A New Ergostane Triterpenoid from a Solid Culture of the Basidiomycete *Inocybe lilacina*

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A new and rare ergostane triterpenoid, 4α-methyl-3α,4β-dihydroxy-ergost-8,24(28)-dien-7,11-dion-25-al (**1**), was isolated from cultures of the basidiomycete *Inocybe lilacina*, and its structure established on the basis of spectral methods (MS, IR, 1D and 2D NMR).

Key words: Ergostane Triterpenoid, *Inocybe lilacina*, Solid Culture

Introduction

Among the many diverse organisms, higher fungi (mushrooms, macromycetes) are a major source of biologically active natural products (Liu, 2005). Triterpenoids and related compounds are fairly common among mushroom metabolites (Zhou *et al*., 2006; Shiao, 2003; Gao, 2008) and are mainly classified into eight groups on the basis of their different carbon frameworks, among which ergostane triterpenoids are relatively rare. The first ergostane triterpenoids of mushroom origin were isolated from *Antrodia cinnamomea* in 1995 (Cherng *et al*., 1995), and no more than 30 compounds have been reported to date. In this paper, we report on the isolation and structure elucidation of a new ergostane triterpenoid from the solid culture of the toxic mushroom *Inocybe lilacina*.

Results and Discussion

Compound **1** (Fig. 1) was obtained as a colourless powder. The HRESI mass spectrum of **1** showed a pseudomolecular ion peak at m/z 493.2928, consistent with the molecular formula $C_{29}H_{42}O_5Na$ and nine degrees of unsaturation. The ^{1}H NMR spectrum (Table I) showed signals for five methyl groups at δ_H 0.72 (s), 1.99 (s), 0.87 (d, $J = 5.5$ Hz), 1.49 (d, $J = 7.5$ Hz), and 1.62 ppm (s), one carboxaldehyde proton at δ_H 9.60 ppm (s), one oxymethine group at δ_H 4.03 ppm (br s), and one terminal methylene group at δ_H 5.06 (br s) and 5.21 ppm (br s). The ^{13}C NMR and HMQC data for **1** supported the presence of three carbonyl groups (δ_C 203.4, 203.0, 204.7 ppm), three sp^2 quaternary carbon atoms (δ_C 155.0, 144.2, 150.6 ppm), one sp^2 methylene carbon atom (δ_C 110.7 ppm), one oxygenated methine group (δ_C 74.5 ppm), and one oxygenated quaternary carbon atom (δ_C 73.9 ppm) as well as two sp^3 quaternary carbon atoms, five methine, eight methylene, and five methyl groups, respectively (Table I). These observations, in combination with the molecular formula, indicated the presence of two OH groups and four rings in **1**.

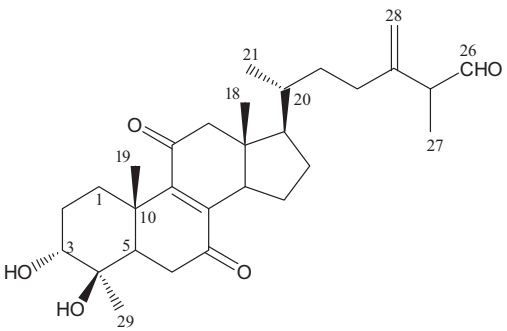

Fig. 1. Chemical structure of compound **1**.

Table I. ^{1}H and ^{13}C NMR data of **1** in pyridine-d_5.

No.	δ_H	δ_C
1	2.09 (dt, $J = 13.0, 3.5$)	29.0
	3.05 (dt, $J = 13.0, 3.5$)	
2	1.92 (m)	26.7
	2.74 (m)	
3	4.03 (br s)	74.5
4		73.9
5	2.65 (m)	44.6
6	2.90 (dd, $J = 13.1, 3.5$)	37.0
	3.17 (d, $J = 13.1$)	
7		203.4
8		155.0
9		144.2
10		40.1
11		203.0
12	2.45 (d, $J = 13.4$)	57.9
	2.96 (d, $J = 13.4$)	
13		47.7
14	2.62 (m)	49.9
15	1.66 (m)	25.9
	2.70 (m)	
16	1.26 (m)	28.2
	1.93 (m)	
17	1.40 (m)	54.3
18	0.72 (s)	12.3
19	1.99 (s)	19.7
20	1.38 (m)	36.3
21	0.87 (d, $J = 5.5$)	18.8
22	1.30 (m)	34.8
	1.75 (m)	
23	2.20 (m)	31.3
	2.39 (m)	
24		150.6
25	3.47 (q, $J = 7.5$)	46.8
26	9.60 (s)	204.7
27	1.49 (d, $J = 7.5$)	17.3
28	5.06 (br s)	110.7
	5.21 (br s)	
29	1.62 (s)	27.5

Chemical shifts (δ) are given in ppm and J in Hz.

Structural elucidation of **1** was accomplished by analysis of COSY and HMBC data (Fig. 2). The hydroxy groups attached at C-3 and C-4 were designated by the HMBC correlations from H-3 (δ_H 4.03 ppm, br s) to C-2 and C-4, and Me-29 (δ_H 1.62 ppm, s) to C-3, C-4, and C-5. The C-25 aldehydic functionality was assigned due to the presence of HMBC correlations from H-25 (δ_H 3.47 ppm, q, $J = 7.5$ Hz) to C-26, and Me-27 (δ_H 1.49 ppm, d, $J = 7.5$ Hz) to C-25 and C-26. The above data suggested a 4-methyl-3,4-dihydroxy-ergost-8,24(28)-dien-7,11-dion-25-al skeleton similar to that of antcin K (Shen *et al*., 2003). The key differences between the two compounds were that the hydroxy-bearing methine carbon atom C-7 and the carboxylic functionality at C-25 in antcin K were replaced by the carbonyl functionality and the aldehydic group in **1**, respectively, which were further supported by the HMBC correlations.

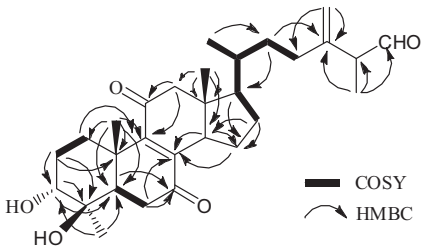

Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

The NOESY correlations of Me-19 to Me-18 and H-1b, H-5 to Me-29 and H-1a, H-1b to H-3 and Me-19, Me-18 to H-20 and H-12b (δ_H 2.96 ppm), H-14 to H-17 and H-12a (δ_H 2.45 ppm), and H-17 to H-14 and Me-21 revealed the relative stereochemistry as shown. The chemical shifts of protons and carbon atoms in the side chain were similar to those of antcin K, which strongly suggested the same 20*S* configuration for **1**. From the above evidence, the structure of **1** was unambiguously characterized as 4α-methyl-3α,4β-dihydroxy-ergost-8,24(28)-dien-7,11-dion-25-al.

Experimental

General experimental procedures

IR spectra were obtained on a Bruker Tensor 27 spectrometer (Bruker Optics, Ettlingen, Germany) with KBr pellets. UV spectra were recorded on a TU-1810 spectrometer (Pgeneral, Beijing, China). Optical rotation was measured on a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). NMR experiments were performed on a 600-MHz Bruker Avance spectrometer (Bruker BioSpin, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a microTOF-QII or a Xevo G2 TOF mass spectrometer (Waters, Milford, MA, USA). Column chromatography was performed using silica gel (200 – 300 mesh; Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). Thin-layer chromatography (TLC) analysis was carried out on silica gel GF_{254}-precoated plates (0.20 – 0.25 mm; Qingdao Marine Chemical Ltd.) with detection by spraying the plates with 10 % H_2SO_4 in ethanol and subsequent heating.

Fermentation

The fungus *I. lilacina* was purchased from China Forestry Culture Collection Center (Beijing, China). The fungal strain was maintained on slants of potato dextrose agar (PDA) at 25 °C for 10 d. Agar plugs were used to inoculate 250-mL Erlenmeyer flasks, each containing 50 mL of medium (0.4 % glucose, 1 % malt extract, and 0.4 % yeast extract), and the final pH value of the medium was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 d. Fermentation was carried out in twelve 1-L Erlenmeyer flasks each containing 160 g rice grains and 240 mL distilled H_2O. Each flask was inoculated with 10 mL of spore and mycelial suspension and incubated at 25 °C without shaking for 60 d.

Isolation

The fermented rice substrate was extracted three times with ethanol (3×1 L) and the organic solvent evaporated to dryness under vacuum to afford 13.6 g of crude extract, which was chromatographed on a silica gel column using CH_2Cl_2/CH_3OH gradient elution to afford five fractions, A – F. Fraction B (74 mg) was further purified on a Sephadex LH-20 column eluted with MeOH and repeated chromatography over silica gel using petroleum ether/EtOAc (100:1 – 30:1, v/v) as eluent to afford **1** (6.8 mg).

4α-Methyl-3α,4β-dihydroxy-ergost-8,24(28)-dien-7, 11-dion-25-al (**1**): Colourless powder. – $[\alpha]_D^{25} = +57^\circ$ ($c = 0.05$, MeOH). – UV (MeOH): $\lambda_{max}(\log \varepsilon) = 270$ (3.56) nm. – ^{1}H and ^{13}C NMR: see Table I. – (+)-ESIMS: $m/z = 493$ $[M + Na]^+$. – (+)-HRESIMS: $m/z = 493.2928$ $[M + Na]^+$ (calcd. for $C_{29}H_{42}O_5Na$, 493.2930).

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