A New Spiroaxane Sesquiterpene from Cultures of the Basidiomycete *Pholiota adiposa*

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A new spiroaxane sesquiterpene (15-hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene, **1**) was isolated from the culture broth of the basidiomycete *Pholiota adiposa*. The structure of **1** was established on the basis of MS, IR, ID, and 2D NMR experiments.

Key words: Spiroaxane Sesquiterpene, Culture Broth, *Pholiota adiposa*, Basidiomycete

Introduction

Pholiota adiposa is an edible and medicinal fungus, which has been found to show antimicrobial activities and high inhibition to Sarcoma-180 and E-cancer in mice, and can also prevent infection from *Staphylococcus aureus*, *Escherichia coli* and *Mycobacterium tuberculosis* [1]. So far, some chemical constituents have been reported [2, 3]. As a part of our search for naturally occurring bioactive metabolites from higher fungi in China [4 – 7], we have carried out a chemical investigation on the cultures of *P. adiposa* which led to the isolation of a new spiroaxane sesquiterpene (**1**). This report deals with the isolation and structure elucidation of **1**.

Experimental Section

General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. The IR spectrum was obtained on a Bruker Tensor 27 instrument with KBr pellets. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers in CD_3OD and $CDCl_3$ with TMS as an internal standard. The EI-MS was recorded with a VG Autospec-3000 spectrometer. The HRESI-MS was 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 basidiomycete *P. adiposa* was collected at Ailao Mountain of Yunnan Province, China, in July 2003 and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). Voucher specimens were deposited in the Herbarium of the Kunming Institute of Botany, CAS.

Fermentation and isolation

The culture medium consisted of potato (peel off) (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 water. Reagent bottles were used as flask (size: 500 mL; volume of media: 300 mL). The pH was adjusted to 6.5 before autoclaving. Fermentation was carried out on a shaker at 22 °C and 150 rpm for 10 days.

The whole culture broth of *P. adiposa* (12 L) was filtered and then extracted twice with EtOAc. The organic layer was concentrated *in vacuo* to give 2.3 g of extract which was chromatographed on a silica gel column and eluted stepwise with $CHCl_3$-MeOH. Fr. II (7.6 mg), eluted with $CHCl_3$-MeOH (100 : 1, v/v), was further purified repeatedly over a Sephadex LH-20 column eluting with MeOH to give compound **1** (2.5 mg).

Identification

15-Hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene (**1**), colorless oil. – $[\alpha]_D^{29.7} = -3.85$ (c = 0.4, MeOH). – IR (KBr): ν = 3424, 2956, 2927, 2869, 1640, 1460, 1379, 1027 cm^{-1}. – ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD and $CDCl_3$): see Table 1. – EI MS (70 eV): *m/z* (%) = 236 (4) $[M]^+$, 222 (2), 219 (13), 203 (7), 175 (18), 163 (9), 159

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Table 1. NMR spectral data for compounds **1** and **2**[a].

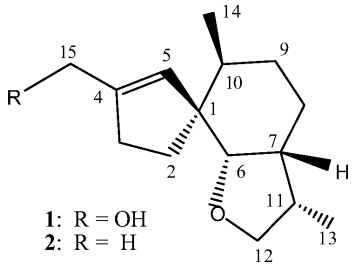
Position	**1** (CD_3OD)		**1** ($CDCl_3$)	**2** ($CDCl_3$)
	^1H	^{13}C	^{13}C	^{13}C
1		57.0	55.7	56.0
2	1.99 (m)	33.7	32.4	32.9
	1.83 (m)			
3	2.30 (m)	33.2	32.4	36.7
4		148.3	146.6	142.8
5	5.47 (s)	127.0	126.3	126.0
6	3.56 (d, 3.0)	89.4	87.6	88.0
7	1.95 (m)	41.4	40.1	40.1
8	1.47 (m)	23.0	21.9	21.9
	1.21 (br d, 3.0)			
9	1.45 (m)	31.7	30.6	30.6
	1.23 (br d, 13.0)			
10	1.67 (m)	36.1	34.6	34.7
11	2.42 (m)	38.9	37.7	37.7
12	3.90 (dd, 10.0, 8.0)	72.6	71.6	71.6
	3.40 (dd, 8.0, 8.0)			
13	0.96 (d, 6.9)	12.0	11.8	11.8
14	0.81 (d, 6.9)	16.8	16.3	16.3
15	4.10 (s)	62.1	62.2	16.8

[a] Chemical shift values δ in ppm, coupling constants J in Hz (in parentheses).

(22), 121 (30), 105 (100), 91 (73). – HRESI-MS: *m/z* = 259.1675 (calcd. 259.1673 for $C_{15}H_{24}O_2Na$, $[M+Na]^+$).

Results and Discussion

Compound **1** was obtained as an oil. Its molecular formula of $C_{15}H_{24}O_2$ was established on the basis of positive FAB-MS, ^{13}C NMR and DEPT spectra and further confirmed by HRESI-MS. Thus, the structure of **1** contains four degrees of unsaturation. The IR spectrum indicated the presence of a hydroxy group at 3424 cm^{-1} and a C=C double bond at 1640 cm^{-1}. The ^1H NMR spectrum (Table 1) exhibited two secondary methyls at δ = 0.96 (d, J = 6.9 Hz, 3H) and 0.81 (d, J = 6.9 Hz, 3H). The ^{13}C NMR and DEPT spectra (Table 1, in CD_3OD) revealed the presence of two sp^2 carbons at δ = 148.3 (C-4) and 127.0 (C-5), one oxygenated methine carbon at δ = 89.4 (C-6) and two oxygenated methylene carbons at δ = 72.6 (C-12) and 62.1 (C-15), as well as two methyl groups at δ = 16.8 (C-14) and 12.0 (C-13), four methylenes at δ = 33.7 (C-2), 33.2 (C-3), 31.7 (C-9) and 23.0 (C-8), three methines at δ = 41.4 (C-7), 38.9 (C-11) and 36.1 (C-10), and one quaternary carbon atom at δ = 57.0 (C-1).



1: R = OH
2: R = H

Fig. 1. The structures of compounds **1** and **2**.

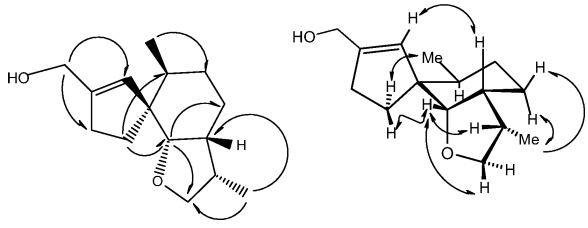


Fig. 2. Key HMBC and ROESY correlations of compound **1**.

Comparison of the ^{13}C NMR data of **1** with those of **2** which was isolated from the unpolar part of the Haitian Vetiver Oil (Fig. 1) implied that they shared the same planar structure except for the hydroxyl substituent at C-15 [8], causing the downfield shifts of C-4 and C-15 from δ = 142.8 and 16.8 in **2** to δ = 146.6 and 62.2 in **1**, respectively. The above assignment was further supported by cross peaks between H-15 (δ = 4.10, s) and C-3, C-4, and C-5 in the HMBC spectrum of **1**. The relative configurations of **1** were determined by comparison with **2** and confirmed by a ROESY experiment. The ROESY correlations (Fig. 2) of 5-H (δ = 5.47, s) with 6-H_{eq} (δ = 3.56, d, J = 3.0 Hz) and 7-H_{ax} (δ = 1.95, m), 6-H_{eq} with 11-H (δ = 2.42, m) and 2-Hβ (δ = 1.99, m), and 10-Me (δ = 0.81, d, J = 6.9 Hz) with 2-Hα (δ = 1.83, m) indicated that H-6, H-7, H-11, and H-10 possessed β-, β-, β-, and α-orientations, respectively. The important NOE between 5-H and 7-H_{ax} made the stereochemistry at the spiro center and the configuration at C-7 obvious. On the basis of the evidence mentioned above, the structure of **1** was therefore deduced to be 15-hydroxy-6α,12-epoxy-7β,10αH, 11βH-spiroax-4-ene.

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