Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2013, **11**(1): 0071–0073
doi: 10.3724/SP.J.1009.2013.00071
Chinese Journal of Natural Medicines

A new menthane-type monoterpene from *Pleurotus eryngii*

LIU Liang-Yan[1, 2], LI Zheng-Hui [1], LIU Ji-Kai [*]

[1] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*;
[2] *Graduate University of Chinese Academy of Sciences, Beijing 100049, China*

Available online 20 Jan. 2013

[ABSTRACT] A new menthane-type monoterpene, pleurolactone (**1**), was isolated from the culture broth of the fungus *Pleurotus eryngii*, along with five known compounds 1, 2-dihydroxymintlactone (**2**), (22*E*, 24*R*)-ergosta-5, 7, 22-trien-3*β*-ol (**3**), (22*E*, 24*R*)-ergosta-7, 22-dien-3*β*, 5*α*, 6*β*-triol (**4**), (22*E*, 24*R*)-ergosta-7, 22-dien-3*β*-ol (**5**), and cerebroside B (**6**). Their structures were identified by extensive spectroscopic analyses.

[KEY WORDS] *Pleurotus eryngii*; Menthane-type monoterpene; Pleurolactone

[CLC Number] R284.1 **[Document code]** A **[Article ID]** 1672-3651(2013)01-0071-03

1 Introduction

Pleurotus eryngii (DC.) Quél. (Pleurotaceae) (King oyster), the largest species of the oyster mushroom genus, is well known for its good taste. Its original distribution is located in Northern Africa, Southern Europe, and Central Asia[1], and in the 1990s', it was introduced in to China. The previous chemical investigation on *P. eryngii* revealed several polysaccharides with various biological activities[2], such as hepatoprotective, hypolipidemic[3], antioxidant[4], antitumor and immunomodulating functions[5]. However, little attention was paid on its small-molecular constituents. As a part of efforts to discover structurally diverse and biologically significant metabolites from higher fungi[6-9], the investigation of the culture broth of *P. eryngii* led to the isolation of a new menthane-type monoterpene, named pleurolactone (**1**), along with five known compounds 1,2-dihydroxymintlactone (**2**)[10], (22*E*, 24*R*)-ergosta-5, 7, 22-trien-3*β*-ol (**3**) [11], (22*E*, 24*R*)-ergosta-7, 22-diene-3*β*, 5*α*, 6*β*-triol (**4**)[12], (22*E*, 24*R*)-ergosta-7, 22-dien-3*β*-ol (**5**)[13], and cerebroside B (**6**)[14]. The structure of **1** was elucidated by extensive spectroscopic analyses, while the known compounds were identified by comparison with the NMR data in the literature. All these compounds were isolated from this fungus for the first time.

[Received on] 20-Dec.-2011
[Research funding] This project was supported by the National Basic Research Program of China (973 Program, No. 2009CB522300), and the National Natural Sciences Foundation of China (Nos. 30830113, U1132607).
[*Corresponding author] LIU Ji-Kai: Prof., Tel: 86-871-5216327. Fax: 86-871- 5219934. E-mail: jkliu@mail.kib.ac.cn
These authors have no conflict of interest to declare.

2 Materials and Methods

The fungus *P. eryngii* was collected in Kunming, and identified by Prof. YANG Zhu-Liang, Kunming Institute of Botany. The culture medium consisted of potato (peeled, 200 g), glucose (20 g), KH_2PO_4 (3 g), and $MgSO_4$ (3 g) in deionized water (1 L). The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out in a shaker (150 $r \cdot min^{-1}$) at 25 °C for 25 days. The culture broth (20 L) was extracted with EtOAc three times, and the organic layer was concentrated under reduced pressure to give a crude extract (19 g). This residue was subjected to column chromatography (CC) over silica gel using a petroleum ether/acetone gradient (1 : 0 → 0 : 1) to afford fractions A—F. Fraction C was separated by CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) to obtain two subfractions, C-1 and C-2. Subfraction C-1 was purified by CC over MCI eluted with acetone, to obtain compound **2** (4 mg). Subfraction C-2 was purified by prep-HPLC (MeCN/H_2O, 0 : 100 → 15 : 85) to yield compound **1** (9 mg). Fraction B was subjected to CC over silica gel eluting with $CHCl_3$/MeOH (30 : 1 → 10 : 1) to provide subfractions B-1 to B-4. Compounds **3** (10 mg) and **5** (10 mg) were obtained from subfraction B-2 and B-3, respectively. Compounds **4** (3 mg) and **7** (20 mg) were obtained from fraction D, by repeated CC.

3 Results

Compound **1** was isolated as a colorless oil with the molecular formula $C_{10}H_{14}O_4$ deduced from the $[M + Na]^+$ peak at *m/z* 221.078 8 (Calcd. 221.078 9) in HRESI-MS. The IR spectrum displayed absorption bands for hydroxyl (3440 cm^{-1}), carbonyl (1 760 cm^{-1}) and alkene (1 630 cm^{-1}) functional groups. The ^{13}C NMR (DEPT) spectra revealed the presence of ten carbons, including one methyl (δ_C 26.4), three methylenes (δ_C 19.1, 31.8, and 119.9), three methines (δ_C 39.7, 77.5 and 82.6), and three quaternary carbons (δ_C 71.7, 136.8, and 170.6). The ^1H NMR spectrum indicated the existence of two olefinic protons (δ_H 6.26, d, J = 3.4 Hz, and 5.52, d, J = 3.4 Hz), belonging to an sp^2 methylene. The above resonances suggested that compound **1** had a similar structure with the known compound 1, 2-dihydroxymintlactone (**2**)[10], which was also obtained from same fungus in this investigation, except for the double bond C_4=C_8 in **2** migrating to C_8=C_{10} in **1**. The elucidation was further supported by HMBC correlations from H-10 to C-9, C-8, and C-4. The relative stereochemistry of compound **1** was determined by the ROESY spectrum, in which the correlations of H-2 with H-7, and H-3 with the 2-OH and H-4 were detected. Therefore, compound **1** was elucidated as shown in Fig. 1.

Pleurotlactone (**1**): colorless oil; $[\alpha]^{24}_D$ +85.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 213 (2.95) nm; IR (KBr) ν_{max} 3440, 1760, 1630 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (positive) *m/z* 221 $[M + Na]^+$, 419 $[2M + Na]^+$; HRESI-MS (positive) *m/z* 221.078 8 (Calcd. for $C_{10}H_{14}O_4Na$, 221.078 9).

1, 2-Dihydroxymintlactone (**2**): colorless oil; ^1H and ^{13}C NMR data, see Table 1. The data were in accordance to those in the literature [10].

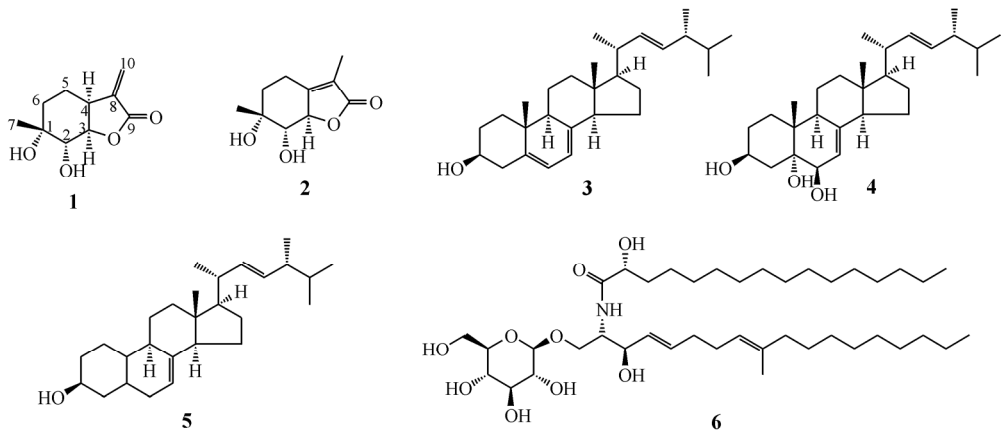

Fig. 1 Structures of compounds 1–6

Table 1 ^1H and ^{13}C NMR spectroscopic data for compounds 1 and 2 in $CDCl_3$

	1		2	
Position	δ_C (mult.)	δ_H (mult., J in Hz)	δ_C (mult.)	δ_H (mult., J in Hz)
1	71.7 (s)		72.5 (s)	
2	77.5 (d)	3.18 (d, 7.9)	79.6 (d)	3.23 (d, 8.5)
3	82.6 (d)	4.62 (t, 7.9)	84.0 (d)	4.85 (d, 8.5)
4	39.7 (d)	3.29 (m)	120.6 (s)	
5a	19.1 (t)	2.18 (m)	21.3 (t)	2.60 (m)
5b		1.84 (m)		2.58 (m)
6a	31.8 (t)	1.70 (dt, 13.8, 4.0)	36.3 (t)	2.09 (dt, 13.8, 3.7)
6b		1.43 (td, 13.8, 4.4)		1.42 (dt, 13.8, 9.3)
7	26.4 (q)	1.26 (s)	25.9 (q)	1.33 (s)
8	136.8 (s)		160.1 (s)	
9	170.6 (s)		175.1 (s)	
10a	119.9 (t)	6.26 (d, 3.4)	8.3 (q)	1.82 (s)
10b		5.52 (d, 3.4)		

(22*E*, 24*R*)-Ergosta-5, 7, 22-trien-3β-ol (**3**): colorless needles; ^1H NMR (400 MHz $CDCl_3$): 5.57 (1H, m,), 5.38 (1H,

m), 5.22 (1H, dd, *J* = 15.3, 7.1 Hz), 5.16 (1H, dd, *J* = 15.3, 7.6 Hz), 3.64 (1H, m), 1.04 (3H, d, *J* = 6.6 Hz), 0.95 (3H, s), 0.92 (3H, d, *J* = 6.8 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.82 (3H, d, J = 6.5 Hz), 0.63 (3H, s); EI-MS: *m/z* 396 ([M]$^+$, 25).

(22*E*, 24*R*)-Ergosta-7, 22-diene-3*β*, 5*α*, 6*β*-triol (**4**): colorless needles; ^{1}H NMR (400 MHz, $CDCl_3$): 5.73 (1H, d, J = 3.4 Hz), 5.24 (1H, dd, *J* = 15.2, 7.3 Hz), 5.18 (1H, dd, *J* = 15.2, 8.2 Hz), 4.83 (1H, m), 4.32 (1H, d, *J* = 3.4 Hz), 3.02 (1H, dd, *J* = 12.6, 11.7 Hz), 1.52 (3H,s), 1.06 (3H, d, *J* = 6.5 Hz), 0.95 (3H, d, *J* = 6.8 Hz), 0.86 (3H, d, *J* = 6.3 Hz), 0.85 (3H, d, *J* = 6.6 Hz), 0.66 (3H, s); EI-MS: *m/z* 412 ([M − 18]$^+$, 7).

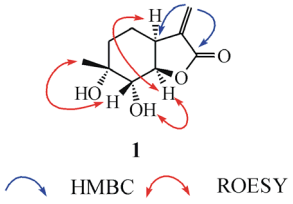

Fig. 2 Key HMBC and ROESY correlations of compound 1

(22*E*, 24*R*)-Ergosta-7, 22-dien-3*β*-ol (**5**): colorless needles; ^{1}H NMR (400 MHz, $CDCl_3$): 5.21 (1H, dd, *J* = 15.3, 7.1 Hz), 5.16 (1H, dd, *J* = 15.3, 7.6 Hz), 5.15 (1H, m), 3.59 (1H, m), 1.01 (3H, d, *J* = 6.6 Hz), 0.91 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.4 Hz), 0.82 (3H, d, *J* = 8.2 Hz), 0.79 (3H, s), 0.54 (3H, s); EI-MS: *m/z* 398 ([M]$^+$, 18).

Cerebroside B (**6**): amorphous powder; ^{1}H NMR (400 MHz, CD_3OD): 5.73 (1H, dt, *J* = 15.4, 6.2 Hz), 5.46 (1H, dd, *J* = 15.4, 7.6 Hz), 5.12 (1H, br.t, *J* = 6.8 Hz), 4.28 (1H, d, *J* = 7.8 Hz), 2.05 (4H, m), 1.96 (2H, t, *J* = 7.6 Hz), 1.58 (3H, s), 0.89 (6H, t, *J* = 6.7 Hz); FAB-MS (neg.): 726 ([M − H]$^-$, 100).

References

[1] Zervakis GI, Venturella G, Papadopoulou K. Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters [J]. *Microbiology*, 2001, **147**(11): 3183-3194.

[2] Guan L, Yang J, and Li N. Study on extraction and purification of polysaccharides from *Pleurotus eryngii* fruit body [J]. *Heilongjiang Med J*, 2001, **24**(3): 403-405.

[3] Chen JJ, Mao D, Yong YY, *et al*. Hepatoprotective and hypolipidemic effects of water-soluble polysaccharidic extract of *Pleurotus eryngii* [J]. *Food Chem*, 2012, **130**(3): 687-694.

[4] Alam N, Yoon KN, Lee KR, *et al*. Phenolic compounds concentration and appraisal of antioxidant and antityrosinase activities from the fruiting bodies of *Pleurotus eryngii* [J]. *AdvEnviron Biol*, 2011, **5**(6): 1104-1113.

[5] Jeong YT, Jeong SC, Gu YA, *et al*. Antitumor and immunomodulating activities of endo-biopolymers obtained from a submerged culture of *Pleurotus eryngii* [J]. *Food Sci Biotechnol*, 2010, **19**(2): 399-404.

[6] Liu JK, *N*-Containing compounds of macromycetes [J]. *Chem Rev*, 2005, **105**(7): 2723-2744.

[7] Liu JK, Natural terphenyls: developments since 1877 [J]. *Chem Rev*, 2006, **106**(6): 2209-2223.

[8] Jiang MY, Zhang L, Liu R, *et al*. Speciosins A−K, oxygenated cyclohexanoids from the basidiomycete *Hexagonia speciosa* [J]. *J Nat Prod*, 2009, **72**(8): 1405-1409.

[9] Liu DZ, Wang F, Liao TG, *et al*. Vibralactone: a lipase inhibitor with an unusual fused *β*-lactone produced by cultures of the basidiomycete *Boreostereum vibrans* [J]. *Org Lett*, 2006, **8**(25): 5749-5752.

[10] Marc S, Sterner O, *et al*. Toxic terpenoids from higher fungi and their possible role in chemical defence systems [J]. *Z Naturforsch C: Biosci*, 1995, **50**(7/8): 473-475.

[11] Nandan CK, Patra P, Bhanja SK, *et al*. Structural characterization of a water-soluble beta-(1→6)-linked D-glucan isolated from the hot water extract of an edible mushroom, *Agaricus bitorquis* [J]. *Carbonhydr Res*, 2008, **343**(18): 3120-3122.

[12] Iorozzi M, Minale L, Riccio R, *et al*. Polar steroids from the marine scallop *Patinopecten yessoensis* [J]. *J Nat Prod*, 1988, **51**(6): 1098-1103.

[13] Keller AC, Maillard MP, Hostettmann K, *et al*. Antimicrobial steroids from the fungus *Fomitopsis pinicola* [J]. *Phytochemistry*, 1996, **41**(4): 1041-1046.

[14] Hu L, Ding ZH, Liu JK, The chemical constituents of basidiomycetes *Boletopsis grisea* [J]. *Acta Bot Yunnan*, 2002, **24**(5): 667-670.

杏鲍菇中一个新的薄荷烷型单萜

刘良燕[1,2]，李正辉[1]，刘吉开[1*]

[1] 中国科学院昆明植物研究所植物化学与西部植物资源持续利用国家重点实验室，昆明 650201；

[2] 中国科学院研究生院，北京 100049

【摘 要】 对侧耳属真菌杏鲍菇（*Pleurotus eryngii*）的发酵液进行了系统的化学成分研究，经过一系列的正相反相硅胶柱色谱和高效液相色谱等现代分离技术，分离得到 2 个薄荷烷型单萜、3 个麦角甾醇和 1 个脑苷酯 B，并通过 MS, IR, NMR 等光谱学方法确定了这些化合物的结构。其中侧耳内酯（**1**）为一个新的薄荷烷型单萜。

【关键词】 杏鲍菇；薄荷烷型单萜；侧耳内酯

【基金项目】 国家基础研究计划（973 计划，No. 2009CB522300），国家自然科学基金（Nos. 30830113, U1132607）资助项目