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Antibacterial Constituents from Fruit Bodies of Ascomyce *Bulgaria inquinans*

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Two ergosterins and two triterpenoids were isolated from the dried fruit bodies of Ascomyce *Bulgaria inquinans*. By means of chemical (hydrolysis) and spectroscopic methods (NMR, EI-MS), their structures were established as betuinic acid (**1**), cerevisterol (**2**), (24*R*)ergosta-7, 22*E*-diene-3β, 5α, 6β-triol-3-*O*-palmitate (**3**) and ursolic acid (**4**). Compound **3** is a new compound.

Key words: *Bulgaria inquinans*, Ergosterin, Cerevisterol, Betuinic acid, Ursolic acid

INTRODUCTION

B. inquinans is a wood-inhabiting Ascomycete growing on freshly felled oak widely distributed in the North of China, which has activities of antibacterial (M.Stadler *et al.*, 1995) and photosensitization (Raymond *et al.*, 1976). Several benzofluoranthrene derivatives, one dihydroxy-perylenequinone (Raymond *et al.*, 1976) and three azaphiones (Stadler *et al.*, 1995), were isolated from the fruit bodies. In this paper, we describe the isolation and structure elucidation of four compounds firstly obtained from the fungus including a new one.

MATERIALS AND METHODS

General experimental procedures

Melting point was measured on a Yanaco-hot-stage without correction. NMR spectra were recorded on Bruker-ARX-300 spectrometer, using TMS as an internal standard. EI-MS was performed on VG-5050E mass spectrometer. The optical rotation was measured on Perkin-Elmer 241 polarimeter. Silica gel for chromatography was purchased from Qingdao Ocean Chemical Group Co. in China.

Plant materials

The fungus was collected at Chang Bai Mountain, Jilin Province, China, in August 2002, and identified by Medicine Industrial Research Institute of Jilin Province. A voucher specimen (NO. 20020801) is deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Dried fruit bodies (7.0 kg) of *B. inquinans* were extracted with 70% ethanol. The extract was concentrated *in vacuo*. Then the extract (1800 g) was partitioned with petroleum ether (3000 mL), $CHCl_3$ (3000 mL), EtOAc (3000 mL) and BuOH (5000 mL) successively. The petroleum ether fraction (60 g) was chromatographied on a silica gel column with gradient elution of PE-EtOAc mixture. Subfraction 8 [PE-EtOAc (100:5), 300.0 mg] was rechromatographied on a silica gel column eluted with PE-EtOAc (100:4) to give compound **1** (20.0 mg). Subfraction 20 [PE-EtOAc (100:40), 500.0 mg] was rechromatographied on a silica gel column eluted with $CHCl_3$-MeOH (100:7) to give compound **2** (18.0 mg). Subfraction 10 [PE-EtOAc (100:7), 700.0 mg] was rechromatographied on a silica gel column to give compound **3** [PE-EtOAc-acetone (100:5:5), 20.0 mg] and compound **4** [PE-EtOAc-acetone (100:8:8), 20.0 mg].

Compound **2**

White needle, m.p. 234-236 °C. $[\alpha]_D^{20}$ = -30.0 (*c* 0.02, MeOH). The ^{1}H-NMR (300 MHz, pyridine-d_5) : see Table I. The ^{13}C-NMR (75 MHz, pyridine-d_5): see Table II.

Compound **3**

White powder, m.p. 156-158 °C. $[\alpha]_D^{20}$ = -10.0 (*c* 0.01, MeOH). The ^{1}H-NMR (300 MHz, pyridine-d_5) : see Table I. The ^{13}C-NMR (75 MHz, pyridine-d_5): see Table II. The EI-MS (70 ev, ral. int %) data of palmitate on compound 3

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Table I. The ^{1}H-NMR data of compounds **2** and **3** (in pyridine-d_5)

Proton No.	2	3
3	4.84 (1H, m)	5.71 (1H, m)
7	5.74 (1H, m)	5.71 (1H, m)
18	0.65 (3H, s)	0.63 (3H, s)
19	1.58 (3H, s)	1.44 (3H, s)
21	1.05 (3H, d, *J*=6.6 Hz)	1.05 (3H, d, *J*=6.6 Hz)
22	5.23 (3H, dd, *J*=15.3, 6.9 Hz)	5.21 (3H, dd, *J*=15.2, 6.9 Hz)
23	5.15 (3H, dd, *J*=15.3, 7.7 Hz)	5.28 (3H, dd, *J*=15.3, 7.7 Hz)
26	0.85 (3H, d, *J*=6.8 Hz)	0.85 (3H, d, *J*=6.7 Hz)
27	0.83 (3H, d, *J*=6.8 Hz)	0.83 (3H, d, *J*=6.7 Hz)
28	0.94 (3H, d, *J*=6.8 Hz)	0.94 (3H, d, *J*=6.8 Hz)

Table II. The ^{13}C-NMR data of compounds **2** and **3** (in pyridine-d_5)

Carbon No.	Cerevisterol	2	3	
			Cerevisterol	Palmitate
1	33.8	33.8	35.0	173.3 (C-1')
2	32.6	32.7	28.1	33.3 (C-2')
3	67.6	67.6	72.2	25.6 (C-3')
4	41.9	42.0	37.7	29.4 (C-4')
5	76.1	76.1	76.0	29.6 (C-5')
6	74.2	74.3	73.9	29.6 (C-6')
7	120.5	120.5	120.2	29.8 (C-7')
8	141.6	141.5	141.6	30.0 (C-8'~13')
9	43.7	43.8	43.7	32.1 (C-14')
10	38.1	38.1	38.0	22.9 (C-15')
11	22.4	22.4	22.3	14.3 (C-16')
12	39.9	38.9	39.8	
13	43.7	43.8	43.5	
14	55.2	55.3	55.2	
15	23.5	23.5	23.5	
16	28.5	28.5	28.5	
17	56.1	56.1	56.0	
18	12.5	12.5	12.5	
19	18.8	18.8	18.6	
20	40.9	40.9	40.9	
21	21.4	21.4	21.4	
22	136.1	136.2	136.2	
23	132.1	132.1	132.1	
24	43.1	43.1	43.5	
25	33.4	33.3	33.3	
26	20.2	20.1	20.1	
27	19.9	19.8	19.8	
28	17.9	17.8	17.8	

after alkaline hydrolysis: *m/z* 256 $[M]^+$ (100), 239 (3), 227 (7), 213 (21), 199 (8), 185 (16), 171 (12), 157 (16), 143 (7), 129 (36), 115 (15).

RESULTS AND DISCUSSION

. 70% ethanol extract of the air-dried fruit bodies of *B. inquinans* was separated using liquid-liquid extraction. The petroleum ether fraction was subjected to various isolation procedures including classical methods of separation followed by chromatographic techniques as described in plant Material. These studies have led to the isolation of four compounds. The structure of compound **2** have been established by using spectral methods (^{1}H-NMR, ^{13}C-NMR and EI-MS) and chemical method (hydrolysis), while the other compounds were identified by comparisons of their spectroscopic (^{1}H-NMR and ^{13}C-NMR) data with those of the corresponding constituents reported in the literature (Junichi *et al.*, 1990; Yoshihisa *et al.*, 1991; Mahoto *et al.*, 1994).

Compound **2** was isolated as white powder, m.p. 234-236 °C It showed a positive reaction with Liebermann-Burchard reagent. The ^{1}H-NMR spectrum gave two tertiary methyl and four secondary methyl signals at δ 0.65 (3H, s, 18-CH_3), 1.58 (3H, s, 19-CH_3), 0.83 (3H, d, J = 6.8 Hz, 27-CH_3), 0.85 (3H, d, J = 6.8 Hz, 26-CH_3), 0.94 (3H, d, J = 6.8 Hz, 28-CH_3), 1.05 (3H, d, J = 6.6 Hz, 21-CH_3).

A pair of double bond signals could be observed at δ_C 132.1 (C-23) and 136.2 (C-22) in ^{13}C-NMR together with a couple of *trans*-configuration proton signals at δ_H 5.15 (1H, dd, J = 15.3, 7.7 Hz, H-23)/5.23 (1H, d, J = 15.3, 6.9 Hz, H-22), respectively. The other pair were observed at δ_C 120.5/141.5 and δ_H 5.74 (1H, m) assigned to C-7/C-8 and H-7, respectively. Three oxygen bearing carbon signals could be observed at δ_C 67.6 (C-3), 74.3 (C-6), 76.1 (C-5), and the proton signals attached to them were at δ_H 4.32 (1H, m, H-6), 4.84 (1H, m, H-3) respectively. The ^{1}H-NMR and ^{13}C-NMR spectra data of compound **2** corresponded to the data of cerevisterol (ergosta-7,22*E*-diene-3β,5α,6β-triol) (Yoshihisa *et al.*, 1991) well, so we identified **2** as cerevisterol.

Compound **3** was isolated as white powder, m.p. 156-158 °C. It showed a positive reaction with Liebermann-Burchard reagent. The ^{1}H-NMR spectrum of **3** was very similar to that of **2** except for chemical shift at δ 5.71 (1H, m, H-3), an additional strong signal including 21 protons at δ 1.25 and another additional methyl signal at δ 0.85 (3H, t), which suggested that the proton of 3-OH should be substituted by a long chain fatty acid.

The ^{13}C-NMR spectrum of **3** was also similar to that of **2**. There were two differences between these two spectrums. Firstly, the signals of C-1 and C-3 were

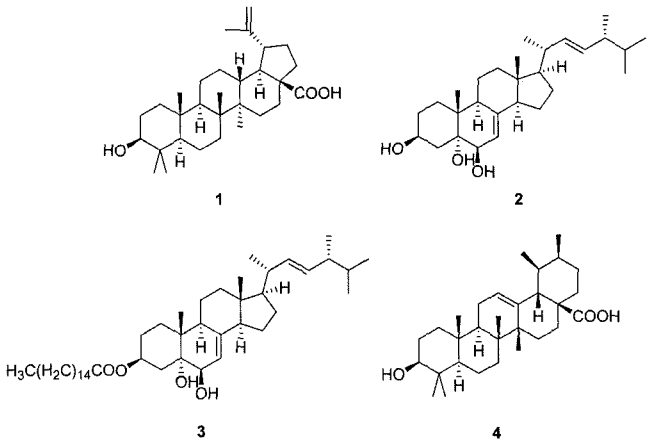

Fig. 1. Structures of compounds **1-4**

downfield-shifted to 35.0 and 72.2 respectively, however, the signal of C-2 was upfield-shifted to 28.1, which further confirmed our conclusion mentioned above that an acyl group was linked to 3-OH. Secondly, an additional carbonyl carbon signal at δ 173.3 and others 8 additional signals at the range of δ 14.3-33.3 (a strong signal at δ 30.0 maybe overlap several carbon signals). As the additional spectrum fraction of **3** was corresponding with palmitate fraction signals in the reference (SUN Hong-xiang *et al.*, 2002), we got the inference that compound **3** was a palmitate-substituted derivative of **2**.

In order to confirm the structure of **3**, we hydrolyzed it with KOH (Dale *et al.*, 1992). The hydrolyzed alkaline solution was neutralizated with HCl and extracted with petroleum ether, a white fatty acid was obtained. Its EI-MS showed a molecular ion at *m/z* 256, corresponding to the palmitate. The Co-TLC which was performed with the residue of the solution and compound **2** (MeOH-$CHCl_3$= 10:1 Rf 0.3) revealed the precursor of compound **3** was identical to compound **2**, cerevisterol.

On the basis of the above analysis, compound **3** was identified as (24*R*)ergosta-7, 22*E*-diene-3β, 5α, 6α-triol-3-O-palmitate, which is a new product.

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