Chemical Constituents of the Basidiomycete *Cortinarius umidicola*

Lin Hu, Jian-Wen Tan, and Ji-Kai Liu*

Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China.
Fax: +86 871 5150227. E-mail: jkliu@mail.kib.ac.cn

* Author for correspondence and reprint requests

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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 $C_7H_8N_2O_2$ by HR-EI-MS ($[M]^+$, 152.0495; calcd. for $C_7H_8N_2O_2$: 152.0585). The IR spectrum displayed 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 δ_H 3.90 appearing in ^1H NMR and one CH_3 signal exhibited at δ_C 56.3 in the ^{13}C NMR spectrum (DEPT). NMR spectra showed signals of an aldehyde group (δ_H 9.75, 1H, s, δ_C 206.7, CH), moreover, the ^{13}C NMR (DEPT) spectrum displayed resonances of three sp^2 quaternary carbons (δ 167.9, C-6; 164.8, C-2, 124.0, C-3) and two sp^2 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 ($[\alpha]_D^{21}$ −13.3°, *c* 0.15, C_5H_5N). Its molecular formula was suggested to be $C_{21}H_{40}O_3$ 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 δ_H 0.85 (3H, *t*, J = 6.4 Hz, H_3-18′), overlapped resonances of

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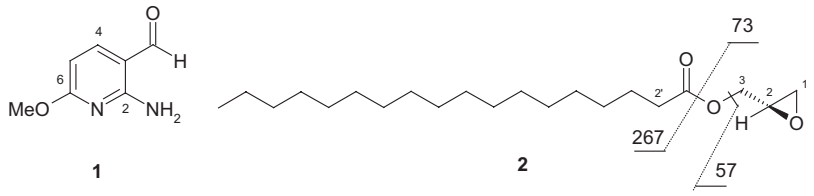

Fig. 1. The structures of 3-aldehyde-2-amino-6-methoxypyridine (**1**) and (*R*)-glycidyl octadecanoate (**2**, the characteristic fragment ions at *m/z* 267, 73, 57).

methylenes at δ_H 1.25–1.41 (28H, *m*, H-4′~17′), δ_C 14.3 (C-18′), overlapped δ_C 25.3, 29.4–30.0 (C-4′~17′), and a signal of ester carbonyl group at δ_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 ($[C_{18}H_{35}O]^+$) 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-β-D-glucopyranosyl-22E,24*R*-5α,α-epidioxyergosta-6,22-diene, (22*E*,24*R*)-ergosta-5,7,22-trien-3β-ol,5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol, (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol, (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one, respectively.

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; tetramethylsilane 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^R RP-18 (40–63 μm, Merck, Darmstadt, Germany). All solvents were distilled before use.

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

Position	δ_C (DEPT)	δ_H	^{1}H-^{1}H COSY (selected)	HMBC (selected)
1	66.8 (*t*)	4.68 (2H, AB_2 coupling system)	H-2	H-2, 3
2	71.0 (*d*)	4.45 (1H, *p*, *J* = 5.5)	H-1, 3	H-1, 3
3	64.3 (*t*)	4.12 (2H, *d*, *J* = 5.5)	H-2	H-1, 2
1′	173.8 (*s*)			H-1, 2′, 3′
2′	34.4 (*t*)	2.35 (2H, *t*, *J* = 7.4)	H-3′	
3′	32.1 (*t*)	1.63 (2H, *p*, *J* = 7.5)	H-2′, 4′	
4′–17′	25.3, 29.4 ~30.0 (*t*)	1.25 ~1.41 (28H, *m*)		
18′	14.3 (*q*)	0.85 (3H, *t*, *J* = 6.4)	H-17′	H-17′, 16′

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 × 3), then with chloroform/methanol (1:1, v/v; 5 l × 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 × 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, 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/H_2O, 85:15, v/v) to provide compound **2** (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 **1** (28 mg).

3-Aldehyde-2-amino-6-methoxypyridine (**1**). Colorless needles (methanol). M.p. 186–187 °C; IR (KBr) ν_{max} cm^{-1}: 3442, 3175, 3025, 2982, 2842, 2725, 2672, 2561, 1685, 1604, 1577,1427, 1302, 1264, 1179, 1169. UV (MeOH) λ_{max} (log ε) nm: 205 (4.32), 252 (4.38). HR-EI-MS: 152.0495 ($C_7H_8N_2O_2$, $[M]^+$: Calc. 152.0585). EI-MS *m/z* (rel. int.,%): 152 ($[M-H]^+$, 100), 151($[M-H]^+$, 30), 137 ($[M-CH_3]^+$, 53), 136 ($[M-H-CH_3]^+$, 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 (500м Hz, CD_3COCD_3) 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*, OCH_3). ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 206.7 (COH), 167.9 (C-6), 164.8 (C-2), 132.9 (C-4), 124.0 (C-3), 114.9 (C-5), 56.3 (OCH_3).

(*R*)-Glycidyl octadecanoate (**2**). White powder. $[\alpha]_D^{21}$ −13.3° (*c* 0.15, C_5H_5N). IR (KBr) ν_{max} cm^{-1}: 2985, 2852, 1715, 1456, 1388, 1258, 1164, 1012, 2672, 721. EI-MS (rel. int., %) *m/z*: 340 ($[M]^+$, <1), 311 ($[M-C_2H_5]^+$, <1), 267 ($[C_{18}H_{35}O]^+$, 5), 134 (5), 111 (14), 98 (51), 83 (50), 74 ($[C_3H_5O_2+H]^+$, 42), 71(62), 57 ($[C_3H_5O]^+$, 100). NMR data are given in Table I.

3-O-β-D-glucopyranosyl-22*E*,24*R*-5α,α-epidioxyergosta-6,22-diene (**3**). White needles ($CHCl_3$/MeOH); m.p. 213~215 °C; IR (KBr) ν_{max} cm^{-1}: 2947, 2832, 1464, 1445, 1380, 1074, 1043, 987, 965, 855; EI-MS (70 eV) *m/z* (rel. int., %): 590 ($[M]^+$, 2), 556 ($[M-O_2]^+$, 2), 492 ($[M-H_2O-CH_3]^+$, 40), 457 (3), 428 ($[M-162]^+$, 4), 410 ($[M-162-H_2O]^+$, 18), 394 ($[M-162-2H_2O]^+$, 14), 378 ($[M-162-H_2O-O_2]^+$, 33), 363 (8), 285 (13), 267(11), 251 (17); ^{13}C NMR (125Hz, $CDCl_3$) 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, $CDCl_3$) 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}-4), 1.28–2.55 (sterol nucleus), 1.06 (3H, *s*, H_3-19), 1.01 (3H, *d*, *J* = 6.4 Hz, H_3-21), 0.94 (3H, d, *J* = 6.8 Hz, H_3-28), 0.84 (3H, *d*, *J* = 6.2 Hz, H_3-26, 27), 0.75 (3H, *s*, H_3-18). NMR data were in accordance with those reported (Yue *et al.*, 2000).

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

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

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

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

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