Chemistry of Natural Compounds, Vol. 45, No. 5, 2009

STEROIDS AND OTHER CONSTITUENTS FROM THE MUSHROOM *Armillaria lueo-virens*

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UDC 547.918

Armillaria luteo-virens (Alb. et Schw. Fr.) Sacc. is a special medicinal and edible mushroom in China. This fungi is mainly distributed in the meadow and grassland of Qinghai-Tibet plateau and is used as a traditional Chinese medicine for the treatment of dizziness, headache, neurasthenia, insomnia, numbness in limbs, and infantile convulsion [1]. However, its chemical constituents have not been reported up until now. There was only a report that a novel lectin was isolated from *A. luteo-virens* [2]. In this paper, we report the isolation and structural elucidation of eleven known compounds **1–11** from the fruiting bodies of *A. luteo-virens*.

The fruiting bodies of *A. luteo-virens* were collected from Qilian County, Qinghai Province in August 2007 and identified by Prof. Zhi-Min Diao, Agriculture and Animal Husbandry College, Qinghai University, Xining, P. R. China, where a voucher specimen (No. AL-10) was deposited.

Extraction and Isolation. The dried and minced fruiting bodies (3 kg) of *A. luteo-virens* were extracted with 95% ethanol three times at room temperature, 7 days each time. After evaporation of the solvent in vacuo, the residue (300 g) was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH successively. The petroleum ether-soluble fraction (96.0 g) was concentrated and subjected to silica gel CC using gradient elution with petroleum ether–EtOAc to give seven fractions. The subfractions were then purified by successive CC on silica gel, which led to the isolation of compounds **1–7** and **9–11**. The EtOAc-soluble fraction (10.0 g) was concentrated and subjected to silica gel CC using gradient elution with petroleum ether–acetone to give seven fractions. The subfractions were then purified by successive CC on silica gel, which led to the isolation of compound **8**.

On the basis of extensive spectroscopic analyses and by comparison with the spectral data reported in the literature, their structures were established as ergosterol (**1**, 4.0 g), 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (**2**, 25 mg), (22*E*,24*R*)-ergosta-7,22-dien-3β-ol (**3**, 14 mg), 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**4**, 11 mg), 5α,6α-epoxy-(22*E*,24*R*)-ergosta-8(14),22-dien-3β,7α-diol (**5**, 40 mg), 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (**6**, 10 mg), *E*-cinnamic acid (**7**, 20 mg), nicotinic acid (**8**, 40 mg), linoleic acid (**9**, 13 mg), linoleic acid ethyl ester (**10**, 7 mg), and 1-linoleoyl glycerol (**11**, 14 mg). In addition, the structure of **6** was confirmed by single-crystal x-ray crystallography (Fig. 1).

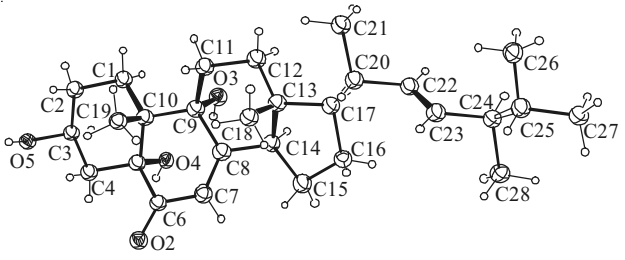

Fig. 1. ORTEP Diagram of the Crystal Structure of **6**.

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0009-3130/09/4505-0759

Ergosterol (1). $C_{28}H_{44}O$, colorless crystals, mp 144–146°C. $[\alpha]_D^{20}$ –27° (*c* 0.43, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3392, 2957, 2871, 1716, 1658, 1456, 1376, 1158, 1049, 975. EI-MS: (*m/z,* %): 396 $[M]^+$ (18), 378 (1), 363 (13), 337 (7), 271 (6), 253 (6), 211 (7), 43 (100). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.57 (1H, m, H-6), 5.39 (1H, m, H-7), 5.22 (1H, dd, J = 15.2, 6.8, H-23), 5.20 (1H, dd, J = 15.2, 7.2, H-22), 3.64 (1H, m, H-3), 1.04 (3H, d, J = 7.2, H-21), 0.95 (3H, s, H-19), 0.92 (3H, d, J = 7.2, H-28), 0.84 (3H, d, J = 6.8, H-27), 0.82 (3H, d, J = 6.8, H-26), 0.63 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 38.4 (C-1), 32.0 (C-2), 70.5 (C-3), 40.8 (C-4), 139.8 (C-5), 116.3 (C-6), 119.6 (C-7), 141.3 (C-8), 46.2 (C-9), 37.0 (C-10), 21.1 (C-11), 39.1 (C-12), 42.9 (C-13), 54.5 (C-14), 21.1 (C-15), 28.3 (C-16), 55.7 (C-17), 12.0 (C-18), 16.3 (C-19), 40.1 (C-20), 21.1 (C-21), 135.6 (C-22), 132.0 (C-23), 42.8 (C-24), 33.1 (C-25), 19.6 (C-26), 19.9 (C-27), 17.6 (C-28) [3].

5α,8α-Epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol (2). $C_{28}H_{44}O_3$, colorless crystals, mp 181–183°C. $[\alpha]_D^{20}$ –30° (*c* 0.62, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3394, 2956, 2872, 1457, 1377, 1043, 1024, 969, 861, 756. EI-MS (*m/z*, %): 428 $[M]^+$ (1), 410 (1), 396 (17), 363 (6), 337 (3), 285 (1), 251 (4), 211 (3), 157 (5), 119 (8), 81 (31), 69 (61), 43 (100). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.51 (1H, d, J = 8.4, H-6), 6.25 (1H, d, J = 8.4, H-7), 5.23 (1H, dd, J = 15.0, 7.8, H-23), 5.15 (1H, dd, J = 15.0, 8.4, H-22), 3.97 (1H, m, H-3), 1.00 (3H, d, J = 6.3, H-21), 0.91 (3H, d, J = 6.9, H-28), 0.88 (3H, s, H-19), 0.84 (3H, d, J = 6.9, H-27), 0.83 (3H, d, J = 6.9, H-26), 0.82 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 34.6 (C-1), 30.0 (C-2), 66.3 (C-3), 36.8 (C-4), 82.1 (C-5), 135.4 (C-6), 130.7 (C-7), 79.4 (C-8), 51.0 (C-9), 36.9 (C-10), 23.3 (C-11), 39.2 (C-12), 44.5 (C-13), 51.6 (C-14), 20.6 (C-15), 28.6 (C-16), 56.1 (C-17), 12.8 (C-18), 18.1 (C-19), 39.7 (C-20), 20.8 (C-21), 135.1 (C-22), 132.2 (C-23), 42.7 (C-24), 33.0 (C-25), 19.9 (C-26), 19.6 (C-27), 17.5 (C-28) [4].

(22*E*,24*R*)-Ergosta-7,22-dien-3β-ol (3). $C_{28}H_{46}O$, colorless crystals, mp 145–148°C. $[\alpha]_D^{20}$ –5° (*c* 0.85, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3341, 2951, 2928, 2870, 1663, 1458, 1377, 1044, 970. EI-MS: (*m/z*, %): 398 $[M]^+$ (4), 273 (7), 217 (8), 255 (9), 246 (2), 229 (3), 213 (4), 107 (15), 69 (24), 43 (100). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.18 (3H, m, H-7, H-22, H-23), 3.60 (1H, m, H-3), 1.01 (3H, d, J = 6.6, H-21), 0.92 (3H, d, J = 6.6, H-28), 0.83 (3H, d, J = 6.9, H-27), 0.81 (3H, d, J = 6.9, H-26), 0.79 (3H, s, H-19), 0.54 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 37.1 (C-1), 29.6 (C-2), 71.0 (C-3), 37.9 (C-4), 40.2 (C-5), 31.4 (C-6), 117.4 (C-7), 139.6 (C-8), 49.5 (C-9), 34.2 (C-10), 21.5 (C-11), 39.4 (C-12), 43.3 (C-13), 55.1 (C-14), 22.9 (C-15), 28.1 (C-16), 55.8 (C-17), 12.1 (C-18), 13.0 (C-19), 40.5 (C-20), 21.1 (C-21), 135.6 (C-22), 131.8 (C-23), 42.8 (C-24), 33.1 (C-25), 19.6 (C-26), 19.9 (C-27), 17.6 (C-28) [5].

3β-Hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (4). $C_{28}H_{42}O_2$, colorless crystals, mp 167–170°C. $[\alpha]_D^{20}$ –14° (*c* 0.61, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3385, 2957, 2870, 1711, 1658, 1623, 1460, 1374, 1066, 973. EI-MS (*m/z*, %): 410 $[M]^+$ (3), 395 (2), 285 (2), 267 (7), 253 (5), 229 (2), 213 (3), 199 (2), 84 (100), 43 (77). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.05 (1H, br.s, H-6), 5.24 (1H, dd, J = 15.3, 6.6, H-23), 5.19 (1H, dd, J = 15.3, 6.3, H-22), 3.67 (1H, m, H-3), 1.35 (3H, s, H-19), 1.05 (3H, d, J = 6.6, H-21), 0.92 (3H, d, J = 6.9, H-28), 0.84 (3H, d, J = 6.9, H-27), 0.83 (3H, d, J = 6.9, H-26), 0.65 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 34.6 (C-1), 30.6 (C-2), 71.9 (C-3), 41.8 (C-4), 161.7 (C-5), 126.7 (C-6), 186.3 (C-7), 134.0 (C-8), 161.1 (C-9), 41.8 (C-10), 24.6 (C-11), 35.5 (C-12), 42.3 (C-13), 48.4 (C-14), 24.7 (C-15), 29.5 (C-16), 53.3 (C-17), 11.9 (C-18), 23.7 (C-19), 40.3 (C-20), 21.1 (C-21), 135.4 (C-22), 132.1 (C-23), 42.8 (C-24), 33.1 (C-25), 19.6 (C-26), 19.9 (C-27), 17.6 (C-28) [6].

5α,6α-Epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3β,7α-diol (5). $C_{28}H_{44}O_3$, colorless crystals, mp 181–182°C. $[\alpha]_D^{20}$ –115° (*c* 0.41, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3400, 2958, 2871, 1720, 1663, 1461, 1377, 1053, 1024, 970, 756. EI-MS (*m/z*, %): 428 $[M]^+$ (1), 410 (4), 392 (1), 377 (4), 285 (3), 267 (5), 43 (100). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.22 (1H, dd, J = 15.2, 7.2, H-23), 5.19 (1H, dd, J = 15.2, 7.6, H-22), 4.42 (1H, br.s, H-7), 3.92 (1H, m, H-3), 3.14 (1H, m, H-6), 1.01 (3H, d, J = 7.2, H-21), 0.91 (3H, d, J = 7.2, H-28), 0.86 (6H, s, H-18, H-19), 0.84 (3H, d, J = 6.8, H-27), 0.82 (3H, d, J = 6.8, H-26). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 32.2 (C-1), 31.0 (C-2), 68.6 (C-3), 39.5 (C-4), 67.8 (C-5), 61.4 (C-6), 65.0 (C-7), 125.2 (C-8), 38.7 (C-9), 35.8 (C-10), 19.0 (C-11), 36.6 (C-12), 42.9 (C-13), 152.5 (C-14), 24.9 (C-15), 27.1 (C-16), 56.8 (C-17), 17.6 (C-18), 16.5 (C-19), 39.2 (C-20), 21.2 (C-21), 135.2 (C-22), 132.2 (C-23), 42.8 (C-24), 33.1 (C-25), 19.6 (C-26), 19.9 (C-27), 18.0 (C-28) [3].

3β,5α,9α-Trihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6-one (6). $C_{28}H_{44}O_4$, colorless crystals, mp 218–219°C. $[\alpha]_D^{20}$ –29° (*c* 0.50, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3343, 2958, 2872, 1678, 1459, 1384, 1071, 969, 756. EI-MS (*m/z*, %): 426 $[M\text{-}H_2O]^+$ (5), 369 (16), 300 (1), 232 (2), 217 (3), 81 (32), 69 (70), 55 (89), 43 (100). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.61 (1H, br.s, H-7), 5.24 (1H, dd, J = 15.3, 6.9, H-23), 5.16 (1H, dd, J = 15.3, 6.6, H-22), 4.04 (1H, m, H-3), 1.02 (3H, d, J = 6.6, H-21), 0.98 (3H, s, H-19), 0.92 (3H, d, J = 6.9, H-28), 0.84 (3H, d, J = 6.6, H-27), 0.82 (3H, d, J = 6.6, H-26), 0.61 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 25.7 (C-1), 30.0 (C-2), 67.4 (C-3), 36.8 (C-4), 79.6 (C-5), 199.0 (C-6), 119.9 (C-7), 165.4 (C-8), 74.9 (C-9), 41.9 (C-10), 29.8 (C-11), 35.1 (C-12), 45.5 (C-13), 52.0 (C-14), 22.6 (C-15), 28.1 (C-16),

56.2 (C-17), 12.5 (C-18), 20.5 (C-19), 40.5 (C-20), 21.3 (C-21), 135.5 (C-22), 132.6 (C-23), 43.0 (C-24), 33.3 (C-25), 20.2 (C-26), 19.6 (C-27), 17.8 (C-28) [7].

***E*-Cinnamic Acid (7)**. $C_9H_8O_2$, colorless crystals, mp 133–135°C. $[\alpha]_D^{20}$ –11° (*c* 0.42, $CHCl_3$). EI-MS (*m/z*, %): 148 $[M]^+$ (73). ^1H and ^{13}C NMR [8].

Nicotinic Acid (8). $C_6H_5NO_2$, white powder. $[\alpha]_D^{20}$ +15° (*c* 0.16, CH_3OH). EI-MS (*m/z*, %): 123 $[M]^+$ (93). ^1H and ^{13}C NMR [9].

Linoleic Acid (9). $C_{18}H_{32}O_2$, yellow oil. EI-MS (*m/z*, %): 280 $[M]^+$ (1). ^1H NMR and ^{13}C NMR [10].

Linoleic Acid Ethyl Ester (10). $C_{20}H_{36}O_2$, yellow oil. EI-MS (*m/z*, %): 308 $[M]^+$ (1). ^1H NMR and ^{13}C NMR [10].

1-Linoleoyl Glycerol (11). $C_{21}H_{38}O_4$, yellow oil. EI-MS (*m/z*, %): 354 $[M]^+$ (1). ^1H NMR and ^{13}C NMR [11].

X-ray Crystallography of Compound 6. Crystal data: $C_{28}H_{44}O_4$, formula wt 444.32, crystal dimensions $0.22 \times 0.20 \times 0.10$ mm, monoclinic, space group $P2_1$, $a = 6.7738\ (11)$ Å, $b = 7.2214\ (11)$ Å, $c = 28.233\ (5)$ Å, $\beta = 95.306\ (3)°$, $V = 1375.1\ (4)$ Å^3, $Z = 3$, $D_c = 1.112$ g/cm^3, $F(000) = 504$. The reflection data were collected on a Bruker Smart Apex CCD diffractometer using graphite-monochromated Mo Kα radiation, $\lambda = 0.71073$ Å. A total of 3452 reflections was collected in the range $0.72° \le \theta \le 25.49°$, of which 1745 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The structure was solved by direct methods using Bruker SHELXS-97 and refined by full-matrix least-squares on F^2 using Bruker SHELXS-97. The final R and R_w factors were 0.0678 and 0.0918, respectively.

ACKNOWLEDGMENT

Financial support from the National Natural Science Foundation of China (NSFC project No. 30460083) is gratefully acknowledged.

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