THE CHEMICAL CONSTITUENTS OF BASIDIOMYCETE *CALODON SUAVEOLENS*

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Abstract Eight compounds have been isolated from the fruiting bodies of the basidiomycete *Calodon suaveolens*. Their structures were established as ergosta-7,22-dien-3β-ol (1), 5α,8α-epidioxyergosta-6,22-dien-3β-ol (2), ergosta-5,7,22-trien-3β-ol (3), 3β-hyohoxy-ergosta-5,22-dien-7-one (4), 4-hydroxybenzonic acid (5), uracil (6), polyozellin (7) and (2S,3R,4E,8E,2′R)-2-N-(2′-hydroxy-palmitoyl)-1-O-β-D-gluco-pyranosyl-9-methyl-4,8-sphingadienine (8) by spectroscopic and chemical methods. All compounds are reported from this fungus for the first time.

Key words *Calodon suaveolens*; chemical constituents; polyozellin; ceramide; ergosterol

蓝柄丽齿菌的化学成分

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摘 要 从担子菌亚门蓝柄丽齿菌(*Calodon suaveolens*)中首次分离得到8个化合物,通过波谱学技术并借助必要的化学方法最终确定结构,它们是:(22E,24R)-麦角甾-7,22-二烯-3β-醇(1),(22E,24R)-5α,8α-过氧麦角甾-6,22-二烯-3β-醇(2),(22E,24R)-麦角甾-5,7,22-三烯-3β-醇(3),(22E,24R)-3β-羟基麦角甾-5,22-二烯-7-酮(4),对羟基苯甲酸(5),尿嘧啶(6),polyozellin(7),(4E,8E)-2-N-(2'-羟基棕榈酰)-1-O-β-D-吡喃葡萄糖基-9-甲基-4,8-sphingadienine(8)。

关键词 蓝柄丽齿菌;化学成分;polyozellin;神经酰胺;麦角甾醇

Introduction

Calodon suaveolens, a fungus belonging to the family Hydnaceae, is mainly distributed in the provinces of Tibet, Sichuan and Yunnan of China[1]. As a part of our search for naturally occurring bioactive metabolites of the higher fungi in Yunnan province, we have carried out a detailed chemical investigation on this fungus and isolated eight known compounds from its EtOH extracts, including ergosta-7, 22-dien-3β-ol (1), 5α, 8α-epidioxyergosta-6, 22-dien-3β-ol (2), ergosta-5, 7, 22-trien-3β-ol (3), 3β-hyohoxy-ergosta-5, 22-dien-7-one (4), 4-hydroxybenzoic acid (5), uracil (6), polyozellin (7) and (2S,3R,4E,8E,2′R)-2-N-(2′-hydroxy-palmitoyl)-1-O-β-D-gluco-pyranosyl-9-methyl-4,8-sphingadienine (8).

Experimental

General

NMR spectra (^{1}H NMR, ^{13}C NMR and DEPT) were recorded on Bruker AV-400 and DXR-500 NMR instruments with TMS as an internal standard. MS spectra (HR-ESI-MS, FAB-MS and EI-MS) were recorded on a VG Atuospec-3000 mass spectrometer. IR spectra were

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measured in KBr pellets on a Bruker Tensor 27 infrared spectrophotometer. TLC was carried out on plates precoated with silica gel GF_{254} (Qingdao Marine Chemical Ltd., P.R.China).

Fungal material

The fresh fruiting bodies of *Calodon suaveolens* were collected from Ailao Mountain of Yunnan Province in China, in July 2003 and identified by Prof. Zang Mu, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, P.R.China. A voucher specimen was deposited at the Herbarium of Kunming Institute of Botany.

Extraction and isolation

The fresh fruiting bodies (dry wt. 1000 g) of *Calodon suaveolens* were extracted successively four times with EtOH and three times with $CHCl_3$/MeOH (1:1) at room temperature. The combined extract was concentrated to dryness *in vacuo* to give a syrup (40 g) which was subjected to silica gel chromatography with a gradient elution of petroleum ether/acetone, subsequently four fractions (A-D) were obtained. Rechromatography on silica gel with petroleum ether/acetone as eluents, fraction A gave compound 1 (28 mg) and compound 3 (20 mg), fraction B gave compound 2 (145 mg) and compound 4 (38 mg). Fraction C was rechromatographed over silica gel using $CHCl_3$/MeOH (97:3) to produce a residue (30 mg), which then was purifiedby preparative TLC to afford compound 5 (13 mg) and compound 6 (9 mg). Fraction D eluted with $CHCl_3$/MeOH (95:5 ~ 90:10) as eluents yielded compound 7 (72 mg) and compound 8 (138 mg), respectively.

Results and Discussion

Compound 1 Ergosta-7, 22-dien-3β-ol[2], $C_{28}H_{46}O$, white needles (from $CHCl_3$). ^{13}C NMR (100 MHz, $CDCl_3$,): δ 139.5 (s, C-8), 135.7 (d, C-22), 131.9 (d, C-23), 117.4 (d, C-7), 71.0 (d, C-3), 56.0 (d, C-17), 55.1 (d, C-14), 49.4 (d, C-9), 43.3 (s, C-13), 42.8 (d, C-24), 40.4 (d, C-20), 40.3 (d, C-5), 39.4 (t, C-12), 38.0 (t, C-4), 37.1 (t, C-1), 34.2 (s, C-10), 33.1 (d, C-25), 31.4 (t, C-6), 29.6 (t, C-2), 28.1 (t, C-16), 22.9 (t, C-15), 21.5 (t, C-11), 21.1 (q, C-21), 19.9 (q, C-28), 19.6 (q, C-27), 17.6 (q, C-26), 13.0 (q, C-19), 12.1 (q, C-18). EI-MS *m/z* (rel.int.): 398 $[M]^+$ (18), 383 $[M-Me]^+$ (5), 300 (16), 273 (35), 271 (100), 255 (58), 246 (31), 229 (35), 213 (33), 107 (63), 69 (35).

Compound 2 5α,8α-Epidioxyergosta-6,22-dien-3β-ol[3], $C_{28}H_{44}O_3$, white needles (from petroleum ether/Me_2CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.4 (d, C-6), 135.2 (d, C-22), 132.4 (d, C-23), 130.7 (d, C-7), 82.1 (s, C-5), 79.4 (s, C-8), 66.5 (d, C-3), 56.2 (d, C-17), 51.7 (d, C-14), 51.1 (d, C-9), 44.6 (s, C-13), 42.8 (d, C-24), 39.7 (d, C-20), 39.3 (t, C-12), 37.0 (t, C-4), 37.0 (s, C-10), 34.7 (t, C-1), 33.1 (d, C-25), 30.1 (t, C-2), 28.6 (t, C-16), 23.4 (t, C-11), 20.9 (q, C-21), 20.6 (t, C-15), 19.9 (q, C-28), 19.6 (q, C-27), 18.2 (q, C-19), 17.5 (q, C-26), 12.9 (q, C-18). EI-MS *m/z* (rel.int): 428 $[M]^+$ (9), 410 $[M-H_2O]^+$ (17), 396 $[M-O_2]^+$ (55), 363 (21), 271 (10), 251 (30), 152 (42), 107 (35), 95 (37), 69 (100), 55 (34).

Compound 3 Ergosta-5, 7, 22-trien-3β-ol[4], $C_{28}H_{44}O$, white needles (from petroleum ether/Me_2CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.4 (s, C-8), 139.8 (s, C-5), 135.7 (d, C-22), 132.0 (d, C-23), 119.6 (d, C-6), 116.4 (d, C-7), 70.5 (d, C-3), 55.8 (d, C-17), 54.6 (d, C-14), 46.3 (d, C-9), 42.9 (d, C-24), 42.9 (s, C-13), 40.8 (t, C-4), 40.4 (d, C-20), 39.1 (t, C-12), 38.4 (t, C-1), 37.1 (s, C-10), 33.1 (d, C-25), 32.0 (t, C-2), 28.3 (t, C-16), 23.0 (t, C-15), 21.1 (t, C-11), 21.1 (t, C-12), 19.9 (q, C-28), 19.6 (q, C-27), 17.6 (q, C-26), 16.3 (q, C-19), 12.0 (q, C-18). EI-MS *m/z* (rel.int.): 396 $[M]^+$ (25), 378 $[M-H_2O]^+$ (3), 363 (19), 337 (17), 271 (33), 253 (20), 157 (25), 143 (27), 105 (34), 95 (34), 81 (59), 69 (100), 55 (53).

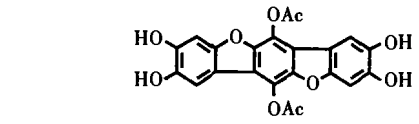
Compound 4 (22*E*,24*R*)-3β-Hydroxy-ergosta-5,22-dien-7-one[5], $C_{28}H_{44}O_2$, colorless needles (from petroleum ether/Me_2CO). ^1H NMR (400 MHz, $CDCl_3$): δ 5.66 (1H, d, *J* = 1.7Hz, H-6), 5.16 (2H, m, H-22 and H-23), 3.65 (1H, m, H-3), 2.47 (1H, m, Heq-4), 2.39 (1H, m, Hax-4), 2.22 (1H, t, *J* = 11.0 Hz, H-8), 1.94 (1H, m, H-20), 1.82 (1H, m, H-24), 1.44 (1H, m, H-25), 1.17 (3H, s, H-19), 0.99 (3H, d, *J* = 6.6 Hz, H-21), 0.89 (3H, d, *J* = 6.8 Hz, H-28), 0.81 (3H, d, *J* = 6.7 Hz, H-26 or H-27), 0.79 (3H, d, *J* = 6.8 Hz, H-27 or H-26), 0.67 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.3 (s, C-7), 165.1 (s, C-5), 135.6 (d, C-22), 131.9 (d, C-23), 126.1 (d, C-6), 70.5 (d, C-3), 54.7 (d, C-17), 50.0 (d, C-9), 49.9 (d, C-14), 45.4 (d, C-8), 43.0 (s, C-13), 42.8 (d, C-24), 41.8 (t, C-4), 39.9 (d, C-20), 38.5 (t, C-12), 38.4 (s, C-10), 36.3 (t, C-1), 33.1 (d, C-25), 31.2 (t, C-2), 28.6 (t, C-16), 26.3 (t, C-15), 21.2 (t, C-11), 21.2 (q, C-21), 19.9 (q, C-28), 19.6 (q, C-27), 17.6 (q, C-26), 17.3 (q, C-19), 12.2 (q, C-18). EI-MS m/z (rel.int.): 412 $[M]^+$ (69), 397 $[M-Me]^+$ (5), 394 $[M-H_2O]^+$ (2), 379 (3), 369 (12), 351 (6), 314 (100), 287 (74), 285 (60), 269 (22), 245 (18), 227 (7).

Compound 5 4-Hydroxybenzonic acid, $C_7H_6O_3$, yellowish crystal (from MeOH). ^1H NMR (500 MHz, CD_3OD): δ 7.86 (1H, dd, *J* = 8.8, 2.2 Hz, H-2 and H-6), 6.80 (1H, dd, *J* = 8.8, 2.2Hz, H-3 and H-5), 4.89 (1H, brs, OH). ^{13}C NMR (125 MHz, CD_3OD): δ 170.1 (s, COOH), 163.4 (s, C-4), 133.0 (d, C-3 and C-5), 122.8 (s, C-1), 116.0 (d, C-2 and C-6). EI-MS *m/z* (rel.int.): 138 $[M]^+$ (94), 121 (100), 93 (24), 65 (8).

Compound 6 Uracil[6], (pyrimidine-2, 4-dione), $C_4H_4N_2O_2$, white amorphous powder (from MeOH). ^1H NMR(400 MHz, DMSO-d_6): δ 10.98(1H, brs, H-3), 10.79 (1H, brs, H-1), 7.36 (1H, d, J = 7.6 Hz, H-6), 5.45(1H, d, J = 7.6 Hz, H-5). ^{13}C NMR(100 MHz, DMSO-d_6): δ 164.5(s, C-4), 151.5 (s, C-2), 142.2 (d, C-6), 100.3 (d, C-5). EI-MS m/z (rel.int.): 112 $[M]^+$(100), 69 (33).

Compound 7 Polyozellin[7], (6, 12-diacetoxy-2, 3, 8, 9-tetrahydroxybenzo[1, 2-b : 4, 5-b']bisbenzofuran), $C_{22}H_{14}O_{10}$, dark green powder (from MeOH). IR (KBr) υ_{max} cm^{-1}: 3405, 1780, 1753, 1616, 1474, 1371, 1310, 1222, 1123. ^1H NMR(400 MHz, DMSO-d_6): δ 9.75(1H, brs, OH), 9.35 (1H, brs, OH), 7.15 (4H, s, H-1, 4, 7 and 10), 2.53(6H, s, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ168.2 (s, C=O), 150.4 (s, C-4a and 10a), 147.1(s, C-3 and C-9), 143.4(s, C-2 and C-8), 137.0(s, C-5a and C-11a), 130.9(s, C-6 and C-12), 116.8(s, C-6a and C-12a), 112.9(s, C-6b and C-12 b), 106.0(d, C-1 and C-7), 98.9 (d, C-4 and C-10), 20.3(q, $COCH_3$). FAB-MS (negative) m/z (rel.int.): 437$[M-1]^-$(100), 394$[M-1-COCH_3]^+$ (25), 352$[M-1-COCH_3-COCH_2]^-$ (56). HR-ESI-MS (negative) m/z (rel.int.): 437.0502$[M-1]^-$, (calcd. for $C_{22}H_{13}O_{10}$ 437.0508).

Compound 8 (2*S*, 3*R*, 4*E*, 8*E*, 2'*R*)-2-N-(2'-hydroxy-palmitoyl) -1 -O-β-D-gluco-pyranosyl-9-methyl-4, 8-sphingadienine[8], $C_{41}H_{77}NO_9$, white amorphous powder (from MeOH). ^1H NMR (500 MHz, CD_3OD): δ 5.73 (1H, dt, J = 15.4, 5.4 Hz, H-5), 5.46 (1H, dd, J = 15.4, 7.6 Hz, H-4), 5.12 (1H, dt, J = 6.8 Hz, H-8), 4.28 (1H, d, J = 7.8 Hz, H-1''), 2.05 (4H, m, H-10, H-7), 1.96 (2H, t, J = 7.5 Hz, H-6), 1.58 (3H, s, H-19), 1.39 (2H, m, H-3'), 1.22 ~ 1.35 (Alphatic -CH_2-), 0.89 (6H, t, J = 6.7 Hz, H-18, H-16'). ^{13}C NMR (125 MHz, CD_3OD): δ 177.2 (s, C-1'), 136.8 (s, C-9), 134.8 (d, C-4), 131.0 (d, C-5), 124.8 (d, C-8), 104.6 (d, C-1''), 77.9 (d, C-3''), 77.8 (d, C-5''), 74.9 (d, C-2''), 73.1 (d, C-2'), 72.8 (d, C-3), 71.5 (d, C-4''), 69.8 (t, C-1), 62.6 (t, C-6''), 54.5 (d, C-2), 40.8 (t, C-10), 35.9 (t, C-3'), 33.8 (t, C-7), 33.1 (t, C-6), 16.2 (q, C-19), 14.5 (q, C-18), 14.5 (q, C-16'). FAB-MS (negative) m/z: 726$[M-1]^-$(100). EI-MS m/z(rel.int.): 276 (4), 262 (16), 222 (14), 180 (4).



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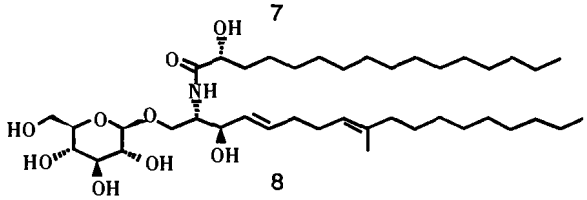

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Fig. 1 Structures of compounds 7 and 8

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