

Two new sesquiterpenoids from basidiomycete *Tyromyces chioneus*

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Abstract: Two new sesquiterpenoids, named as tyromols A and B (**1** and **2**), were isolated from cultures of basidiomycete *Tyromyces chioneus*, along with two previously reported 15-hydroxy-6 α , 12-epoxy-7 β , 10 α H, 11 β H-spiroax-4-ene (**3**) and agripilol C (**4**). Compounds **1**–**4** were separated and purified by silica gel, RP-18, Sephadex LH-20 column chromatography. Their structures were elucidated on the basis of extensive spectroscopic analysis including IR, MS, 1D and 2D NMR experiments.

Key words: *Tyromyces chioneus*; sesquiterpenoid; tyromol A; tyromol B

CLC number: R284

Document code: A

Article ID: 0513-4870 (2014) 11-1578-04

担子菌薄皮干酪菌中两个新的倍半萜

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摘要: 采用硅胶、RP-18、Sephadex LH-20 等多种材料进行分离纯化, 通过理化方法和波谱分析进行结构鉴定, 从担子菌薄皮干酪菌 (*Tyromyces chioneus*) 发酵液的乙酸乙酯提取部位分离并鉴定了 4 个化合物, 它们分别为: tyromol A (**1**)、tyromol B (**2**)、15-hydroxy-6 α , 12-epoxy-7 β , 10 α H, 11 β H-spiroax-4-ene (**3**) 和 agripilol C (**4**)。其中化合物 **1** 和 **2** 为新的倍半萜, 化合物 **3** 和 **4** 均为首次从该真菌中分离得到。

关键词: 薄皮干酪菌; 倍半萜; tyromol A; tyromol B

The mushroom *Tyromyces chioneus* belongs to the family Polyporaceae, which has a wide distribution in most parts of China, such as Hebei, Shanxi and Heilongjiang provinces. Previous chemical investigation on this fungus only reported a cadinane sesquiterpenoid, which was demonstrated to exhibit significant anti-HIV-1 activity^[1]. In the course of searching for more biologically active compounds^[2–5], two new sesquiterpenoids, tyromol A (**1**) and tyromol B (**2**),

together with two previously known sesquiterpenoids **3** and **4**, were obtained (Figure 1) from cultures of *T. chioneus*. Herein, we report the isolation and structural elucidation of these compounds.

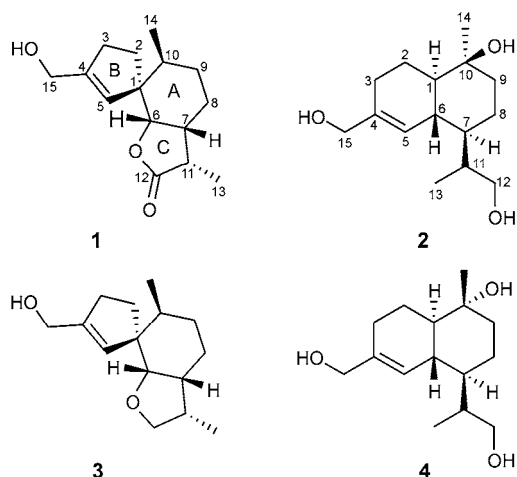
Results and discussion

Compound **1** was isolated as white powder. Its molecular formula C₁₅H₂₂O₃ was deduced from HR-ESI-MS at m/z 273.1459 [M+Na]⁺ (calcd. 273.1466), requiring for five degrees of unsaturation. The IR spectrum showed absorption bands for OH (3428 cm⁻¹), C=O (1770 cm⁻¹) and C=C (1632 cm⁻¹) moieties. The ¹H NMR spectrum (Table 1) exhibited two secondary methyls (δ_H 1.14, d, J = 7.2 Hz; δ_H 0.81, d, J = 6.8 Hz),

Received 2014-06-20; Accepted 2014-07-22.

Project supported by the National Natural Science Foundation of China (U1132607, 81102346); Youth Innovation Promotion Association of CAS (2011312D11019).

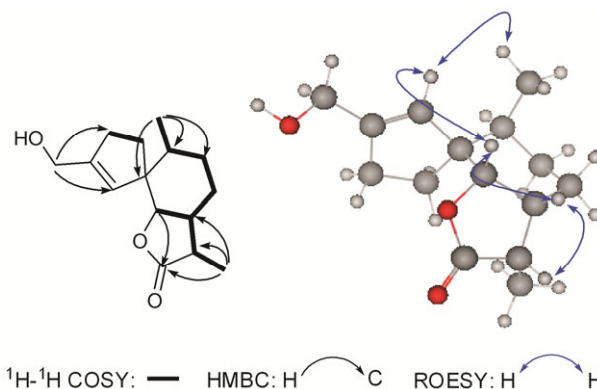
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**Figure 1** Structures of compounds 1–4

three protons attached to two oxygenated carbons separately (δ_{H} 4.23, 2H, dd, $J = 14.5, 10.0$ Hz; δ_{H} 4.04, 1H, d, $J = 3.4$ Hz), and one olefinic proton (δ_{H} 5.48, br s). The ^{13}C NMR and DEPT spectra (Table 1) revealed the presence of one double bond (δ_{C} 148.7, C-4; δ_{C} 123.2, C-5), one oxygenated methine carbon (δ_{C} 85.9, C-6), one oxygenated methylene carbon (δ_{C} 62.1, C-15) and one carbonyl carbon (δ_{C} 179.6, C-12), as well as two methyl groups (δ_{C} 16.0, C-14; δ_{C} 9.1,

C-13), four methylenes (δ_{C} 32.3, C-2; δ_{C} 32.2, C-3; δ_{C} 29.3, C-9; δ_{C} 23.3, C-8), three methines (δ_{C} 42.8, C-11; δ_{C} 38.5, C-7; δ_{C} 34.2, C-10), and one quaternary carbon (δ_{C} 55.0, C-1).

The ^1H - ^1H COSY spectrum displayed signals establishing two fragments as shown in Figure 2. The HMBC correlations from H-10 and H-6 to C-1 established a substituted hexatomic ring A, while the HMBC correlations from H-3 and H-15 to C-4 and from H-2 and H-5 to C-1 constructed a five-membered carbon ring B. In addition, the HMBC correlations

**Figure 2** Key ^1H - ^1H COSY, HMBC and ROESY correlations of **1****Table 1** ^1H and ^{13}C NMR spectroscopic data of **1–2** at 600/150 MHz, respectively. Chemical shift values δ in ppm, coupling constants J in Hz (in parentheses)

Position	1		2		
	^1H (CDCl_3)	^{13}C (CDCl_3)	^1H (CDCl_3)	^{13}C (CDCl_3)	^1H (CD_3OD)
1		55.0, qC	1.59, overlap	46.1, CH	1.43, m
2	2.12, m	32.3, CH_2	1.66, m	20.4, CH_2	1.52, m
	1.96, m		1.59, overlap		1.42, m
3	2.34, m	32.2, CH_2	2.12, m	26.8, CH_2	1.96, m
			2.08, m		1.90, m
4		148.7, qC		137.5, qC	
5	5.48, br s	123.2, CH	5.84, d (5.5)	125.6, CH	5.70, d (5.4)
6	4.04, d (3.4)	85.9, CH	2.45, m	33.4, CH	2.35, m
7	2.36, m	38.5, CH	1.59, overlap	38.7, CH	1.45, m
8	1.68, m	23.3, CH_2	1.48, m	19.7, CH_2	1.39, m
			1.27, m		1.08, m
9	1.48, m	29.3, CH_2	1.53, m	34.3, CH_2	1.37, m
			1.49, m		1.29, m
10	1.69, m	34.2, CH		72.2, qC	
11	2.75, m	42.8, CH	2.01, m	35.0, CH	1.83, m
12		179.6, qC	4.01, br s	67.3, CH_2	3.21, m
13	1.14, d (7.2)	9.1, CH_3	0.89, d (7.0)	10.4, CH_3	0.75, d (7.0)
14	0.81, d (6.8)	16.0, CH_3	1.23, s	29.5, CH_3	1.07, s
15	4.23, dd (14.5, 10.0)	62.1, CH_2	3.50, dd (7.5, 3.2)	67.0, CH_2	3.76, d (5.4)
OH-10					3.96, s
OH-12					4.37, t (5.2)
OH-15					4.65, t (5.7)

from H-6, H-11, and H-13 to C-12 suggested a five-membered lactone ring C. Therefore, the planar structure of **1** was established, which was similar to that of 15-hydroxy-6 α , 12-epoxy-7 β , 10 α H, 11 β H-spiroax-4-ene (**3**)^[6], except that C-12 was oxygenated to a carbonyl carbon, as supported by the HMBC correlations from H-6, H-7, and H-13 to δ_C 179.6 (s, C-12). The relative configuration of **1** was assigned on the basis of an ROESY experiment (Figure 2). ROESY correlations of H-5/H-6, H-5/H-14, and H-6/H-7 suggested that H-5, H-6, H-7, and Me-14 were at the same side, while the ROESY correlations of H-7/H-11 suggested that H-11 should be at the same side with H-7. Thus, the structure of compound **1** was established and named as tyromol A.

Compound **2** was also obtained as white powder. Its molecular formula was determined to be C₁₅H₂₆O₃ by HR-ESI-MS, corresponding to three degrees of unsaturation. The IR spectrum showed absorption bands for a hydroxy group (3 427 cm⁻¹) and double bonds (1 634 cm⁻¹). The ¹³C NMR and DEPT spectra (Table 1) showed 15 carbon signals that attributed to two methyls, six methylenes, five methines, and two quaternary carbons. The ¹H and ¹³C NMR spectra of **2** were similar to those of agripilol C (**4**)^[7]. Careful comparison of their NMR data indicated that they were a pair of isomers, with the only difference assigned to the configuration of H₃-14. The HMBC spectrum suggested that its planar structure was identical to that of compound **4**. In the ROSEY spectrum, correlations of H₃-14/H-1/H-7 and OH-10/H-6 indicated the α -orientation of H₃-14 and β -orientation of OH-10. Thus, compound **2** (tyromol B) was established as shown.

The known compounds **3** and **4** were purified as white powders, and characterized as 15-hydroxy-6 α , 12-epoxy-7 β , 10 α H, 11 β H-spiroax-4-ene (**3**)^[6], and agripilol C (**4**)^[7] by comparison of their ¹H and ¹³C NMR data with those reported in the literature. Compounds **3** and **4** were isolated from this fungus for the first time.

Experimental section

General experimental procedures Optical rotations were measured on a Jasco-P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with an Avance III 600 MHz instrument. HR-ESI-MS were measured on an API QSTAR Pulsar mass spectrometer. Silica gel (200–300

mesh and 80–100 mesh, Qingdao Marine Chemical Inc., China), RP-18 gel (40–75 μ m, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots visualized by heating silica gel plates immersed in vanillin-H₂SO₄ in EtOH.

Fungal material and cultivation conditions

The fungus *T. chioneus* was collected from the Ailao Mountain in Yunnan Province, People's Republic of China, in July 2003. The fungus was identified by Prof. Mu Zang at the Kunming Institute of Botany. The voucher specimen was deposited at the Herbarium of Kunming Institute of Botany (No. HFC 20110812X). Culture medium: glucose (5%), pork peptone (0.15%), yeast (0.5%), KH₂PO₄ (0.05%), MgSO₄ (0.05%). The fermentation was carried out on a shaker at 24 and 150 r·min⁻¹ for 20 d.

Extraction and isolation The culture broth (20 L) was extracted three times with EtOAc (3 \times 10 L). The combined EtOAc extracts were evaporated *in vacuo* to give a residue (10.0 g). The residue was subjected to silica gel CC with a gradient elution system of petroleum ether–acetone (30 1 \rightarrow 0 1) to obtain six fractions (A–F). Fraction C was eluted with petroleum ether–acetone (5 1). It was then subjected to Sephadex LH-20 CC (CHCl₃–MeOH, 1 1) and silica gel CC with petroleum ether–acetone (4 1) to afford **2** (2.0 mg) and **4** (3.0 mg). Fraction E was separated by repeated silica gel CC (petroleum ether–acetone, 10 1 \rightarrow 0 1) to yield fractions E01–E03. Fraction E02 was chromatographed on a RP-18 column (MeOH–H₂O, 30%) and then purified by CC on silica gel with petroleum ether–acetone (2 1) to give **1** (1.0 mg) and **3** (2.0 mg).

Tyromol A (1): C₁₅H₂₂O₃, white powder; [α]_D¹⁵ +16.7 (*c* 0.10, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 240 (2.1), 199 (1.8) nm; IR (KBr) ν_{\max} 3 428, 2 923, 1 770, 1 632, 1 169 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Table 1; HR-ESI-MS *m/z* 273.145 9 [M+Na]⁺ (calcd. for C₁₅H₂₂O₃Na 273.146 6).

Tyromol B (2): C₁₅H₂₆O₃, white powder; [α]_D¹⁵ +8.8 (*c* 0.10, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 239 (1.9) nm; IR (KBr) ν_{\max} 3 427, 2 926, 1 634, 1 384, 1 035 cm⁻¹; ¹H NMR (CDCl₃ and CD₃OD, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Table 1; ESI-MS (positive)

m/z 277 $[M+Na]^+$; HR-ESI-MS m/z 277.177 4 $[M+Na]^+$
(calcd. for $C_{15}H_{26}O_3Na$ 277.177 9).

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