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陆地革菌的化学成分研究

周忠玉[1,2*]，谭建文[1]，刘吉开[2]

(1. 中国科学院华南植物园，中国科学院植物资源保护与可持续利用重点实验室，广州 510650；2. 中国科学院昆明植物研究所，植物化学与西部植物资源持续利用国家重点实验室，昆明 650204)

摘要： 从陆地革菌(Thelephora terrestris)子实体中分离得到9个已知化合物，经波谱学分析鉴定为：(22E,24R)-麦角甾-7,22-二烯-3β-醇(**1**)，(22E,24R)-麦角甾-7,22-二烯-3β,5α,6β-三醇(**2**)，(22E,24R)-麦角甾-4,6,8(14),22-四烯-3-酮(**3**)，24-亚甲基羊毛甾-8-烯-3β-醇(**4**)，熊果酸(**5**)，木栓酮(**6**)，cerebroside B(**7**)，(2S,3S,4R,2′R)-2-(2′-羟基二十二碳酰氨基)-十八碳烷-1,3,4-三醇(**8**)，(2S,3S,4R,2′R)-2-(2′-羟基二十三碳酰氨基)-十八碳烷-1,3,4-三醇(**9**)。

关键词： 陆地革菌；化学成分；麦角甾醇；三萜；神经酰胺

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Chemical Constituents of *Thelephora terrestris*

ZHOU Zhong-yu[1,2*], TAN Jian-wen[1], LIU Ji-kai[2]

(1. Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China; 2. State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China)

Abstract: Nine known compounds have been isolated from the fruiting bodies of Thelephora terrestris. Their structures were established as (22E,24R)-ergosta-7,22-dien-3β-ol (**1**), cerevisterol (**2**), (22E,24R)-ergosta-4,6,8(14),22-tetraen-3-one (**3**), 24-methylenelanost-8-en-3β-ol (**4**), ursolic acid (**5**), friedelin (**6**), cerebroside B (**7**), (2S,3S,4R,2′R)-2-(2′-hydroxydocosanoylamino) octadecane-1,3,4-triol (**8**), and (2S,3S,4R,2′R)-2-(2′-hydroxytricosanoylamino) octadecane-1,3,4-triol (**9**).

Key words: Thelephora terrestris; Chemical constituents; Ergosterols; Triterpenoid; Ceramide

The genus Thelephora belongs to the order Thelephorales and family Thelephoraceae, and is a rich source in p-terphenyl compounds[1-6]. Previous investigations of basidiomycetes of this genus reported the isolation of ganbajunins A-G from T. ganbajun[1,3], aurantiotinin A from T. aurantiotincta[2], and terrestrins A-G, ganbajunin B, thelephantins F and H from T. terrestris[7]. As part of our search for naturally occurring bioactive substances from higher fungi in China, we investigated the constituents of the fruiting bodies of T. terrestris collected from Yunnan province in China and isolated a series of structurally diverse compounds, including three ergosterols (**1-3**), three triterpenoids (**4-6**), and three ceramides (**7-9**).

1 Experiment

1.1 Instrument

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were measured in Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on a Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals.

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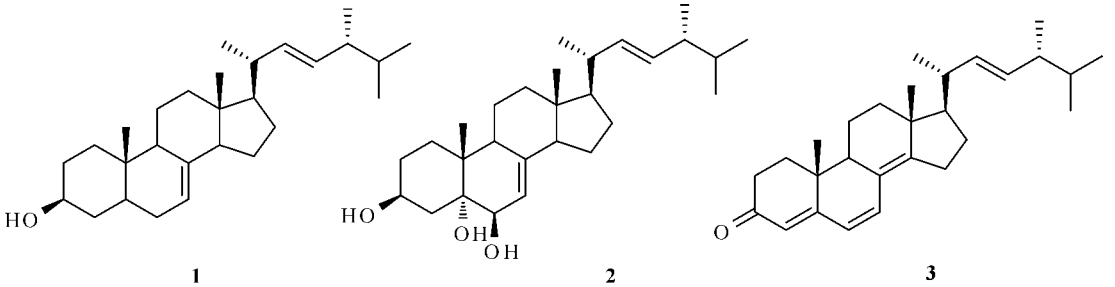
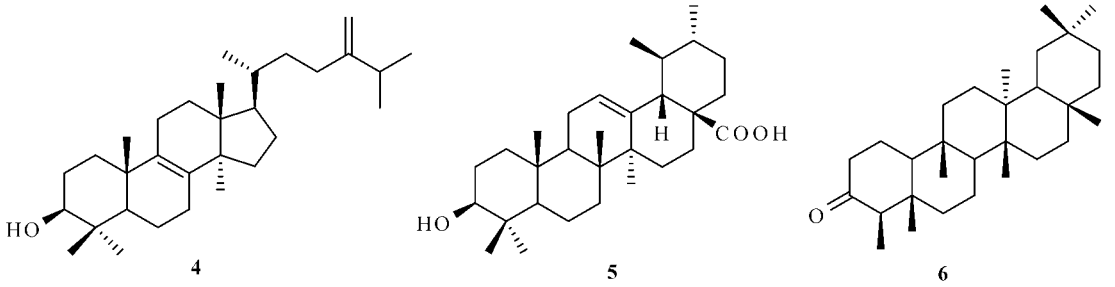
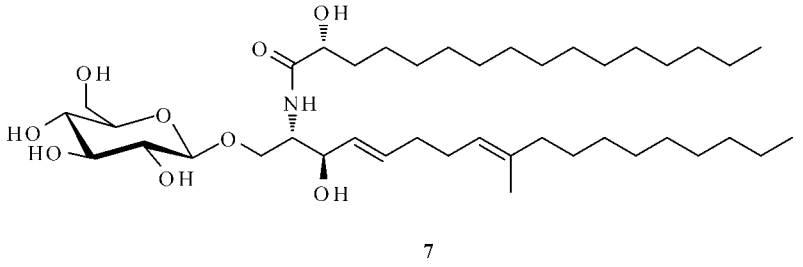
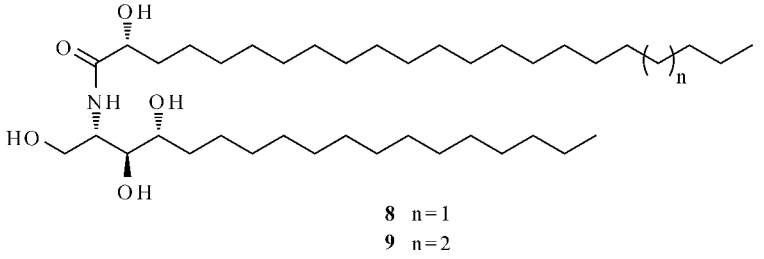
* Corresponding author. E-mail: zhouzhongyu@scbg.ac.cn

FAB-MS were recorded with a VG Autospec-3000 spectrometer. EI-MS were recorded with a VG Autospec-3000 spectrometer. ESI-MS and HRESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4 mm × 150 mm) column. Preparative MPLC was performed on Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605 and manager C-615. Silica gel (200 – 300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), RP-C18 silica gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.









1.2 Fungal material

The fungus T. terrestris was collected from Ailao Mountain of Yunnan Province, China, in July 2005, and identified by Prof. Mu Zang, Kunming Institute of Botany. The voucher specimen was deposited at the Herbarium of Kunming Institute of Botany, Chinese

Academy of Sciences.

1.3 Extraction and isolation

Dry fruiting bodies (120 g) were extracted three times with a mixture of chloroform and methanol (1∶1, V/V). The solvent was evaporated in vacuo to give a crude extract (8 g), which was applied to silica gel column chromatography (200–300 mesh) eluted with a petroleum ether-acetone gradient (100∶0–0∶100 gradient system) to afford fractions A–G. Fraction A, eluted with petroleum ether-acetone (95∶5, V/V), was separated by silica gel (petroleum ether-ethyl acetate, 5∶1) and Sephadex LH-20 ($CHCl_3$-MeOH, 1∶1, V/V) column chromatography to obtain **3** (1.8 mg) and **6** (2.3 mg). Fraction B, eluted with petroleum ether-acetone (90∶10, V/V), was subjected to silica gel column chromatography and eluted with pure $CHCl_3$ to give subfractions B1 and B2. After repeated silica gel and Sephadex LH-20 ($CHCl_3$-MeOH, 1∶1, V/V) column chromatography, compounds **1** (9.6 mg) from B1 and **4** (7.2 mg) from B2 were obtained. Fraction C, eluted with petroleum ether-acetone (80∶20, V/V), was divided into subfractions C1 and C2 by passage through a silica gel column using $CHCl_3$-MeOH (50∶1–10∶1). Subfraction C1 was subjected to Sephadex LH-20 ($CHCl_3$-MeOH, 1∶1, V/V) and reversed phase silica gel (MeOH-H_2O, 30%–50%) column chromato-graphy to provide **2** (5.6 mg). Subfraction C2 was further separated by repeated reversed phase silica gel column chromatography (MeOH-H_2O, 25%–50%) to afford **5** (3.1 mg). Repeated column chromatography over silica gel and Sephadex LH-20 gave a mixture (19.3 mg) of **8** and **9** from fraction D, eluted with petroleum ether-acetone (70∶30, V/V), and **7** (10.2 mg) from fraction E, eluted with petroleum ether-acetone (50∶50, V/V), respectively.

2 Structure identification

(22*E*,24*R*)-Ergosta-7,22-dien-3β-ol (1) [8]

$C_{28}H_{46}O$; colorless needles; EI-MS m/z (%): 398 $[M]^+$ (34), 383 $[M-Me]^+$ (15), 300 (20), 271 (100), 255 (76); ^1H NMR ($CDCl_3$, 400 MHz): δ 5.12 (2H, m, H-7, H-22, H-23), 3.58 (1H, m, H-3), 0.99 (3H, d, J = 6.6 Hz, H-21), 0.88 (3H, d, J = 6.8 Hz, H-28), 0.82 (3H, d, J = 6.3 Hz, H-27), 0.80 (3H, d, J = 6.3 Hz, H-26), 0.77 (3H, s, H-19), 0.52 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 37.1 (t, C-1), 31.4 (t, C-2), 71.0 (d, C-3), 38.0 (t, C-4), 40.2 (d, C-5), 29.6 (t, C-6), 117.4 (d, C-7), 139.6 (s, C-8), 49.4 (d, C-9), 34.2 (s, C-10), 21.5 (t, C-11), 39.4 (t, C-12), 43.3 (s, C-13), 55.1 (d, C-14), 22.9 (t, C-15), 28.1 (t, C-16), 56.0 (d, C-17), 12.1 (q, C-18), 13.0 (q, C-19), 40.5 (d, C-20), 21.1 (q, C-21), 135.7 (d, C-22), 131.8 (d, C-23), 42.8 (d, C-24), 33.1 (d, C-25), 19.9 (q, C-26), 19.6 (q, C-27), 17.7 (q, C-28).

Cerevisterol (2) [9] $C_{28}H_{46}O_3$; colorless needles; EI-MS m/z (%): 430 $[M]^+$, 412 $[M-H_2O]^+$ (35), 394 $[M-2H_2O]^+$ (37), 379 (65), 376 $[M-3H_2O]^+$ (15), 269 (33), 251 (62), 69 (100); ^1H NMR (C_5D_5N, 400 MHz): δ 5.74 (1H, s, H-7), 5.24 (1H, dd, J = 15.3, 7.4 Hz, H-23), 5.16 (1H, dd, J = 15.3, 8.3 Hz, H-22), 4.84 (1H, m, H-3), 4.32 (1H, br. d, J = 4.8 Hz, H-6), 1.52 (3H, s, H-19), 1.07 (3H, d, J = 6.4 Hz, H-21), 0.94 (3H, d, J = 6.8 Hz, H-28), 0.85 (3H, d, J = 6.7 Hz, H-27), 0.84 (3H, d, J = 6.7 Hz, H-26), 0.67 (3H, s, H-18); ^{13}C NMR (C_5D_5N, 100 MHz): δ 33.8 (t, C-1), 32.6 (t, C-2), 67.6 (d, C-3), 42.0 (t, C-4), 76.5 (s, C-5), 74.3 (d, C-6), 120.5 (d, C-7), 141.6 (s, C-8), 43.8 (d, C-9), 38.1 (s, C-10), 22.4 (t, C-11), 40.1 (t, C-12), 43.9 (s, C-13), 55.2 (d, C-14), 23.5 (t, C-15), 28.2 (t, C-16), 56.5 (d, C-17), 12.3 (q, C-18), 18.8 (q, C-19), 40.7 (d, C-20), 21.4 (q, C-21), 136.2 (d, C-22), 132.5 (d, C-23), 43.0 (d, C-24), 33.1 (d, C-25), 19.9 (q, C-26), 20.1 (q, C-27), 17.6 (q, C-28).

(22*E*,24*R*)-Ergosta-4,6,8(14),22-tetraen-3-one (3) [10] $C_{28}H_{40}O$; yellow needles; EI-MS m/z (%): 392 $[M]^+$ (15), 377 (3), 349 (4), 268 (100), 253 (30), 214 (26), 173 (23), 69 (47); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.58 (1H, d, J = 9.4 Hz, H-7), 6.00 (1H, d, J = 9.4 Hz, H-6), 5.70 (1H, s, H-4), 5.24 (1H, dd, J = 15.2, 7.2 Hz, H-23), 5.18 (1H, dd, J = 15.2, 7.2 Hz, H-22), 1.21–2.53 (18H, m, steroid necleus), 1.03 (3H, d, J = 6.6 Hz, H-21), 0.97 (3H, m, H-19), 0.93 (3H, s, H-18), 0.90 (3H, d, J = 6.8 Hz, H-28), 0.82 (3H, d, J = 6.8 Hz,

H-27), 0.78 (3H, d, J = 6.6 Hz, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 34.1 (t, C-1), 19.0 (t, C-2), 199.3 (s, C-3), 123.0 (d, C-4), 164.2 (s, C-5), 124.5 (d, C-6), 133.9 (d, C-7), 124.3 (s, C-8), 44.0 (d, C-9), 36.8 (s, C-10), 25.4 (t, C-11), 34.2 (t, C-12), 44.0 (s, C-13), 156.0 (s, C-14), 35.7 (t, C-15), 27.7 (t, C-16), 55.8 (d, C-17), 16.7 (q, C-18), 18.9 (q, C-19), 39.2 (d, C-20), 21.2 (q, C-21), 135.0 (d, C-22), 132.6 (d, C-23), 42.9 (d, C-24), 33.1 (d, C-25), 19.7 (q, C-26), 20.0 (q, C-27), 17.6 (q, C-28).

24-Methylenelanost-8-en-3β-ol (4) [11] $C_{31}H_{52}O$; white needles; EI-MS m/z (%): 440 [M]$^+$ (43), 425 (100), 407 (52), 341 (18), 259 (39), 241 (35), 187 (25), 69 (77); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.72 (1H, s, H-31a), 4.66 (1H, s, H-31b), 3.24 (1H, dd, J = 11.5, 4.4 Hz, H-3), 1.03 (3H, d, J = 6.6 Hz, H-26 or H-27), 1.02 (3H, d, J = 6.6 Hz, H-27 or H-26), 1.00 (3H, s, H-19), 0.98 (3H, s, H-30), 0.92 (3H, d, J = 6.6 Hz, H-21), 0.88 (3H, s, H-28), 0.81 (3H, s, H-29), 0.69 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 35.6 (t, C-1), 27.9 (t, C-2), 79.0 (d, C-3), 38.9 (s, C-4), 50.5 (d, C-5), 18.3 (t, C-6), 28.2 (t, C-7), 134.5 (s, C-8), 134.5 (s, C-9), 37.1 (s, C-10), 21.0 (t, C-11), 26.5 (t, C-12), 44.6 (s, C-13), 49.9 (s, C-14), 31.1 (t, C-15 or 16), 30.9 (t, C-16 or 15), 50.5 (d, C-17), 26.5 (q, C-18), 18.7 (q, C-19), 36.5 (d, C-20), 19.2 (q, C-21), 35.1 (t, C-22), 31.3 (t, C-23), 156.9 (s, C-24), 33.9 (d, C-25), 22.0 (q, C-26), 21.9 (q, C-27), 15.4 (q, C-28), 28.0 (q, C-29), 24.3 (q, C-30), 106.0 (t, C-31).

Ursolic acid (5) [12] $C_{30}H_{48}O_3$; white amorphous powder; EI-MS m/z (%): 456 [M]$^+$ (1), 248 (100), 203 (45), 133 (35); ^{1}H NMR ($CDCl_3$/CD_3OD, 400 MHz): δ 5.19 (1H, t, J = 3.5 Hz, H-12), 3.36 (1H, dd, J = 10.1, 5.7 Hz, H-3), 1.04 (3H, s, H-23), 0.94 (3H, s, H-27), 0.89 (3H, J = 6.6 Hz, H-29), 0.86 (3H, s, H-26), 0.82 (3H, J = 6.2 Hz, H-30), 0.76 (3H, s, H-24), 0.73 (3H, s, H-25); ^{13}C NMR ($CDCl_3$/CD_3OD, 100 MHz): δ 38.5 (t, C-1), 26.9 (t, C-2), 78.9 (d, C-3), 38.6 (s, C-4), 55.1 (d, C-5), 18.2 (t, C-6), 32.9 (t, C-7), 39.4 (s, C-8), 47.5 (d, C-9), 36.9 (s, C-10), 23.2 (t, C-11), 125.4 (d, C-12), 138.1 (s, C-13), 42.0 (s, C-14), 27.9 (t, C-15), 24.1 (t, C-16), 47.7 (s, C-17), 52.7 (d, C-18), 39.0 (d, C-19), 38.8 (d, C-20), 30.6 (t, C-21), 36.7 (t, C-22), 28.0 (q, C-23), 15.5 (q, C-24), 15.3 (q, C-25), 16.9 (q, C-26), 23.4 (q, C-27), 180.6 (s, C-28), 16.8 (q, C-29), 21.1 (q, C-30).

Friedelin (6) [13] $C_{30}H_{50}O$; colorless needles; EI-MS m/z (%): 426 [M]$^+$ (57), 411 (21), 218 (56), 205 (64), 191 (33); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16 (3H, s, H-28), 1.02 (3H, s, H-27), 0.98 (3H, s, H-26), 0.97 (3H, s, H-29), 0.93 (3H, s, H-30), 0.85 (3H, J = 6.6 Hz, H-23), 0.85 (3H, s, H-25), 0.70 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.3 (t, C-1), 41.5 (t, C-2), 213.2 (s, C-3), 58.2 (d, C-4), 42.1 (d, C-5), 41.3 (t, C-6), 18.2 (t, C-7), 53.1 (d, C-8), 37.4 (s, C-9), 59.4 (d, C-10), 35.4 (t, C-11), 30.5 (t, C-12), 39.7 (s, C-13), 38.3 (s, C-14), 32.7 (t, C-15), 36.0 (t, C-16), 30.0 (s, C-17), 42.8 (d, C-18), 35.3 (t, C-19), 28.2 (s, C-20), 32.4 (t, C-21), 39.2 (t, C-22), 6.8 (q, C-23), 14.6 (q, C-24), 17.9 (q, C-25), 20.2 (q, C-26), 18.7 (q, C-27), 32.1 (q, C-28), 31.8 (q, C-29), 35.0 (q, C-30).

Cerebroside B (7) [14] $C_{41}H_{77}NO_9$; white amorphous powder; FAB-MS (neg.) m/z (%): 726 [M − H]$^-$, 565 [M − 162]$^-$; ^{1}H NMR (C_5D_5N, 400 MHz): δ 8.35 (1H, d, J = 8.4 Hz, NH), 5.73 (2H, m, H-4, H-5), 5.23 (1H, m, H-8), 4.90 (1H, d, J = 7.8 Hz, H-1′′), 4.75 (1H, m, H-2), 4.69 (1H, dd, J = 10.7, 5.4 Hz, H-1a), 4.57 (1H, m, H-2′′), 4.48 (1H, d, J = 11.8 Hz, H-6a′′), 4.33 (1H, dd, J = 11.8, 5.0 Hz, H-6b′′), 4.20 (2H, m, H-1, H-3′′), 4.03 (1H, m, H-2′′), 3.89 (1H, m, H-5′′), 2.14 (4H, m, H-6, H-7), 2.00 (3H, m, H-10, H-3′), 1.74 (1H, br. s, H-3′), 1.61 (3H, s, H-19), 1.37 (2H, m, H-11), 1.25 (8H, m, H-12 ~ 15), 0.89 (6H, t, J = 6.7 Hz, H-18, H-16′); ^{13}C NMR (C_5D_5N, 100 MHz): δ 69.8 (t, C-1), 54.5 (d, C-2), 72.8 (d, C-3), 131.6 (d, C-4), 132.2 (d, C-5), 32.8 (t, C-6), 31.9 (t, C-7), 124.0 (d, C-8), 136.1 (s, C-9), 39.8 (t, C-10), 28.1 (t, C-11), 29.8-29.4 (t, C-12 ~ 15, C-4′ ~ 13′), 31.9 (t, C-16), 22.7 (t, C-17), 14.1 (q, C-18, C-16′), 15.9 (q, C-19), 175.4 (s, C-1′), 72.2 (d, C-2′), 35.4 (t, C-3′), 31.5 (t, C-14′), 22.9 (t, C-15′), 105.2 (d, C-1′′), 74.9 (d, C-2′′), 77.9 (d, C-3′′), 71.5 (d, C-4′′), 77.9 (d, C-5′′), 62.5 (t, C-6′′).

(2*S*,3*S*,4*R*,2′*R*)-2-(2′-Hydroxydocosanoylamino) octadecane-1,3,4-triol (8)[15]　$C_{40}H_{81}NO_5$; white amorphous powder; FAB-MS (neg.) m/z (%): 654 $[M-H]^-$; ^{1}H NMR (C_5D_5N, 400 MHz): δ 8.60 (1H, d, J = 9.1 Hz, NH), 5.13 (1H, m, H-2), 4.63 (1H, dd, J = 7.7, 4.0 Hz, H-2′), 4.51 (1H, dd, J = 10.6, 4.6 Hz, H-1a), 4.45 (1H, dd, J = 10.6, 4.8 Hz, H-1b), 4.35 (1H, dd, J = 6.6, 4.0 Hz, H-3), 4.29 (1H, m, H-4), 2.23 (1H, m, H-3′a), 2.05 (1H, m, H-3′b), 1.92 (2H, m, H-5), 1.76 (2H, m, H-4′), 1.71 (2H, m, H-6), 0.86 (6H, t, J = 7.0 Hz, H-18, H-22′); ^{13}C NMR (C_5D_5N, 100 MHz): δ 62.2 (t, C-1), 53.1 (d, C-2), 76.8 (d, C-3), 73.1 (d, C-4), 34.2 (t, C-5), 26.7 (t, C-6), 29.7-30.4 (t, C-7 ~ 16, C-5′ ~ 20′), 22.7 (t, C-17, C-21′), 14.3 (q, C-18, C-22′), 175.5 (s, C-1′), 72.6 (d, C-2′), 35.7 (t, C-3′), 25.9 (t, C-4′).

(2*S*,3*S*,4*R*,2′*R*)-2-(2′-Hydroxytricosanoylamino) octadecane-1,3,4-triol (9)[15]　$C_{41}H_{83}NO_5$; white amorphous powder; FAB-MS (neg.) m/z (%): 668 $[M-H]^-$; ^{1}H NMR (C_5D_5N, 400 MHz): δ 8.58 (1H, d, J = 8.9 Hz, NH), 5.12 (1H, m, H-2), 4.62 (1H, dd, J = 7.8, 4.0 Hz, H-2′), 4.52 (1H, dd, J = 11.0, 4.6 Hz, H-1a), 4.43 (1H, dd, J = 11.0, 4.6 Hz, H-1b), 4.36 (1H, dd, J = 6.5, 4.2 Hz, H-3), 4.28 (1H, m, H-4), 2.26 (1H, m, H-3′a), 2.04 (1H, m, H-3′b), 1.93 (2H, m, H-5), 1.77 (2H, m, H-4′), 1.70 (2H, m, H-6), 0.86 (6H, t, J = 7.0 Hz, H-18, H-23′); ^{13}C NMR (C_5D_5N, 100 MHz): δ 62.1 (t, C-1), 53.1 (d, C-2), 76.7 (d, C-3), 73.1 (d, C-4), 34.2 (t, C-5), 26.7 (t, C-6), 29.7-30.4 (t, C-7 ~ 16, C-5′ ~ 21′), 22.7 (t, C-17, C-22′), 14.3 (q, C-18, C-23′), 175.5 (s, C-1′), 72.6 (d, C-2′), 35.8 (t, C-3′), 25.9 (t, C-4′).

3 Results and discussion

Compounds **1**-**9** are reported from this fungus for the first time. Previous phytochemical study on T. terrestris have led to the isolation of a series of p-terphenyls and three pregnane-type steriods[1,16]. However, the characteristic components of p-terphenyls in this fungus were not isolated in our experiments, which could be resulted from T. terrestris specimens′ regional differences.

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