-15′), 1.61 (3H, s, H-12′), 1.58 (3H, s, H-13′); ^{13}C NMR (125 MHz, CD_3COCD_3), 205.6 (C-1′), 176.4 (C-14′), 156.3 (C-1), 150.2 (C-4), 136.4 (C-7′), 131.6 (C-11′), 125.6 (C-5), 125.0 (C-10′), 124.3 (C-6′), 120.0 (C-2), 119.3 (C-6), 115.4 (C-3), 40.5 (C-2′), 40.3 (C-8′), 40.1 (C-3′), 32.6 (C-4′), 27.3 (C-9′), 26.1 (C-5′), 25.7 (C-12′), 17.7 (C-13′), 16.1 (C-15′); positive FABMS *m*/*z* (rel int) 361 ($[M + H]^+$, 100), 342 (26), 278 (28); HRESIMS *m*/*z* 383.1833 $[M - H + Na]^-$ (calcd for $C_{21}H_{28}O_5Na$, 383.1834).

Bioassay. Cytotoxicity assay using Hep-2 cells was performed according to standard procedures for the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reduction method.[11,12] Cisplatin was used as positive control. The optical densities (OD) were read at 540 nm. The IC_{50} values were calculated as the concentration of the sample that decreased the number of viable cells by 50% in comparison to the control.

Acknowledgment. This study was partially supported by a grant from the Hong Kong Research Grants Council.

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NP060218K