Chemical Constituents of the Basidiomycete *Cortinarius umidicola*

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A new natural pyridine derivative (3-aldehyde-2-amino-6-methoxypyridine, 1) together with (R)-glycidyl octadecanoate (2) and five ergostane-type sterols (3–7) were isolated from the fruiting bodies of the basidiomycete *Cortinarius umidicola* Kauffm. Their structures were established by spectral methods (MS, IR, 1D and 2D-NMR experiments).

Key words: *Cortinarius umidicola*, Basidiomycete, 3-Aldehyde-2-amino-6-methoxypyridine

Introduction

The basidiomycete *Cortinarius umidicola* grows under pine trees in a mountainous region near Kunming. Its property of edibility and toxicity has not been understood and the chemical constituents have not been reported. As part of our continuing research on basidiomycete-derived bioactive secondary metabolites of higher fungi in Yunnan Province, China, the chemical constituents of *C. umidicola* were investigated. From methanol and methanol/chloroform (1:1, v/v) extracts of the fruiting bodies, a new natural pyridine derivative: 3-aldehyde-2-amino-6-methoxypyridine (1) was isolated. In addition, (R)-glycidyl octadecanoate (2), together with five ergostane-type sterols (3–7) as common metabolites of fungal species, were isolated from this fungus material. This report describes the structure elucidation of the new compound (1) based on spectroscopic evidences.

Results and Discussion

Compound 1 was obtained as colorless needles, m.p. 186–187 °C. Its molecular formula was determined to be C₇H₈N₂O₂ by HR-EI-MS ([M]⁺, 152.0495; calcd. for C₇H₈N₂O₂: 152.0585). The IR spectrum exhibited sharp absorptions of an amino-group at 3442 and 3175 cm⁻¹, characteristic absorptions of a heterocycle at 3025, 1604, 1577 cm⁻¹, and aldehyde group (2824, 2725, 1648 cm⁻¹). Evidence of the existence of one methoxy group was provided by the presence of one singlet at δH 3.90 appearing in ¹H NMR and one CH₃ signal exhibited at δC 56.3 in the ¹³C NMR spectrum (DEPT). NMR spectra showed signals of an aldehyde group (δH 9.75, 1H, s, δC 206.7, CH), moreover, the ¹³C NMR (DEPT) spectrum displayed resonances of three sp² quaternary carbons (δ 167.9, C-6; 164.8, C-2, 124.0, C-3) and two sp² methines (132.9, C-4; 114.9, C-5). Taking the molecular formula into account, the above spectral data revealed that 1 should contain pyridine ring with an aminogroup, an aldehyde and a methoxy group. The coupling constant (J = 7.1 Hz) between two protons proposed that they are located in vicinal position in the pyridine ring (Yu and Yang, 1999). The correlation peaks between H-4 (δ 8.02, 1H, dd, J = 7.1, 1.6, 1.8 Hz) and C-6 (δ 167.9), C-2 (δ 164.8) and the aldehyde carbonyl: H-5 (δ 7.04, 1H, d, J = 7.1Hz) and C-3 (δ 124.0). Protons of methoxyl and C-6 in the HMBC spectrum suggested that the aldehyde carbonyl, methoxyl group, and the aminogroup were substituted at C-3, C-6, C-2, respectively. Therefore, the structure of compound 1 was deduced to be 3-Aldehyde-2-amino-6-methoxypyridine as shown in Fig. 1.

Compound 2 was isolated as an optically active amorphous solid ([α]D² -13.3°, c 0.15, C₅H₅N). Its molecular formula was suggested to be C₂₁H₄₀O₃ by analysis of the EI-MS spectrum ([M]⁺ at m/z 340) and NMR data. The IR spectrum exhibited a strong absorption of an ester carbonyl group at 1715 cm⁻¹ and a characteristic band of long aliphatic chain (721 cm⁻¹). Existence of a long alkyl chain in the molecule was suggested according to signals in the NMR spectra of a terminal methyl at δH 0.85 (3H, t, J = 6.4 Hz, H₃-18'), overlapped resonances of...
methylenes at $\delta_H$ 1.25–1.41 (28H, $m$, H-4‘–17’), $\delta_C$ 14.3 (C-18’), overlapped $\delta_C$ 25.3, 29.4–30.0 (C-4‘–17’), and a signal of ester carbonyl group at $\delta_C$ 173.8 (C-1’). In addition, the $^{13}$C NMR (DEPT) revealed signals of an oxygenated methine, two oxygenated methylenes, indicative of the presence of glycidyl group, if taking the molecular formula and degree of unsaturation of 2 into account. The fragment ion at $m/z$ 267, 73, 57). In addition, the13C NMR (DEPT) revealed signals of an oxygenated methine, two oxygenated methylenes, indicative of the presence of glycidyl group, if taking the molecular formula and degree of unsaturation of 2 into account. The fragment ion at $m/z$ 267 ([C18H35O]+) formed by loss of the glycidyl group from the molecular ion in EI-MS spectrum confirmed the above assumption and indicated the long chain fatty acid was octadecanoic acid. All the spectral evidence supported that 2 is glycidyl octadecanoate. The absolute configuration of the chiral carbon (C-2) was identified to be $R$, according to the negative optical rotation, which was consistent with that of ($R$)-epoxy-glycidyl butyrate ($[\alpha]_D^{20}$ $30.00$, c neat) (Acros Organics, Geel, Belgium, 2002–2003).

Comparison of the physicochemical properties with the reported data allowed to identify compounds 3–7, isolated from the same fungus, as 3-O-$\beta$-d-glucopyranosyl-22E,24R-5α,α-epidioxyergosta-6,22-diene, (22E,24R)-ergosta-5,7,22-trien-3β-ol, 8α-epidioxy-(22E,24R)-ergosta-6,22-dien-3β-ol, (22E,24R)-ergosta-7,22-dien-3β,5α,6β-triol, (22E,24R)-ergosta-4,6,8(14),22-tetraen-3-one, respectively.

Table I. $^1$H and $^{13}$C NMR data for 2 ($\delta$ in ppm, $J$ in Hz, in pyridine-$d_5$).

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$ (DEPT)</th>
<th>$\delta_H$</th>
<th>$^1$H–$^1$H COSY (selected)</th>
<th>HMBC (selected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.8 (t)</td>
<td>4.68</td>
<td>H-2</td>
<td>H-2, 3</td>
</tr>
<tr>
<td>2</td>
<td>71.0 (d)</td>
<td>4.45 (1H, $p$, $J = 5.5$)</td>
<td>H-1, 3</td>
<td>H-1, 3</td>
</tr>
<tr>
<td>3</td>
<td>64.3 (t)</td>
<td>4.12 (2H, $d$, $J = 5.5$)</td>
<td>H-2</td>
<td>H-1, 2</td>
</tr>
<tr>
<td>1’</td>
<td>173.8 (s)</td>
<td>2.35 (2H, $t$, $J = 7.4$)</td>
<td>H-3’</td>
<td>H-1, 2’, 3’</td>
</tr>
<tr>
<td>2’</td>
<td>34.4 (t)</td>
<td>2.35 (2H, $t$, $J = 7.4$)</td>
<td>H-3’</td>
<td>H-1, 2’, 3’</td>
</tr>
<tr>
<td>3’</td>
<td>32.1 (t)</td>
<td>1.63 (2H, $p$, $J = 7.5$)</td>
<td>H-2’, 4’</td>
<td></td>
</tr>
<tr>
<td>4‘–17’</td>
<td>25.3, 29.4 ~30.0 (t)</td>
<td>1.25 ~1.41 (28H, $m$)</td>
<td>H-17’</td>
<td>H-17’, 16’</td>
</tr>
<tr>
<td>18’</td>
<td>14.3 (q)</td>
<td>0.85 (3H, $t$, $J = 6.4$)</td>
<td>H-17’</td>
<td>H-17’, 16’</td>
</tr>
</tbody>
</table>

Experimental

General

Melting points were obtained on an XRC-1 apparatus (Sichuan University, Sichuan, People’s Republic of China). Optical rotations were taken on a Horiba SEPA-300 automatic polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR) spectra ($^1$H, $^{13}$C, and two-dimensional NMR) were acquired on DRX-500 NMR instruments (Bruker, Karlsruhe, Germany) at 500 MHz for $^1$H and 125 MHz for $^{13}$C NMR; tetramethysilane was used as an internal standard and coupling constants were represented in Hertz. Mass spectra were measured with a VG Autospec3000 mass spectrometer (VG, Manchester, England). Infrared (IR) spectra were obtained in KBr pellets on a Bio-Rad FTS-135 IR spectrophotometer (Bio-Rad, Richmond, CA).

Material

Column chromatography (CC) was performed on silica gel (200–300 mesh; Qindao Marine Chemical Ltd., Qindao, People’s Republic of China). Reversed-phase column chromatography was carried out on LiChroprep® RP-18 (40–63 μm, Merck, Darmstadt, Germany). All solvents were distilled before use.
The fresh fruiting bodies of *C. umidicola* were collected in Kunming, P. R. China in August 2002. A voucher specimen (HKAS 41152) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and isolation**

Fresh fruiting bodies of *C. umidicola* (25 kg) were soaked in 95% ethanol at room temperature to inactivate enzymes. After filtration, the fruiting bodies were dried by air and finely crushed. The dried powders were extracted exhaustively with methanol (5 l x 3), then with chloroform/methanol (1:1, v/v; 5 l x 4) at room temperature. After concentrated in vacuo, the combined extracts were partitioned between water and ethyl acetate. The organic layer was concentrated under reduced pressure to afford a dark brown gum (120 g), which was subjected to a silica gel column (15 x 80 cm) eluted with petroleum ether containing increasing amounts of acetone. Twelve fractions were collected. Fractions eluted with petroleum ether/acetone (100:1, 50:1, 20:1, 9:1, 8:2, 7:3, 6:4, 55:1) were collected. Fractions eluted with petroleum ether/acetone (9:1, v/v) afforded 7 (7.8 mg), 4 (110 mg), 5 (98 mg), 6 (10 mg), 3 (6.7 mg), respectively, by recrystallization. The fraction eluted with petroleum ether/acetone (9:1, v/v) was further chromatographed on a RP-18 column (eluents: MeOH/H2O, 85:15, v/v) to provide compound 1 (5.1 mg). The methanol-soluble fraction of the aqueous partition phase was subjected to RP-18 chromatography and eluted with 50% methanol to provide I (28 mg).

3-Aldehyde-2-amino-6-methoxyprpyridine (1). Colorless needles (methanol). M.p. 186-187 °C; IR (KBr) vmax cm⁻¹: 3442, 3175, 3025, 2982, 2842, 2725, 2672, 2561, 1685, 1604, 1577,1472, 1302, 1264, 1179, 1169. UV (MeOH) λmax (log ε) nm: 205 (4.32), 252 (4.38). HR-ESIMS: 152.0495 (C7H9N2O2 [M]+: Calc. 152.0585). EI-MS m/z (rel. int, %): 152 ([M–H]+, 100), 151([M–H]+, 30), 137 ([M–CH3]+, 53), 136 ([M–H–CH3]+, 30), 121, 107, 92, 77, 62, 55. Negative FAB-MS m/z (rel. int, %): 243 ([M–H+Gly]+, 70), 151 ([M–H]+, 100). 1H NMR (500m Hz, CD2COCO3) δ: 9.75 (1H, s), 8.02 (1H, dd, J = 7.11, 1.63, 1.83 Hz, H-4), 7.04 (1H, J = 7.1Hz, H-6), 3.90 (3H, s, OCH3). 13C NMR (125 MHz, CD2COCO3) δ: 167.9 (COH), 167.9 (C-6), 164.8 (C-2), 132.9 (C-4), 124.0 (C-3), 114.9 (C-5), 56.3 (OCH3).

(R)-Glycidyl octadecanoate (2). White powder. [α]D20 = -13.3° (c 0.15, C5H12N). IR (KBr) vmax cm⁻¹: 2985, 2852, 1715, 1456, 1388, 1258, 1012, 2672, 721. EI-MS (rel. int, %) m/z: 340 ([M]+, 1), 311 ([M–C2H5]+, 1), 267 ([C13H14O]+, 5), 134 (5), 111 (14), 98 (51), 83 (50), 74 ([C2H5O2]+, 42), 71(62), 57 ([C2H5O]+, 100). NMR data are given in Table I. 3-O-β-D-gluopyranosyl-22E,24R-5α,α-epidioxy-ergosta-6,22-diene (3). White needles (CHCl3/ MeOH); m.p. 213–215 °C; IR (KBr) vmax cm⁻¹: 2947, 2832, 1464, 1445, 1380, 1074, 987, 965, 855; EI-MS (70 eV) m/z (rel. int, %): 590 ([M]+, 2), 556 ([M–O]+, 2), 492 ([M–H2O–CH3]+, 40), 457 (3), 428 ([M–162]+, 4), 410 ([M–162–H2O]+, 18), 394 ([M–162–2H2O]+, 14), 378 ([M–162–H2O–O2]+, 33), 363 (8), 285 (13), 267(11), 251 (17); 13C NMR (125Hz, CDCl3) δ: 135.3 (C-6, 22), 132.3 (C-23), 131.0 (C-7), 103.0 (C-1’), 82.0 (C-5), 79.3 (C-8), 78.6 (C-4’), 78.3 (C-5’), 75.3 (C-2’), 73.8 (C-3), 71.5 (C-4’), 62.7 (C-6’), 56.3 (C-17), 52.0 (C-14), 51.8 (C-9), 44.7 (C-13), 43.0 (C-24), 40.0 (C-20), 39.5 (C-4’), 37.4 (C-10, 12), 35.1 (C-1), 34.6 (C-2), 33.3 (C-25), 29.0 (C-16), 23.6 (C-15), 21.1 (C-11), 21.09 (C-21), 20.2 (C-27),19.9 (C-26), 18.1 (C-19), 17.9 (C-28), 13.0 (C-18); 1H NMR(500Hz, CDCl3) δ: 6.48 (1H, d, J = 8.5 Hz, H-6), 6.21 (1H, d, J = 8.5 Hz, H-7), 5.25 (1H, dd, J = 15.3, 8.1 Hz, H-22), 5.17 (1H, dd, J = 15.3, 8.1 Hz, H-23), 4.91 (1H, d, J = 8.7 Hz, H-1’), 4.42 (1H, dd, J = 11.7, 4.8 Hz, H-6a), 4.38 (1H, dd, J = 11.7, 4.8 Hz, H-6b), 4.28 (1H, t, J = 9.1 Hz, H-4’), 4.17 (1H, t, J = 8.9 Hz, H-3’), 4.02 (1H, t, J = 8.0 Hz, H-2’), 3.83 (1H, m, H-5’), 2.52 (1H, dd, J = 10.2, 9.5 Hz, H-ax), 1.28–2.55 (sterol nucleus), 1.06 (3H, s, H-19), 1.01 (3H, d, J = 6.4 Hz, H-3’), 0.94 (3H, d, J = 6.8 Hz, H-3’), 0.84 (3H, d, J = 6.2 Hz, H-3’), 0.75 (3H, s, H-18). NMR data were in accordance with those reported (Yue et al., 2000).

(22E,24R)-ergosta-5,7,22-trien-3β-ol (= ergosteryl, 4). White needles; MS, NMR data are in consistence of those reported (Mishra et al., 1996).

5α,8α-epidioxy-(22E,24R)-ergosta-6,22-dien-3β-ol (5). White needles; MS, NMR data are in consistence of those reported (Ishizuka et al., 1997).

(22E,24R)-ergosta-7,22-dien-3β,5α,6β-triol (= cerylsterol, 6). White needles; MS, NMR data are in consistence of those reported (Iorizzi et al., 1988).

(22E,24R)-ergosta-4,6,8(14),22-tetraen-3-one (7). Orange needles; MS, NMR data are in consistence of those reported (Kjобаяши et al., 1992).
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