Two Novel Secoergosterols from the Fungus *Tylopilus plumbeoviolaceus*

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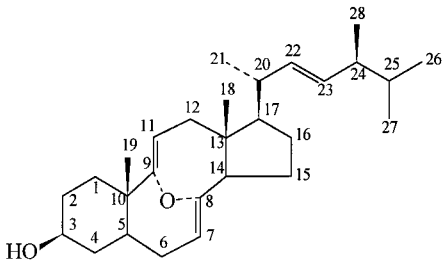
Two novel secoergosterols, 3β-hydroxy-8α,9α-oxido-8,9-secoergosta-7,9(11),22-triene (tylopiol A) (**1**) and 3β-hydroxy-8α,9α-oxido-8,9-secoergosta-7,22-dien-12-one (tylopiol B) (**2**), were isolated from the fresh fruit bodies of *Tylopilus plumbeoviolaceus*, along with three known compounds, ergosta-7,22-dien-3β-ol, uridine, and allitol. Their structures were elucidated by NMR techniques, including ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, and MS. The structure and stereochemistry of compound **1** were demonstrated by X-ray crystallography.

Tylopilus plumbeoviolaceus (Snell. et Dick.) Sing., a fungus belonging to the family Strobilomycetaceae (Boletales), is distributed widely in the central area of Yunnan Province of the People's Republic of China. It is edible, but very bitter. The chemical constituents of this species have not been investigated previously. In the present study on the chemical constituents of *T. plumbeoviolaceus*, two new compounds named tylopiol A (**1**) and tylopiol B (**2**) were isolated, together with three known compounds, ergosta-7,22-dien-3β-ol,[1,2] uridine,[3] and allitol.[4,5] In this paper, the characterization and structure elucidation of compounds **1** and **2** are reported. The structures of compounds **1** and **2** are based on the ergostane skeleton, in which the bond between C-8 and C-9 is cleaved to form an enol ether oriented in the α position. Although this type of modified skeleton is very rare, a similar ergostane compound, jereisterol A, has been isolated from a marine sponge.[6,7]



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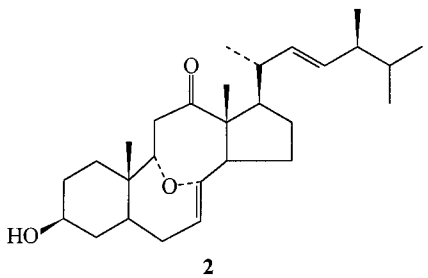

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The molecular formula of tylopiol A (**1**) was determined to be $C_{28}H_{44}O_2$ based on HREIMS at *m*/*z* 412.3336 [M]$^+$ and the ^{13}C NMR spectrum. Compound **1** exhibited signals for six methyl carbons, seven methylenes, 11 methines, and four quaternary carbons in the ^{13}C NMR spectrum (see Table 1). Comparison of the ^{1}H and ^{13}C NMR spectra with reference data[1,5] indicated that **1** is an ergosterol derivative. In the ^{1}H NMR spectrum, six methyl signals appeared at δ 1.07 (3H, s), 1.03 (3H, d, J = 6.6 Hz), 0.88 (3H, d, J = 6.7 Hz), 0.80 (3H, d, J = 6.6 Hz), 0.77 (3H, d, J = 5.5 Hz), and 0.49 (3H, s), and two olefinic proton signals in the ring system appeared at δ 4.89 (1H, t) and 4.50 (1H, t). Altogether, the ^{13}C NMR spectrum exhibited six olefinic carbons. Among these, the signals at δ 135.1 (CH) and 132.4 (CH) were assigned as a C-22−C-23 double bond from the HMBC spectrum, in which long-range couplings were observed for H-21 [δ 1.03 (3H, d)] to C-22 [δ 135.1 (CH)] and for H-28 [δ 0.88 (3H, d)] to C-23 [δ 132.4 (CH)]. The two downfield olefinic carbon signals [δ 154.9 (s) and 166.1 (s)] suggested that these quaternary carbons were attached

Table 1. ^{13}C NMR Spectral Data for Compounds **1** and **2** (in pyridine-d_5 at 100 MHz)

carbon	**1**	**2**
1	38.2 t	35.7 t
2	31.4 t	30.3 t
3	70.8 d	70.7 d
4	38.3 t	40.1 t
5	37.6 d	38.0 d
6	32.7 t	38.7 t
7	106.8 d	119.8 d
8	154.9 s	163.1 s
9	166.1 s	58.9 d
10	41.5 s	49.7 s
11	107.2 d	36.7 t
12	35.8 t	213.7 s
13	44.2 s	51.3 s
14	59.4 d	61.7 d
15	22.0 t	27.3 t
16	26.5 t	29.7 t
17	53.3 d	61.7 d
18	12.4 q	14.9 q
19	17.6 q	20.8 q
20	39.7 d	40.6 d
21	22.0 q	20.1 q
22	135.1 d	134.5 d
23	132.4 d	132.9 d
24	43.0 d	42.9 d
25	33.1 d	33.1 d
26	20.0 q	19.9 q
27	19.7 q	19.6 q
28	17.5 q	17.6 q

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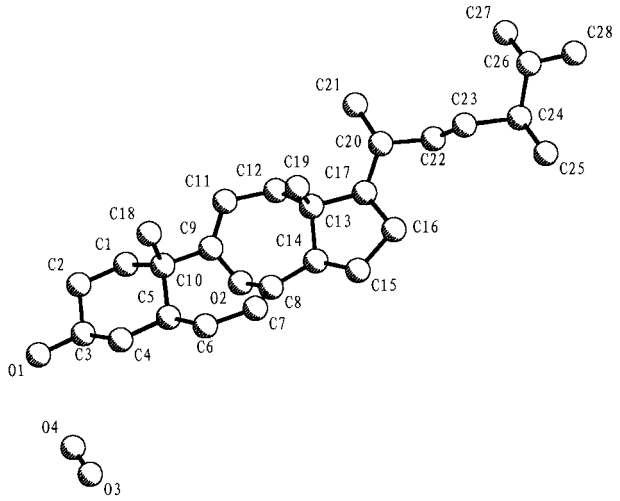

Figure 1. The crystal structure of compound **1**.

to an oxygen atom forming a dienol ether structure. In the HMBC spectrum, long-range couplings were observed for H-11 [δ 4.89 (1H, t)] to C-9 [δ 166.1 (quaternary carbon)], H-12 [δ 2.20 (2H, dd)] to C-9, and H-19 [δ 1.07 (3H, s)] to C-9. In addition, long-range couplings were observed for H-7 [δ 4.50 (1H, t)] to C-8 [δ 154.9 (quaternary carbon)] and H-14 [δ 2.36 (1H, dd)] to C-8. Thus, the two double bonds were assigned between C-7 and C-8 and between C-9 and C-11, respectively, and the oxygen atom was fixed between C-8 and C-9. In addition, the downfield chemical shift of H-5 to δ 2.77 indicated that the oxygen function and H-5 were located on same face of the molecule, so that the 8,9-oxido bridge was in the α position.[6] The stereochemistry at the remaining chiral centers in **1** was identical to that of jereisterol A, as supported by its ^{1}H NMR, ^{1}H$-^1$H COSY, and ROESY spectra. Thus, compound **1** was elucidated as 3β-hydroxy-8α,9α-oxido-8,9-secoergosta-7,9(11),22-triene. Finally, the structure of **1** was demonstrated unambiguously by X-ray crystallographic analysis, which confirmed its proposed configuration, the results of which are shown in Figure 1.

Tylopiol B (**2**) gave a molecular ion at *m/z* 428.3273 $[M]^+$ in the HREIMS. From this and the fragment ions, it was possible to deduce a molecular formula of $C_{28}H_{44}O_3$, which revealed that it was an analogue of **1**, in which a carbon−carbon double bond was hydrogenated and a methine was oxidized to a ketone. The absorption of the carbonyl group at 1689 cm^{-1} in the IR spectrum and the absence of any significant UV absorption suggested that the carbonyl group is not conjugated with a double bond in **2**. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1** except for the presence of the carbonyl group and the absence of the dienol ether in **2**. In the ^{13}C NMR spectrum, only four olefinic carbon signals were observed, which was in accord with the calculated degrees of unsaturation. In contrast to **1**, C-9 and C-11 in **2** were assigned as a methine and a methylene group, respectively, from the HMBC spectrum. In addition, long-range couplings were observed for H-18 [δ 1.14 (3H, s)] to the ketone carbonyl carbon [δ 213.7 (C-12)], to the olefinic carbon [δ 163.1 (C-8)], to C-20 [δ 40.6 (CH)], and for H-11α [δ 2.42 (1H, dd)] to C-19 [δ 20.8 (CH_3)], to C-9 [δ 58.9 (CH)], to C-8 [δ 163.1 (quaternary carbon)], and to C-12 [δ 213.7 (quaternary carbon)]. These observations not only indicated the absence of any olefinic linkage between C-9 and C-11, but also confirmed the presence of a ketone at the C-12 position. The stereochemistry at the other chiral centers in **2** was identical to that of **1**, as supported by its ^{1}H, ^{1}H$-^1$H COSY, and ROESY NMR spectra. Therefore, the structure of compound **2** was determined to be 3β-hydroxy-8α,9α-oxido-8,9-secoergosta-7,22-dien-12-one.

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. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers with TMS as internal standard. MS data were recorded on a VG Autospec-3000 spectrometer. X-ray analysis was performed with a MAC DIP-2030K diffractometer.

Plant Material. The fresh fruit bodies of *T. plumbeoviolaceus* were collected in Nanhua County of Yunnan Province in August 1998, and identified by Prof. Pei-Gui Liu, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China, where a voucher specimen (no. HKAS 32820) is deposited.

Extraction and Isolation. Fresh fruit bodies (485 g, after extraction and drying) of *T. plumbeoviolaceus* were cut and extracted with EtOH and $CHCl_3-CH_3OH$ (1:1) sequentially at the room temperature. The combined extract was concentrated under vacuum. Then, the residue was suspended in water and partitioned with EtOAc. The EtOAc extract was concentrated in vacuo to give a residue (32 g), which was chromatographed on a Si gel column (200−300 mesh, 600 g) and eluted with a $CHCl_3-Me_2CO$ mixture containing increasing amount of Me_2CO to afford eight fractions. Fraction 3 (1.3 g) yielded ergosta-7,22-dien-3-ol as colorless needles (822 mg). Fraction 4 (105 mg) was rechromatographed on a Si gel column and eluted with petroleum ether−acetone (100:8) to give compound **1** (12 mg). Fraction 5 (98 mg) was purified further by passage via a Si gel column, eluted with petroleum ether−acetone (90:10) and petroleum ether−ethyl acetate (80:20), to afford compound **2** (9 mg). The water layer was concentrated in vacuo to give a residue (210 g), which was chromatographed on a Si gel column (200−300 mesh, 1,200 g) eluted with $CHCl_3-MeOH$ (90:10) of increasing concentration of MeOH to afford seven fractions. Fraction 2 (87 mg) was rechromatographed on a Si gel column and eluted with EtOAc−MeOH (15:1) to afford uridine (13 mg). Fractions 4−7 (13.6 g) gave allitol (1.2 g) as colorless needles.

Tylopiol A (1): colorless needles (petroleum ether−acetone); mp 117−119 °C; $[\alpha]^{25}_D$ +106.3° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3337, 3034, 2957, 2873, 1688, 1458, 1371, 1201, 1172, 1076 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.18 (2H, dd, J = 4.8, 2.2 Hz, H-22, H-23), 4.89 (1H, t, J = 6.6 Hz, H-11), 4.50 (1H, t, J = 2.6 Hz, H-7), 3.67 (1H, m, H-3), 2.77 (1H, tt, J = 12.6, 2.8 Hz, H-5), 2.36 (1H, dd, J = 6.9, 13.9 Hz, H-14), 2.20 (2H, dd, J = 3.3, 6.8 Hz, H-12), 2.10 (1H, m, H-20), 2.05 (1H, dd, J = 2.7, 18.4 Hz, H-6β), 1.92 (1H, m, H-6α), 1.82 (1H, m, H-24), 1.68 (1H, m, H-16a), 1.63 (1H, m, H-4α), 1.46 (1H, m, H-25), 1.42 (1H, m, H-17), 1.29 (1H, m, H-16b), 1.23 (1H, m, H-4β), 1.07 (3H, s, H-19), 1.03 (3H, d, J = 6.6 Hz, H-21), 0.88 (3H, d, J = 6.7 Hz, H-28), 0.80 (3H, d, J = 6.6 Hz, H-26), 0.77 (3H, d, J = 5.5 Hz, H-27), 0.49 (3H, s, H-18); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 412 $[M]^+$,[6] 287 (1), 206 (2), 205 (1), 187 (2), 124 (29), 108 (36), 55 (100); HREIMS *m/z* 412.3336 $[M]^+$ (calcd for $C_{28}H_{44}O_2$, 412.3341).

Tylopiol B (2): colorless needles (petroleum ether−acetone); mp 192−194 °C; $[\alpha]^{25}_D$ +17.7° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3526, 3407, 2871, 1689, 1453, 1372, 1257, 1190, 1087 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.45 (1H, t, J = 3.5 Hz, H-7), 5.26 (1H, dd, J = 7.8, 15.2 Hz, H-23), 5.12 (1H, dd, J = 8.2, 15.3 Hz, H-22), 3.56 (1H, m H-3), 3.50 (1H, t, J = 8.9 Hz, H-9), 2.42 (1H, dd, J = 13.9, 8.4 Hz, H-11α), 2.20 (1H, m, overlap, H-5), 2.19 (1H, dd, J = 3.6, 16.0 Hz, H-6β), 1.79 (1H, m, H-6α), 1.57 (1H, m, overlap, H-11β), 1.15 (3H, s, H-19), 1.14 (3H, s, H-18), 0.95 (3H, d, J = 6.5 Hz, H-21), 0.89 (3H, d, J = 6.8 Hz,

H-28), 0.81 (3H, d, $J = 6.6$ Hz, H-26), 0.79 (3H, d, $J = 6.6$ Hz, H-27); ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 428 $[M]^+$,[27] 410 $[M - H_2O]^+$,[4] 303 [M − side chain]$^+$,[50] 285 [M − side chain − $H_2O]^+$,[44] 205 (7), 107 $[C_8H_{11}]^+$,[63] 55 $[C_3H_3O]^+$ (100); HREIMS *m/z* 428.3273 $[M]^+$ (calcd for $C_{28}H_{44}O_3$, 428.3290).

Ergosta-7,22-dien-3*β*-ol: colorless needles (petroleum ether−acetone); mp 170−172 °C; $[\alpha]^{20}_D$ −23.6° (*c* 0.27, $CHCl_3$); exhibited spectral data (IR, ^{1}H NMR, ^{13}C NMR, EIMS) consistent with literature values.[1]

Uridine: colorless needles (MeOH); mp 166.5−168 °C; exhibited spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, MS) consistent with literature values.[2]

Allitol: colorless needles (MeOH−H_2O); mp 154.5−156 °C; $[\alpha]^{25}_D$ 0° (*c* 0.6, MeOH); exhibited spectral data (^{1}H NMR, ^{13}C NMR, EIMS) consistent with literature data.[3,4]

X-ray Crystal Structure Analysis of Tylopiol A (1).[8] Crystal data: $C_{28}H_{44}O_2$, MW = 412.64; monoclinic, space group C_2; $a = 10.575(3)$ Å, $b = 7.560(1)$ Å, $c = 33.396(8)$ Å, $\beta = 90.601(17)°$, $V = 2669.8(11)$ Å^3, $Z = 4$, $D_{calc} = 1.064$ g/cm^3, Mo Kα ($\lambda = 0.71069$ Å). The data were collected on a MAC DIP-2030K diffractometer at 22 °C, with graphite-monochromated, Mo Kα radiation using a colorless crystal with dimensions of 0.07 × 0.10 × 0.50 mm, maximum 2θ value of 42.5°, independent reflections: 1289, observed number of reflections: 1132 [$|F|^2 \geq 8\sigma(|F|^2)$]. The structure was solved by the direct method SHELX-86[9] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[10] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R = 0.097$, $R_w = 0.077$.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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