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CYTOTOXIC STEROIDS FROM THE MUSHROOM *AGARICUS BLAZEI*

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Key Word Index—*Agaricus blazei*; Agaricaceae; cytotoxicity; steroids.

Abstract—Four steroids were isolated from the fruiting bodies of *Agaricus blazei*. Among them, three compounds **2**, **3** and **6** were cytotoxic against HeLa cells. Compound **2** was a novel steroid.

INTRODUCTION

The Basidiomycete fungus, *Agaricus blazei* (Japanese name: Himematsutake) is known as a home remedy having many physiological activities. In the course of our research on the compounds active against HeLa cells, four steroids were obtained. This paper describes the isolation, structure and cytotoxicity of these compounds.

RESULTS AND DISCUSSION

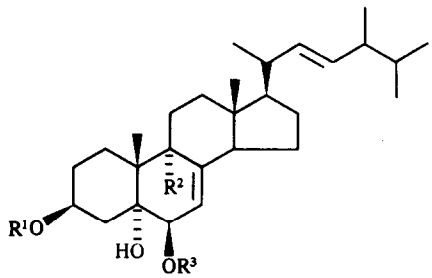
Fresh fruiting bodies of *A. blazei* were extracted with acetone. The extract was concentrated and partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated and separated into its acidic, basic and neutral fractions. Repeated chromatography of the neutral fractions using silica gel afforded compounds **1**–**3** and **6**.

Compound **1** showed ion peaks at FDMS *m/z* 430 $[M]^+$, 412 $[M-H_2O]^+$ (base peak) and EIMS *m/z* 412 $[M-H_2O]^+$. The ^1H NMR data are shown in Table 1. All the data of **1** agreed with those of cerevisterol [1–8].

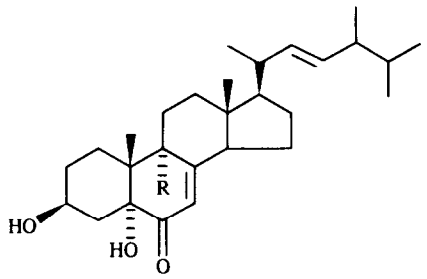
Compound **2** showed a molecular ion peak at *m/z* 444 by the FDMS and has the molecular formula $C_{29}H_{48}O_3$ from the high resolution mass spectrum of the dehydrated peak of the molecular ion, *m/z* 426.3508 $[M-H_2O]^+$ (426.3498, calcd $C_{29}H_{46}O_2$). The ^1H NMR data of **2** are similar to those of cerevisterol **1**. However, compound **2** possesses a methoxy group instead of a hydroxy group in cerevisterol **1**. Acetylation of **2** with pyridine and acetic anhydride gave a monoacetate **4**. From the chemical shift of H-3 proton of **4**, it is obvious that the hydroxy group of C-3 was acetylated. Table 2 shows ^{13}C NMR data of compound **2** and cerevisterol **1**, which are quite similar except for the chemical shift of C-6. In addition, the NOE appeared at H-4β (2.12 ppm, *dd*, $J = 12.91$, 11.81), H-6(3.16 ppm) and H-7(5.39 ppm) by irradiation at methoxy protons (3.38 ppm) in the NOE difference experiments. These results allow us to conclude that the methoxy group is attached at C-6 and the stereochemistry of the methoxy group is β. Therefore, the structure of **2** was determined to be 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene.

Compound **6**; FDMS, *m/z* 444$[M]^+$ (base peak). EIMS, *m/z* 426 $[M-H_2O]^+$, 390$[M-3H_2O]^+$. IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1620.

Compound **3**; FDMS, *m/z* 446 $[M]^+$, 428$[M-H_2O]^+$ (base peak). Acetylation of **3** afforded a diacetate, **5**.



	R^1	R^2	R^3
1	H	H	H
2	H	H	Me
3	H	OH	H
4	Ac	H	Me
5	Ac	OH	Ac



	R
6	OH
7	H

Table 1. ^{1}H NMR data for compounds **1**–**6**

	δ ppm [multiplicity, J (Hz)]*					
H	**1**	**2**	**3**	**4**	**5**	**6**
3	4.07 (*m*)	4.03 (*m*)	3.76 (*m*)	5.13 (*m*)	5.16 (*m*)	4.06 (*m*)
6	3.62 (*d*, 4.81)	3.16 (*d*, 4.95)	3.56 (*m*)	3.16 (*d*, 4.81)	4.95 (*dd*, 4.89, 2.24)	
7	5.35 (*dd*, 4.81, 2.37)	5.39 (*dd*, 4.95, 2.47)	5.12 (*dd*, 5.49, 2.20)	5.40 (*dd*, 4.81, 2.20)	5.30 (*dd*, 4.89, 2.20)	5.65 (*d*, 2.25)
18	0.59 (*s*)	0.59 (*s*)	0.54 (*s*)	0.59 (*s*)	0.61 (*s*)	0.62 (*s*)
19	1.09 (*s*)	0.99 (*s*)	0.95 (*s*)	1.01 (*s*)	1.13 (*s*)	1.01 (*s*)
21	1.03 (*d*, 6.72)	1.01 (*d*, 6.59)	0.97 (*d*, 6.59)	1.02 (*d*, 6.59)	1.02 (*d*, 6.35)	1.03 (*d*, 6.54)
22	5.16 (*dd*, 15.39, 8.09)	5.15 (*dd*, 15.38, 7.69)	5.17 (*dd*, 15.39, 7.70)	5.18 (*dd*, 15.39, 7.33)	5.20 (*m*)	5.16 (*dd*, 15.10, 7.89)
23	5.23 (*dd*, 15.39, 7.04)	5.21 (*dd*, 15.38, 7.00)	5.23 (*dd*, 15.39, 6.96)	5.22 (*dd*, 15.39, 6.96)		5.24 (*dd*, 15.10, 7.32)
26	0.82 (*d*, 6.34)	0.81 (*d*, 6.59)	0.79 (*d*, 6.59)	0.82 (*d*, 6.59)	0.82 (*d*, 6.84)	0.82 (*d*, 6.76)
27	0.84 (*d*, 6.92)	0.83 (*d*, 6.32)	0.80 (*d*, 6.60)	0.84 (*d*, 6.59)	0.84 (*d*, 6.59)	0.84 (*d*, 6.54)
28	0.92 (*d*, 6.64)	0.91 (*d*, 6.87)	0.88 (*d*, 6.96)	0.91 (*d*, 6.96)	0.92 (*d*, 6.84)	0.92 (*d*, 6.76)
OMe		3.38 (*s*)		3.37 (*s*)		
Ac				2.04 (*s*)	2.04 (*s*) 2.07 (*s*)	

*In $CDCl_3$ except for compound **3** (in DMSO-d_6).

The data mentioned above and the ^{1}H NMR data in Table 1 for **6**, **3** and **5** are in good agreement with those of the compounds which have been isolated from the fungus *Polyporus versicolor* as cytotoxic principles to hepatoma cells [8].

The minimum concentrations giving complete growth inhibition of HeLa cells for **6** was 8 μg/ml, for **2** was 16 μg/ml, for **7** was 32 μg/ml and for **3** was 63 μg/ml. Compound **7** was prepared from ergosterol [8, 9]. It is interesting that cerevisterol **1** exhibited no activity and its C-6 methoxy derivative **2** exhibited a strong activity and its C-6 oxo-one **7** a moderate one. Against hepatoma cells, similar results have been obtained; the C-6 methoxy derivative of **3** has the most potent toxicity, followed by **6**, then **3** [8].

EXPERIMENTAL

Mps: uncorr.; ^{1}H NMR: 400 MHz, TMS as int. standard; ^{13}C NMR: 100 MHz.

Extraction and isolation. Fresh fruiting bodies of *A. blazei* (5 kg) were homogenized and extracted with Me_2CO (10 l). The extract was coned and partitioned between EtOAc and H_2O. The EtOAc layer (2 l) was then washed with satd aq. $NaHCO_3$ and 1 M HCl, and dried with Na_2SO_4. The residue (14.9 g) obtained after removing the EtOAc was chromatographed on the column of silica gel [Wakogel C-200, 450 g, eluted with $CHCl_3$–MeOH (47:3)] and further purified by repeated prep. TLC.

Cerevisterol (3β,5α,6β-*trihydroxyergosta*-7,22-*diene*), **1**. Compound **1** was crystallized from $CHCl_3$ as colourless plates (2.3 mg), mp 225–228°. $[\alpha]_D^{20}$ −94° (pyridine; *c* 0.29). FDMS *m/z*: 430 $[M]^+$, 412 $[M-H_2O]^+$ (base peak). EIMS *m/z*: 412 $[M-H_2O]^+$. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1020.

3β,5α-*Dihydroxy*-6β-*methoxyergosta*-7,22-*diene* **2**. Compound **2** was obtained as a colourless syrup (3.4 mg). $[\alpha]_D^{20}$ −61° ($CHCl_3$; *c* 1.19). FDMS *m/z*: 444 $[M]^+$ (base peak). EIMS *m/z*: 426 $[M-H_2O]^+$, 411 $[M-H_2O-Me]^+$, 393 $[M-2H_2O-Me]^+$. IR ν_{max}^{film} cm^{-1}: 3400, 1640.

3β-*Acetoxy*-5α-*hydroxy*-6β-*methoxyergosta*-7,22-*diene*, **4**. Compound **2** was acetylated with acetic anhydride and pyridine to give **4** as colourless needles, mp 105–107°. $[\alpha]_D^{20}$ −13° ($CHCl_3$; *c* 0.23). EIMS *m/z*: 486.3660 (M^+, $C_{31}H_{50}O_4$), 468 $[M-H_2O]^+$, 453 $[M-H_2O-Me]^+$, 408 $[M-H_2O-AcOH]^+$, 393 $[M-H_2O-AcOH-Me]^+$, 376 $[M-H_2O-AcOH-MeOH]^+$. IR ν_{max}^{KBr} cm^{-1}: 3440, 1730, 1710.

3β,5α,9α-*Trihydroxyergosta*-7,22-*diene*-6-*one*, **6**. Compound **6** was crystallized from $CHCl_3$–hexane as colourless needles (5.7 mg), mp 226–230°. $[\alpha]_D^{20}$ −60° ($CHCl_3$; *c* 0.14).

3β,5α,6β,9α-*tetrahydroxyergosta*-7,22-*diene*, **3**. Compound **3**

Table 2. ^{13}C NMR data for compounds **1** and **2**

C	δ ppm (in $CDCl_3$) 1	2
1	32.79	32.77
2	30.44	30.83
3	67.23	67.86
4	39.28	39.38
5	75.88	76.20
6	73.06	82.43
7	117.33	115.00
8	143.22	143.69
9	43.16	43.89
10	37.00	37.26
11	22.00	22.17
12	38.89	39.52
13	43.64	43.89
14	54.69	54.98
15	22.93	22.90
16	27.99	27.93
17	55.93	56.03
18	12.28	12.32
19	18.37	18.33
20	40.44	40.38
21	19.62	19.66
22	131.88	132.15
23	135.30	135.46
24	42.81	42.85
25	33.09	33.50
26	19.94	19.95
27	21.11	21.12
28	17.58	17.62
OMe	—	58.25

was crystallized from $CHCl_3$–MeOH as colourless needles (6.3 mg), mp 225~227°. $[\alpha]_D^{20}$ −140° (pyridine, *c* 1.20). IR ν_{max}^{KBr} cm^{-1}: 3300, 1650.

3β,6β-Diacetoxy-5α,9α-dihydroxyergosta-7,22-diene, **5**. Compound **3** was acetylated with acetic anhydride and pyridine to give **5** as colourless needles, mp 190–193°. $[\alpha]_D^{20}$ −133° ($CHCl_3$; *c* 0.070). FDMS *m/z*: 530[M]$^+$ (base peak). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720.

Bioassay against HeLa cells. HeLa S_3 cells were maintained in monolayers in Eagle's minimum essential medium (MEM) containing 10% bovine serum and kanamycin (60 μg/ml) at 37°. HeLa S_3 cells (1×10^4) in 200 μl of each of the medium containing various concns of the compounds for testing were placed in a well of a tissue culture plate (96 wells) and incubated for 72 hr at 37° in a 5% CO_2–95% air atmosphere. The cells were fixed with MeOH, stained by the Giemsa's method, and counted.

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