A New 18(13 → 12β)-*abeo*-Lanostadiene Triterpenoid from *Ganoderma fornicatum*

by **Ying Qiao**[a])[b]), **Xian-Min Zhang**[a]), **Xue-Chang Dong**[b]), and **Ming-Hua Qiu***[a])

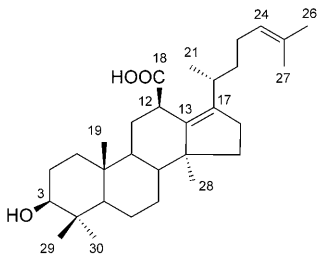
[a]) State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, P. R. China (fax: +86-871-5150227; e-mail: mhchiu@public.km.yn.cn or mhchiu@mail.kib.ac.cn)

[b]) School of Chemistry and Biotechnology, Yunnan Nationalities University, Kunming 650031, P.R. China

A new triterpenoid, fornicatin C (=(3β)-3-hydroxy-18(13 → 12β)-*abeo*-lanosta-13(17),24-dien-18-oic acid; **1**), was isolated from the fruiting bodies of *Ganoderma fornicatum*, together with the known compounds fornicatin A (**2**) and fornicatin B (**3**), among other constituents. The structure of **1** was elucidated by means of spectroscopic techniques, and those of **2** and **3** were identified by comparing their spectroscopic data with those reported in the literature.

Introduction. – *Ganoderma lucidum* is a well-known woody mushroom called '*lingzhi*' in China, and '*reishi*' in Japan. Although *Ganoderma* species have been used for millennia in Chinese and Japanese traditional medicine for the treatment of several types of diseases, systematic research of their chemical constituents started only some two decades ago. So far, more than 130 highly oxygenated lanostane-type triterpenoids have been isolated from their fruiting bodies, spores, and culture mycelia, including common fungal steroids derived from ergosterol. Some of these compounds were shown to exhibit diverse bioactivities.

As part of our recent investigation into constituents of *Ganoderma fornicatum*, a similarly famous traditional folk medicine, we herein describe the isolation and structure elucidation of the new triterpenoid fornicatin C (**1**), which was obtained from the fruiting bodies of '*lingzhi*', together with the known compounds fornicatin A (**2**), fornicatin B (**3**) [1], as well as ergosterol [2], ergosterol peroxide [3], polycarpol [4], and tsugaric acid A [5].



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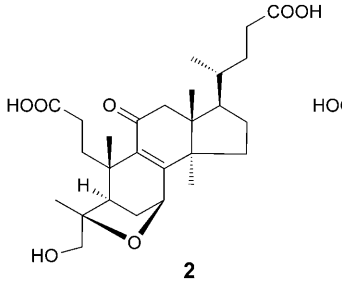

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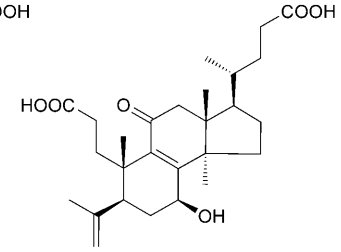

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Table. *^{1}H- and ^{13}C-NMR Data of* **1**. At 500/125 MHz, resp., in C_5D_5N; δ in ppm, J in Hz.

Position	δ(C)	δ(H)	Position	δ(C)	δ(H)
CH_2(1)	34.07	1.88–1.93 (*m*, H_α), 1.27–1.32 (*m*, H_β)	CH_2(16)	31.27	1.82–1.90 (*m*, H_α), 1.12–1.19 (*m*, H_β)
CH_2(2)	28.51	1.85–1.94 (*m*, H_α), 1.23–1.26 (*m*, H_β)	C(17)	137.33	–
H–C(3)	78.34	3.43 (*dd*, J=4.2, 3.9, H_α)	C(18)	179.17	–
C(4)	39.42	–	Me(19)	19.70	1.15 (*s*)
H–C(5)	52.35	1.12–1.19 (*m*, H_α)	H–C(20)	35.40	1.85–1.94 (*m*)
CH_2(6)	19.12	1.82–1.88 (*m*, H_α), 1.50–1.56 (*m*, H_β)	Me(21)	18.58	1.02 (*d*, J=5.4)
CH_2(7)	22.54	1.55–1.59 (*m*, H_α), 1.00–1.04 (*m*, H_β)	CH_2(22)	35.10	1.48–1.56 (*m*, H_α), 1.24–1.32 (*m*, H_β)
H–C(8)	51.24	2.45 (*t*, J=8.7, 2.5)	CH_2(23)	23.19	2.02–2.07 (*m*, H_α), 1.80–1.89 (*m*, H_β)
H–C((9)	44.93	1.80–1.91 (*m*)	H–C(24)	125.60	5.24 (*t*, J=5.1)
C(10)	35.70	–	C(25)	131.50	–
CH_2(11)	27.06	2.11–2.17 (*m*, H_α), 2.02–2.07 (*m*, H_β)	Me(26)	25.76	1.59 (*s*)
H–C(12)	48.95	2.38–2.41 (*m*, H_α)	Me(27)	17.70	1.59 (*s*)
C(13)	145.21	–	Me(28)	26.68	1.00 (*s*)
C(14)	47.85	–	Me(29)	16.40	1.06 (*s*)
CH_2(15)	33.77	2.37–2.41 (*m*, H_α), 2.28–2.31 (*m*, H_β)	Me(30)	28.67	1.23 (*s*)

Results and Discussion. – The dried, chipped fruiting bodies of *G. fornicatum* were extracted with EtOH. The extract was filtered, concentrated *in vacuo* to a suitable volume, suspended in H_2O, and then successively extracted with petroleum ether, AcOEt, and BuOH. Repeated column chromatography of the AcOEt extract finally yielded **1**.

Compound **1** was obtained as a crystalline, high-melting (m.p. > 300°), and optically active ($[\alpha]_D^{19} = +19.6$ (c=0.3, MeOH)) solid. In the FAB mass spectrum, the $[M+H]^+$ peak was observed at *m/z* 457, in accord with the molecular formula $C_{30}H_{48}O_3$, as confirmed by HR-ESI-MS and NMR analyses. The IR spectrum of **1** displayed absorptions for a OH (3390) and COOH (3200–2500 and 1682 cm^{-1}) groups. The ^{1}H-NMR and ^{13}C-NMR data (*Table*) indicated the presence of a triterpenoid. HMBC and HMQC experiments allowed the assignment of all H- and C-atoms, and ROESY data provided the relative configuration of **1**.

The ^{1}H-NMR spectrum of **1** (*Table*) exhibited six Me *singlet*s at δ(H) 1.00, 1.06, 1.12, 1.23, 1.59, 1.59 (3 H each), and one Me *doublet* at δ(H) 1.02 (J=5.4 Hz). The signal at δ(H) 3.43 (*dd*, J=4.2, 3.9 Hz, H_α–C(3)) indicated a β-oriented 3-OH group, as in tsugaric acid A [5]. The ^{13}C-NMR (DEPT) spectra of **1** showed signals for 30 C-atoms: seven Me, nine CH_2, and seven CH groups, and seven quaternary C-atoms. The olefinic signals at δ(C) 144.21 and 137.33, and at 125.60 and 131.50, corresponded to C=C bonds between C(13) and C(17), and between C(24) and C(25), respectively. In addition, a COOH group was inferred from the signal at δ(C) 179.17.

Comparison of the ^{13}C-NMR spectrum of **1** with that of tsugaric acid A revealed that the CH_2(12) group present in ganoderic acid B had disappeared in **1**. Instead, a

methine signal was observed at δ(C) 48.95 (*d*), giving rise to the tetrasubstituted C(13)=C(17) bond. In the HMBC spectrum of **1** (*Figure*), the signal at δ(H) 2.03 (H–C(20)) was correlated with the signal at δ(C) 137.33 (C(17)); the signals at δ(H) 2.40 and 2.31 (CH_2(15)) were correlated with δ(C) 137.33 (C(17)) and 145.21 (C(13)), which supported the presence of a C(13)=C(17) bond. These data suggested a 18(13 → 12β)-*abeo*-lanostadiene triterpenoid, with a structure very similar to that of ananosic acid A [6].

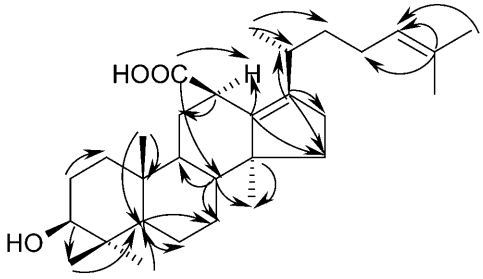

Figure. *Key HMBC correlations for* **1**

From the above results, the structure of compound **1** was identified as (3β)-3-hydroxy-18(13 → 12β)-*abeo*-lanosta-13(17),24-dien-18-oic acid[1]). As far as we know, compound **1** is a novel triterpenoid, and was given the name *fornicatin C*.

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Experimental Part

General. Melting points (m.p.): *XRB-1* hot-stage micro-melting-point apparatus; uncorrected. Optical rotations: *DIP-370* automatic polarimeter. IR Spectra: *Bio-Rad FTS-135* infrared spectrophotometer; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. FAB-MS: *Autospec-3000* spectrometer; in *m*/*z*. HR-ESI-MS: *API QSTAR Pulsar I* spectrometer.

Plant Material. The fruiting bodies of *Ganoderma fornicatum* were purchased from the Kunming medicinal market, Yunnan Province, P.R. China, in August 2004. The plant material was identified by Prof. *Yang Zhuliang*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUN No. 040823) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried, chipped fruiting bodies of *G. fornicatum* (8 kg) were extracted with EtOH. The extract was filtered, concentrated *in vacuo* to a suitable volume, suspended in H_2O, and then extracted with petroleum ether (PE), AcOEt, and BuOH, in this order. The AcOEt extract was concentrated *in vacuo* to afford a residue (109 g), which was subjected to column chromatography (CC) (SiO_2; $CHCl_3$ → $CHCl_3$/MeOH 1:1): six fractions (*Fr. 1*–*Fr. 6*). *Fr. 1* (37.6 g) was repeatedly subjected to CC (SiO_2; PE → PE/acetone 3:1) to afford ergosterol (20.57 g) and ergosterol peroxide (313 mg).

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[1]) Alternative name: (3β,5α,12β)-17-[(1*R*)-1,5-dimethylhex-4-en-1-yl]-3-hydroxy-4,4,10,14-tetramethylgon-13(17)-ene-12-carboxylic acid.

Fr. 2 (9.5 g) was rechromatographed (SiO_2; PE/acetone 10:1, 5:1, and 2:1) to yield polycarpol (21 mg). *Fr. 3* (5.7 g) was also subjected to CC (SiO_2; PE/acetone 2:1 and 1:2), and the resulting subfraction *Fr. 3.4* (2.8 g) was separated by reverse-phase CC (C_{18}; 40 → 65% aq. MeOH) to give tsugaric acid A (12 mg) and **1** (13 mg). *Fr. 4* (22.1 g) was repeatedly subjected to CC (SiO_2; $CHCl_3$ → $CHCl_3$/MeOH 20:1) to afford **2** (129 mg) and **3** (327 mg).

Fornicatin C (=*(3β)-3-Hydroxy-18(13 → 12β)*-abeo-*lanosta-13(17),24-dien-18-oic Acid*; **1**)[1]). Crystalline solid. M.p. >300°. $[\alpha]_D^{19} = 19.6$ ($c = 0.3$, MeOH). IR (KBr): 3390 (br., OH), 3200–2500 (COOH), 1682 (C=O), 1253, 1014. ^{1}H- and ^{13}C-NMR: see *Table*. FAB-MS (pos.): 457 ($[M+H]^+$). HR-ESI-MS: 456.3503 (M^+, $C_{30}H_{48}O_3^+$; calc. 456.3604), 479.3503 ($[M+Na]^+$, $C_{30}H_{48}NaO_3^+$; calc. 479.3501.

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