云 南 植 物 研 究 2006, 28 (3): 315~318
Acta Botanica Yunnanica

绿盖粉孢牛肝菌中一个新的甾体糖苷*

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摘要: 从绿盖粉孢牛肝菌 (*Tylopilus virens*) 中分离得到一个新的麦角甾烷型甾体糖苷, 其化学结构通过波谱学方法鉴定为: (22*E*, 24*R*)-麦角甾-7, 22-二烯-5α, 6β-二醇-3β-*O*-[3-(3-苯基丙酰氧基)]-β-D-葡萄吡喃糖苷, 命名为 tylopiloside (**1**), 同时, 其苷元 cerevisterol (**2**) 也从该菌中分离得到。值得注意的是, 这种糖片段上有芳环取代的烷酰氧基基团的麦角甾烷型甾体糖苷为真菌中首次报道。

关键词: 绿盖粉孢牛肝菌; 甾体糖苷; tylopiloside
中图分类号: Q 946 **文献标识码**: A **文章编号**: 0253-2700(2006)03-315-04

A New Steroidal Glycoside from the Fruiting Bodies of *Tylopilus virens* (Boletaceae)

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Abstract: A new ergostane-type steroidal glycoside, named tylopiloside (**1**), was isolated from the fruiting bodies of *Tylopilus virens* together with its aglycone, cerevisterol (**2**). The structure of the new compound was elucidated as (22*E*, 24*R*)-ergosta-7, 22-dien-5α, 6β-diol-3β-*O*-[3-(3-phenylpropanoyloxy)]-β-D-glucopyranoside on the basis of spectroscopic analysis. It is worth while to note that the steroidal glycoside possessing an aromatic ring substituted alkanoyl group at its glucose moiety was found for the first time in fungi.

Key words: *Tylopilus virens*; Steroidal glycoside; Tylopiloside

The genus *Tylopilus* belonging to Boletaceae has not been paid much attention chemically. A few years ago, a steroidal glycoside from *T. neofelleus* (Takaishi *et al*, 1989) and two novel secoergosterols from *T. plumbeoviolaceus* (Wu *et al*, 2000) were isolated. In our previous work, we have reported a steroidal glycoside, tuberoside from *Tuber indicum* (Gao *et al*, 2001), and two ergosteryl esters, 3β, 5α-dihydroxy-ergosta-7, 22-dien-6β-yl oleate and 3β, 5α-dihydroxy-ergosta-22-en-7-one-6β-yl oleate from *Tricholomopsis rutilans* (Wang *et al*, 2005). In continuing our studies on macromycetes-derived bioactive secondary metabolites, we investigated the constituents of the inedible mushroom *T. virens* (Chiu) Hongo and isolated a new ergostane-type steroidal glycoside, tylopiloside (**1**), together with its aglycone, cerevisterol (**2**). To the best of our knowledge, ergostane-type steroidal glycosides are relatively rare, and no more than ten analogues were isolated in fungi so far (Bok *et al*, 1999; Gao *et al*, 2001; Shiao *et al*, 1989; Takai-

* Foundation item: This project was supported by the National Natural Science Foundation of China (30470027 and 30225048)
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Received date: 2005-01-09, Accepted date: 2005-02-13
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shi *et al*, 1989; Yue *et al*, 2001). This report describes the isolation and structural elucidation of tylopiloside (**1**).

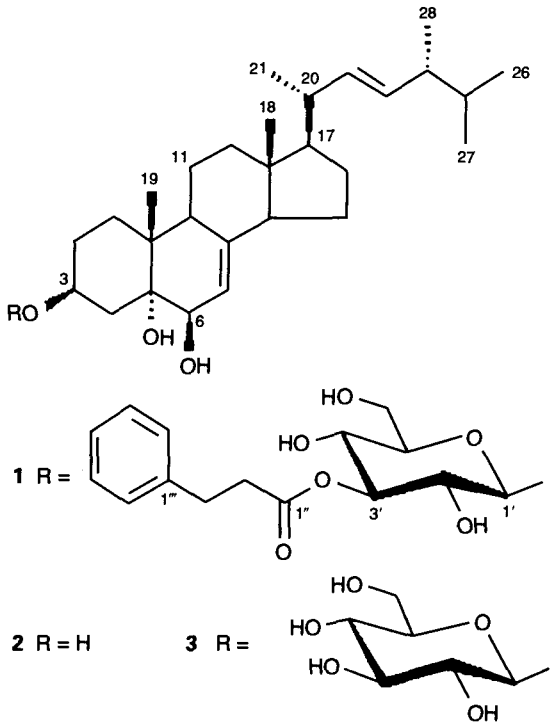

Fig. 1 Structures of tylopiloside (**1**), cerevisterol (**2**) and tuberoside (**3**)

Tylopiloside (**1**), obtained as colorless needles, showed a quasimolecular ion peak at *m/z* 723 corresponding to $[M-H]^-$ in the negative FAB-MS. The molecular formula of **1** was determined to be $C_{43}H_{64}O_9$ by positive HR-ESI-MS at *m/z* 747.4429 (calcd. for $C_{43}H_{64}O_9Na$ 747.4448). The ^1H NMR spectrum (Table 1) of **1** displayed two tertiary methyls at δ 0.65 (3H, s, Me-18) and 1.37 (3H, s, Me-19), four secondary methyls at δ 0.85 (3H, d, J = 6.6 Hz, Me-26), 0.86 (3H, d, J = 6.6 Hz, Me-27), 0.95 (3H, d, J = 6.8 Hz, Me-28) and 1.05 (3H, d, J = 6.5 Hz, Me-21), characteristic of the ergosterol skeleton, and a β-D-glucopyranosyl moiety which exhibited its anomeric proton at δ 4.96 (1H, d, J = 7.8 Hz, H-1′) and typical coupling constants ^3J = 9.5 Hz of H-2′ and H-3′, H-3′ and H-4′, H-4′ and H-5′, as well as a mono-substituted phenyl signals at δ 7.18 (2H, d, J = 7.8 Hz, H-2‴ and H-6‴), 7.23 (2H, dd, J = 7.8, 7.1 Hz, H-3‴ and H-5‴) and 7.14 (1H, t, J = 7.1 Hz, H-4‴). The ^{13}C NMR spectrum (Table 1) of **1** showed total forty-three carbon signals, twenty-eight of which were in accordance with those of cerevisterol (**2**) except the signal differences to some extent at δ 75.6 (d, C-3), 29.9 (t, C-2) and 37.9 (t, C-4), six carbon signals at δ 102.2 (d, C-1′), 73.2 (d, C-2′), 79.5 (d, C-3′), 69.3 (d, C-4′), 78.2 (d, C-5′) and 62.1 (t, C-6′) among which were assignable to be a β-D-glucopyranosyl moiety. The remaining nine carbon signals, including an ester carbonyl carbon at δ 173.0 (s, C-1″), two methenes at δ 36.5 (t, C-2″) and 31.3 (t, C-3″), and a mono-substituted phenyl group at δ 141.4 (s, C-1‴), 128.8 (2 × d, C-2‴ and C-6‴), 128.7 (2 × d, C-3‴ and C-5‴) and 126.5 (d, C-4‴), were elucidated as 3-phenylpropanoyloxy moiety. The presence of a series of characteristic fragmental ion peaks in negative FAB-MS of **1** *m/z* at 723, 591, 311 and 149 further validated the above inference. The pattern of fragmentation was rationalized in Fig. 2. In HMBC spectrum (Fig. 2) of **1**, appearance of an important correlation peak between δ_H 4.96 (1H, d, J = 7.8 Hz, H-1′) and δ_C 75.6 (d, C-3) suggested that the glucose moiety was attached to the C-3 position of aglycone, and the ^{13}C NMR differences between a downfield shift of the signal at δ 75.6 (d, C-3, +8.0 ppm), and upfield shifts of the signals at δ 29.9 (t, C-2, -2.7 ppm), 37.9 (t, C-4, -4.1 ppm) from those of aglycone (**2**) were explained by glycosylation shifts. In the same way, a key correlation between δ_H 5.82 (1H, t, J = 9.5 Hz, H-3′) and the ester carbonyl carbon δ_C 173.0 (s, C-1″) indicated that the 3-phenylpropanoyloxy moiety was linked with the C-3′ position of the glucose, and the ^{13}C NMR differences between a downfield shift of the signal at δ 79.5 (d, C-3′, +0.9 ppm), and upfield shifts of the signals at δ 73.2 (d, C-2′, -2.2 ppm), 69.3 (d, C-4′, -2.5 ppm) from those of tuberoside (**3**) (Gao *et al*, 2001) were interpreted by esterification shifts. In the light of the evi-

dences mentioned above, the structure of **1** was therefore elucidated as ergosta-7, 22-dien-5α, 6β-diol-3β-O-[3-(3-phenylpropanoyloxy)]-β-D-glucopyranoside, named tylopiloside.

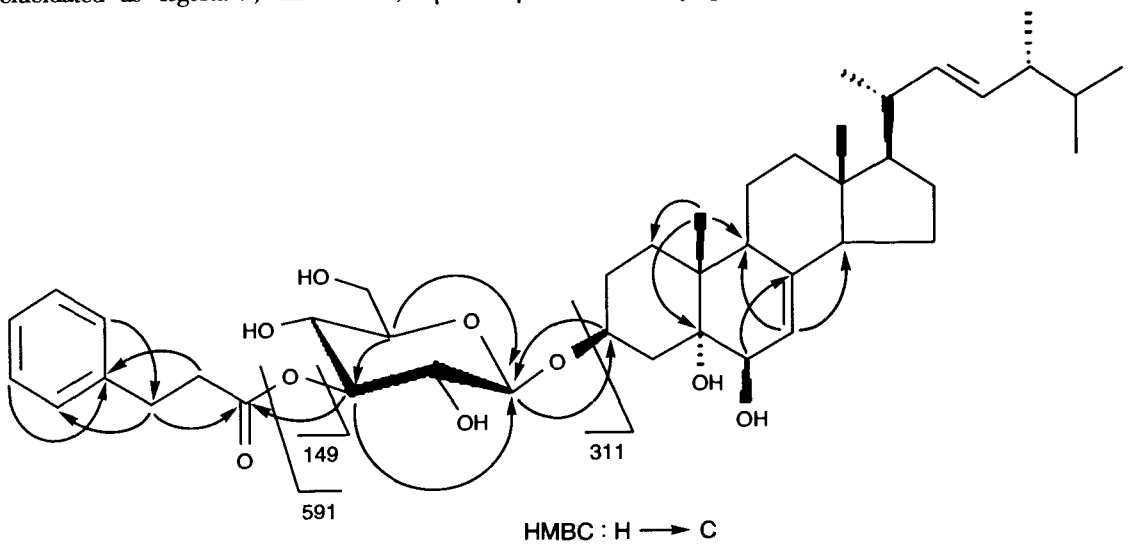

Fig. 2 HMBC correlations and MS fragmentation of tylopiloside (**1**)

Table 1 NMR (pyridine-d_5) data for tylopiloside (**1**), cerevisterol (**2**) and tuberoside (**3**)[a]

No.	**1**		**2**	**3**	No.	**1**		**2**	**3**
	C	H	C	C		C	H	C	C
1	33.5 (t)	2.05*, 1.60*	33.8 (t)	33.6 (t)	22	136.2 (d)	5.17 (dd,15.3,8.3)	136.2 (d)	136.2 (d)
2	29.9 (t)	2.23*, 1.89*	32.6 (t)	30.1 (t)	23	132.2 (d)	5.24 (dd,15.3,7.4)	132.1 (d)	132.2 (d)
3	75.6 (d)	4.88 (m)	67.6 (d)	75.6 (d)	24	43.1 (d)	1.88*	43.1 (d)	43.1 (d)
4	37.9 (t)	2.81 (dd,12.7,11.8), 2.57 (dd,12.7,3.2)	42.0 (t)	38.1 (t)	25	33.4 (d)	1.47*	33.4 (d)	33.4 (d)
					26	19.9 (q)	0.85 (d, 6.6)	19.8 (q)	19.8 (q)
5	75.9 (s)		76.2 (s)	75.9 (s)	27	20.2 (q)	0.86 (d, 6.6)	20.2 (q)	20.1 (q)
6	74.2 (d)	4.27 (br d, 4.8)	74.3 (d)	74.2 (d)	28	17.9 (q)	0.95 (d, 6.8)	17.8 (q)	17.8 (q)
7	120.4 (d)	5.73 (br d, 4.8)	120.5 (d)	120.3 (d)	1′	102.2 (d)	4.96 (d, 7.8)		102.6 (d)
8	141.4 (s)		141.6 (s)	141.4 (s)	2′	73.2 (d)	4.08 (dd,9.5,7.8)		75.4 (d)
9	43.6 (d)	2.50 (br t, 9.3)	43.8 (d)	43.7 (d)	3′	79.5 (d)	5.82 (t, 9.5)		78.6 (d)
10	38.1 (s)		38.1 (s)	38.1 (s)	4′	69.3 (d)	4.38*		71.8 (d)
11	22.4 (t)	1.63*	22.4 (t)	22.4 (t)	5′	78.2 (d)	3.66 (dt, 9.5, 3.2)		78.1 (d)
12	39.9 (t)	2.04*, 1.28*	39.9 (t)	39.9 (t)	6′	62.1 (t)	4.38*		62.9 (t)
13	43.8 (s)		43.8 (s)	43.8 (s)	1″	173.0 (s)			
14	55.3 (d)	1.91*	55.3 (d)	55.2 (d)	2″	36.5 (t)	2.73 (dt, 8.0, 7.8),		
15	23.5 (t)	1.57*	23.5 (t)	23.5 (t)			2.67 (dt, 8.0, 7.8)		
16	28.5 (t)	1.69*	28.5 (t)	28.4 (t)	3″	31.3 (t)	2.99 (t, 7.8)		
17	56.2 (d)	1.22*	56.2 (d)	56.3 (d)	1‴	141.4 (s)			
18	12.5 (q)	0.65 (s)	12.5 (q)	12.5 (q)	2‴,6‴	128.8 (d)[b]	7.18 (d, 7.8)		
19	18.5 (q)	1.37 (s)	18.8 (q)	18.5 (q)	3‴,5‴	128.7 (d)[b]	7.23 (dd, 7.8, 7.1)		
20	40.8 (d)	2.01*	40.8 (d)	40.7 (d)	4‴	126.5 (d)	7.14 (t, 7.1)		
21	21.4 (q)	1.05 (d, 6.5)	21.4 (q)	21.4 (q)					

[a] These data were cited from ref. (Gao *et al*, 2001). [b] Exchangeable. * Overlapped signals. Assignments are based on extensive 2D NMR experiments.

The stereochemistry of the side chain was determined by comparison of the ^{13}C NMR data with those of the reported (22*E*, 24*R*)-ergosterol derivatives (Wright *et al*, 1978). Although both 24α- and 24β-alkyl configurations have been found to occur naturally, in general, fungi only produce sterols with the 24α-methyl configuration (24*R* if a $\triangle^{22}$ unsaturated side-chain, 24*S* if a saturated side-chain) indicating a phylogenetic significance of the configuration at C-24 (Goad *et al*, 1974).

Comparison of the physicochemical properties with the reported data allowed us to identify the known sterol **2** as cerevisterol, isolated from the same mushroom (Gao *et al*, 2001).

Experimental

General Experimental Procedures Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker DRX-500 spectrometer in pyridine-d_5 solvent (δ_H 8.710 ppm, δ_C 149.90 ppm). FAB-MS and EI-MS were recorded with a VG Autospec-3000 spectrometer. HRESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200 - 300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Fungal Material The fresh fruiting bodies of *T. virens* were purchased at market in Nanhua County of Yunnan Province, China, in August 2005 and identified by Prof. ZANG Mu, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, CAS.

Extraction and Isolation The fresh fruiting bodies of *T. virens* (1500 g) were immersed in 80% acetone and left at r. t. for several days. Then the acetone extraction was concentrated and partitioned between EtOAc and water. The EtOAc extract (11.0 g) was applied on a silica gel column and eluted stepwise with $CHCl_3$/MeOH solvent system. Fraction 1 from $CHCl_3$/MeOH (9:1, v/v) was subjected on a silica gel column eluting with $CHCl_3$/MeOH (50:1, v/v) to give a residue (60 mg) mainly containing **1**, which was further purified on a Sephadex LH-20 column eluting with $CHCl_3$/MeOH (1:1, v/v), to yield pure compound **1** (32.5 mg) as colorless needles. Fraction 2 from $CHCl_3$/MeOH (8:1, v/v) was subjected on a silica gel column eluting with $CHCl_3$/MeOH (45:1, v/v) to afford compound **2** (6.0 mg).

Tylopiloside (1) $C_{43}H_{64}O_9$, colorless needles; mp: 187 -190℃ (MeOH); $[\alpha]_D^{26}$ - 75.5° (*c* 0.32, pyridine); R_f = 0.50 ($CHCl_3$: MeOH = 14 : 1); IR (KBr): 3426, 3088, 3064, 3028, 2956, 2928, 2870, 1727, 1653, 1497, 1456, 1381, 1296, 1164, 1079, 1046, 972 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5): see Table 1; FAB-MS (neg.) *m/z*: 723 [M-H]$^-$, 591, 311, 149; HR-ESI-MS (pos.) *m/z*: 747.4429 [M ($C_{43}H_{64}O_9$) + Na], calcd. 747.4448.

Cerevisterol (2) $C_{28}H_{46}O_3$, colorless crystalline solid; R_f = 0.43 ($CHCl_3$: MeOH = 14 : 1); ^1H NMR (pyridine-d_5) δ 5.74 (1H, br d, *J* = 4.8 Hz, H-7), 5.23 (1H, dd, *J* = 15.2, 7.2 Hz, H-23), 5.16 (1H, dd, *J* = 15.2, 8.0 Hz, H-22), 4.84 (1H, m, H-3), 4.33 (1H, br d, *J* = 4.8 Hz, H-6), 3.04 (1H, dd, *J* = 13.0, 11.6 Hz, H-4β), 1.53 (3H, s, Me-19), 1.05 (3H, d, *J* = 6.6 Hz, Me-21), 0.94 (3H, d, *J* = 6.8 Hz, Me-28), 0.85 (3H, d, *J* = 6.7 Hz, Me-27), 0.84 (3H, d, *J* = 6.7 Hz, Me-26), 0.65 (3H, s, Me-18); ^{13}C NMR (pyridine-d_5): see Table 1; EI-MS *m/z*: 412 [M-H_2O]$^+$ (13), 397 (6), 394 (19), 383 (10), 379 (21), 376 (15), 269 (15), 251 (57), 69 (100).

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