云 南 植 物 研 究 2007, **29** (3): 371~374
Acta Botanica Yunnanica

担子菌地星菌丝体中一个新的甾醇酯*

邵红军[1,2], 房立真[1,2], 杨婉秋[1,2], 王 飞[1,2], 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, 云南 昆明 650204;
2 中国科学院研究生院, 北京 100049)

摘要: 从担子菌地星 (*Astraeus hygrometricus*) 发酵培养菌丝体中分离得到一个新的多羟基甾醇酯, 其化学结构通过波谱学方法包括二维核磁共振鉴定为: 3β, 5α-二羟基-(22*E*, 24*R*)-麦角甾醇-7, 22-二烯-6α-棕榈酸酯。同时还从该菌中分离得到其它三个甾醇类化合物。

关键词: 地星; 甾醇酯; 担子菌
中图分类号: Q 946 文献标识码: A 文章编号: 0253-2700 (2007) 03-371-04

A New Steryl Ester from the Culture Mycelia of the Basidiomycete *Astraeus hygrometricus* (Astraceae)

SHAO Hong-Jun[1,2], FANG Li-Zhen[1,2], YANG Wan-Qiu[1,2], WANG Fei[1,2], LIU Ji-Kai[1**]
(1 *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650204, China;
2 *Graduate School of Chinese Academy of Sciences*, Beijing 100049, China)

Abstract: A new steryl ester with a polyhydroxylated ergostane-type nucleus, 3β, 5α-dihydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6α-yl palmitate (**1**), together with three known compounds (**2-4**) was isolated from the culture mycelia of the basidiomycete *Astraeus hygrometricus*. The structure of compound **1** was elucidated on the basis of extensive spectroscopic methods (IR, HR-FAB-MS, 1D and 2D NMR).

Key words: *Astraeus hygrometricus*; Steryl ester; Basidiomycete

Astraeus hygrometricus (Sclerodermatales, Basidiomycete), a mycorrhizal fungus, is widely distributed in China. It is also used as a remedy for haemastatic and inflammation in traditional Chinese medicine (Mao, 2000). Three triterpenes and a splenocyte activity glucan have previously been isolated from the fruiting bodies of this fungus (Takaishi *et al*. 1987; Chakrabory *et al*. 2004). To the best of our knowledge, there are no chemical investigations on the culture broth of this fungus. In our continuing studies on the basidiomycete-derived secondary metabolites (Liu, 2002, 2005, 2006; Shao *et al*. 2005; Wang and Liu, 2005), we have been isolated a new steryl ester with a polyhydroxylated ergostane-type nucleus, 3β, 5α-dihydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6α-yl palmitate (**1**) (Fig. 1), as well as three known compounds (22*E*, 24*R*)-5α, 8α-epidioxyergosta-3, 22-dien-3β-ol (**2**), (22*E*, 24*R*)-ergosta-4, 6, 8 (14), 22-tetraen-3β-one (**3**), and (22*E*, 24*R*)-ergosta-7, 22-dien-3β-ol (**4**) from the culture mycelia of the fungus. It is noted that there are few naturally occurring polyhydroxylated steryl esters reported up to date (Wang and Liu, 2005; Yang *et al*. 2003; Zhang *et al*. 2005). This paper deals with the isolation and struc-

* Foundation item: This work was supported by the National Natural Science Foundation of China (30470027 and 30225048) and the Natural Science Foundation of Yunnan Province (2005C0011R and 2005C0052M).
** Corresponding author. Tel: +86-871-5216327; E-mail: jkliu@mail.kib.ac.cn
Received date: 2006-12-05, Accepted date: 2007-02-04
作者简介: 邵红军 (1977-) 男, 博士研究生, 主要从事高等真菌化学和活性研究。

ture elucidation of the new steryl ester (**1**).

Results and Discussion

Compound **1** was obtained as a colorless oily solid. The molecular formula was determined to be $C_{44}H_{76}O_4$ by ^{13}C-NMR data and HR-FAB-MS (calc. for $[M+H]^+$: 669.5821; found: 669.5814). Its IR spectrum revealed the presence of hydroxyl groups (3 431 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) which assigned with aid of the ^{1}H-^{1}H COSY spectrum (Fig. 2), exhibited two tertiary methyl signals at δ1.01 (s, H-19) and 0.56 (s, H-18), four secondary methyl signals at δ1.02 (d, J = 6.6, H-21), 0.91 (d, J = 6.9, H-28), 0.83 (d, J = 7.2, H-27) and 0.82 (d, J = 7.2, H-26), a pair of 1, 2-disubstituted olefinic protons at δ5.22 (dd, J = 15.2, 7.4, H-23) and 5.15 (dd, J = 15.2, 8.0, H-22), and a trisubstituted olefinic proton at δ4.88 (s, H-7). Its ^{13}C-NMR analyzed together with the DEPT and HMQC spectra also showed signals due to an oxygenated quaternary carbon at δ75.2 (s, C-5), two oxygenated methane carbons at δ73.7 (d, C-6) and 67.3 (d, C-3), and four olefinic carbons at δ144.2 (s, C-8), 135.4 (d, C-22), 132.2 (d, C-23) and 115.2 (d, C-7). Based on these data, **1** was suggested to be an ergosta-7, 22-dien-3β, 5α, 6-triol derivatives. Meanwhile in ^{1}H-NMR spectrum, a characteristic downfield signal of H-6 (δ5.27, s) caused by acylation effect distinctly indicated that the palmitate moiety was located at 6α position of the sterol nucleus. This was also supported by a cross peak observed between H-19 and H-6β in the ROSEY spectrum. These data were in good agreement with those reported for ergosta-7, 22-dien-3β, 5α, 6α-triol and its derivatives (Ayer *et al*. 1992; Chen *et al*. 1991; Goldstein and Frye, 1996). Furthermore, the ^{1}H- and ^{13}C-NMR of compound **1** revealed a terminal methyl signal at (δ0.88 (t, J = 6.8, H-16′)/14.1 (q, C-6′)), a methylene group in α-position to an ester function at (δ2.30 (t, J = 7.6, H-2′)/34.6 (t, C-2′)), a carbonyl carbon at δ173.4 (s, C-1′), and other signals at (δ1.64 (m, H-3′), 1.25-1.34 (overlapped), and δ31.4 (t, C-14′), 25.1 (t, C-3′), 22.7 (t, C-15′), 29.2-29.8 (t)), which showed to be a saturated long-chain fatty-acid ester moiety. By comparison with the data in literature (Zhang *et al*. 2005), those data indicated that the saturated long-chain fatty-acid was palmitate. It was also proved by the EI-MS data, which displayed fragment ion peaks at m/z 412 (67 $[M-C_{16}H_{32}O_2]^+$), 394 (34 $[M-C_{16}H_{32}O_2-H_2O]^+$) and 376 (27 $[M-C_{16}H_{32}O_2-2H_2O]^+$). The linked position of the palmitate moiety was further confirmed by the clearly correlation between H-6β (δ5.27, s) and C-1′ (δ173.4, s) in the HMBC spectrum (Fig. 2). The geometry of the Δ^{22}-double bond was determined to be E from the coupling constant (J = 15.2) between H-22 and H-23. The stereochemistry at C-20 and C-24 was deduced to be R and R, respectively, by comparison of ^{1}H- and ^{13}C-NMR data with those of ergosterol (Wright *et al*. 1978). From all above data, the structure of **1** was assigned as 3β, 5α-dihydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6α-yl palmitate.

Table 1 ^{1}H- and ^{13}C-NMR data of Compound **1** ($CDCl_3$).

Position	δ_C	δ_H	Position	δ_C	δ_H
1	31.9 (t)	1.25 (m)	19	17.9 (q)	1.01 (s)
2	30.8 (t)	1.85 (m)	20	40.3 (d)	2.02 (m)
3	67.3 (d)	4.00 (m)	21	21.1 (q)	1.02 (d, J = 6.6)
4	39.2 (t)	2.04 (m), 1.92 (m)	22	135.4 (d)	5.15 (dd, J = 15.2, 8.0)
5	75.2 (s)	–	23	132.2 (d)	5.22 (dd, J = 15.2, 7.4)
6	73.7 (d)	5.27 (s)	24	42.9 (d)	1.85 (m)
7	115.2 (d)	4.88 (s)	25	33.1 (d)	1.47 (m)
8	144.2 (s)	–	26	19.6 (q)	0.82 (d, J = 7.2)
9	43.5 (d)	2.09 (m)	27	19.9 (q)	0.83 (d, J = 7.2)
10	39.0 (s)	–	28	17.6 (q)	0.91 (d, J = 6.9)
11	21.3 (t)	1.57 (m)	1′	173.4 (s)	–
12	39.6 (t)	1.52 (m), 1.30 (m)	2′	34.6 (t)	2.36 (t, J = 7.6)
13	43.8 (s)	–	3′	25.1 (t)	1.64 (m)
14	54.8 (d)	1.90 (m)	4-13′	29.2-29.8 (t)	1.25-1.34 (m)
15	22.7 (t)	1.40 (m)	14′	31.4 (t)	1.25-1.34 (m)
16	28.0 (t)	1.73 (m)	15′	22.7 (t)	1.25-1.34 (m)
17	55.9 (d)	1.28 (m)	16′	14.1 (q)	0.88 (t, J = 6.8)
18	12.2 (q)	0.56 (s)			

Assignment made on the basis of ^{1}H-^{1}H COSY, HMQC and HMBC data.

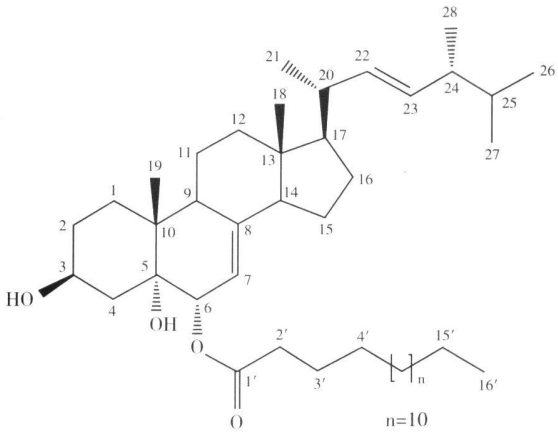

Fig. 1 The structure of compound **1**

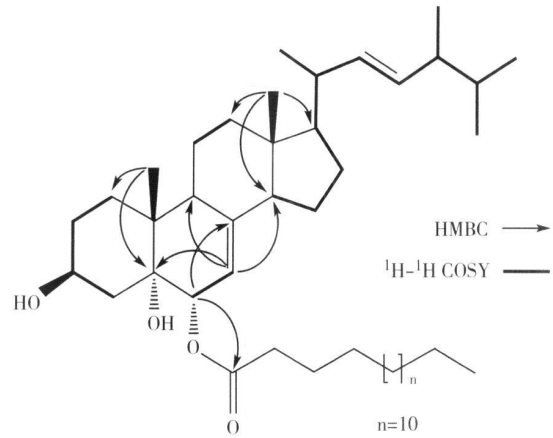

Fig. 2 ^{1}H-^{1}H COSY and key HMBC correlations of compound **1**

Compounds **2**, **3**, and **4** were also isolated from the same fungus, and identified as (22*E*, 24*R*)-5α, 8α-epidioxyergosta-3, 22-dien-3β-ol (**2**), (22*E*, 24*R*)-ergosta-4, 6, 8 (14), 22-tetraen-3β-one (**3**), and (22*E*, 24*R*)-ergosta-7, 22-dien-3β-ol (**4**), respeetively, according to the physical and spectroscopic data in literatures (Yue *et al*. 2001; Gao *et al*. 2001; Lu *et al*. 1985).

Experimental

General Optical rotation was measured on a Horiba SEPA-300 spectropolarimeter. IR spectrum was obtained with a Bruker Tensor 27 spectrometer, with KBr pellets, in cm^{-1}. 1D and 2D-NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers in $CDCl_3$, δ in ppm, *J* in Hz. EI-MS was recorded with a Thermo Finnigan Trace DSQ spectrometer, HR-FAB-MS was recorded with a VG Autospec-3000 spectrometer.

Silica gel (200-300 mesh, Qingdao Marine Chemical Inc, Qingdao. P. R. China), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. Pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Inc, Qingdao. P. R. China) were used for TLC. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Mushroom Material and Culture The fungus *A. hygrometricus* was collected at the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China, in September 2001, and identified by Dr. Fu-Qiang Yu, Kunming Institute of Botany. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. Culture medium: potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, $MgSO_4$ 1.5 g, citric acid 0.1 g and thiamin hydrochloride 10 mg in 1 L of deionized H_2O (pH 6.5 before autoclaving). The culture liquid was fermented at 25℃ for 20 days on a rotary shaker (150 r/min).

Extraction and Isolation The dried mycelia (106 g) filtered from culture broth (25 L) were successively extracted with $CHCl_3$/MeOH (1∶1). The extract was evaporated in vacuo and the oily residue (8.0 g) was subjected to CC (silica gel, petroleum ether/Me_2CO (9∶1 → 1∶1)). The fraction (0.36 g) from petroleum ether/Me_2CO (20∶1) was further purified by CC (silica gel, petroleum ether/AcOEt 20∶1; Sephadex LH-20, $CHCl_3$/MeOH 1∶1) to afford the pure compound **3** (10 mg) and **4** (13 mg). The fraction (0.51 g) from petroleum ether/Me_2CO (10∶1) was further isolated by CC (silica gel, $CHCl_3$/AcOEt 20∶1) to give the pure compound **1** (6 mg) and **2** (20 mg).

Compound **1**, $C_{44}H_{76}O_4$, colorless oily soild; $[\alpha]_D^{24.4}$ = + 46 (*c* 0.17, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3431, 2956, 2925, 2852, 1710, 1629, 1461, 1381, 1187; ^{1}H- and ^{13}C-NMR data: see Table 1; EI-MS: 412 (67 [M-$C_{16}H_{32}O_2$]$^+$), 394 (34 [M-$C_{16}H_{32}O_2$-H_2O]$^+$), 376 (27 [M-$C_{16}H_{32}O_2$-$2H_2O$]$^+$), 251 (100), 157 (48), 129 (30), 109 (15), 69 (56); HR-FAB-MS (pos.): 669.5814 ([M+H]$^+$, $C_{44}H_{77}O_4^+$, calc. 669.5821).

References:

Ayer WA, Ma YT, 1992. Sirosterol and dehydroazasirosterol, unusual steroidal adducts from a *Sirococcus* species [J]. *Can J Chem*, **70**: 1905—1913

Chakrabory I, Mondal S, Pramanik M, 2004. Structural investigation of a water-soluble glucan from an edible mushroom *Astraeus hygrometricus* [J]. *Carbohydr Res*, **399**: 2249—2254

Chen RY, Wang YH, Yu DQ, 1991. Studies on the chemical constituents of the spores from *Ganoderma lucidum* [J]. *Acta Bot Sin*, **33**: 65—68

Gao JM, Dong ZJ, Liu JK, 2001. A new ceramide from the basidiomycete *Russula cyanoxantha* [J]. *Lipids*, **36**: 175—180

Goldstein AS, Frye LL, 1996. Synthesis and bioevaluation of delta 7-5-desaturase inhibitors, an enzyme late in the biosynthesis of the fungal sterol ergosterol [J]. *J Med Chem*, **39**: 5092—5099

Liu JK, 2002. Biologically active substances from mushrooms in Yunnan, China [J]. *Heterocycles*, **57**: 157—167

Liu JK, 2005. N-containing compounds of macromycetes [J]. *Chem*

Rev, **105**: 2723—2744
Liu JK, 2006. Natural terphenyls: developments since 1877 [J]. *Chem Rev*, **106**: 2209—2223
Lu W, Adachi I, Kano K *et al*. 1985. Platelet aggregation potentiators from cho-rei [J]. *Chem Pharm Bull*, **33**: 5083—5087
Mao XL (卯晓岚), 2000. The Macrofungai in China (中国大型真菌) [M]. Zhengzhou: Henan Sciences & Technology Press, 522
Shao HJ, Qing C, Wang F *et al*. 2005. A new cytotoxic lanostane triterpenoid from the basidiomycete *Hebeloma versipelle* [J]. *J Antibiot*, **58**: 828—831
Takaishi Y, Murakami Y, Ohashi T *et al*. 1987. Three triterpenes from *Astraeus hygrometricus* [J]. *Phytochemistry*, **8**: 2341—2344
Wang F, Liu JK, 2005. Two new steryl esters from the basidiomycete *Tricholomopsis rutilans* [J]. *Steroids*, **70**: 127—130
Wright JLC, Mcinnes AG, Shimizu S *et al*. 1978. Identification of C-24 alkyl epimers of marine sterols by ^{13}C nuclear magnetic resonance spectroscopy [J]. *Can J Chem*, **56**: 1898—1903
Yang SP, Xu J, Yue JM, 2003. Sterols from the fungus *Catathelasma imperiale* [J]. *Chin J Chem*, **10**: 161—165
Yue JM, Chen SN, Lin ZW *et al*. 2001. Sterols from the fungus *Lactarium volemus* [J]. *Phytochemistry*, **56**: 801—806
Zhang P, Li X, Li N *et al*. 2005. Antibacterial constituents from fruit bodies of ascomyce *Bulgaria inquinans* [J]. *Arch Pharm Res*, **28**: 889—891

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