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A new sesquiterpenoid metabolite from *Psilocybe samuiensis*

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A novel 2,3-secoaromadendrane-type sesquiterpenoid metabolite, psilosamuiensin A (**1**), was isolated from the broth of *Psilocybe samuiensis*. The structure of psilosamuiensin A was established by spectroscopic data and its configurations were confirmed by single crystal X-ray crystallographic analysis. This is the first report of psilosamuiensin A found in the genus *Psilocybes*.

Keywords: *Psilocybe samuiensis*; magic mushroom; hallucinogenic mushroom; sesquiterpene; secoaromadendrane

1. Introduction

Magic mushrooms refer to several families of mushrooms that grow naturally, producing hallucinogenic properties [1]. These mushrooms belong to several genera including *Psilocybe*, *Panaeolus*, *Copelandia*, *Conocybe*, *Gymnopilus*, *Inocybe*, and *Pluteus*, while the majority belongs to the genus *Psilocybe* [2]. Earlier chemical investigations on the mushrooms reported the isolation of alkaloids psilocybin, psilocin [3–6], baeocystin, norbaeocystin [7], and aeruginascine [8]. Other compounds include ergosterol, ergosterol peroxide and α,α -trehalose [9]. Allen reported the occurrence of several species of hallucinogenic mushrooms in Thailand including a new bluing *Psilocybe* species, *P. samuiensis* [10–12]. The alkaloid content of both naturally occurring and *in vitro* cultivated fruit bodies of *P. samuiensis* was

analyzed by HPLC and the results revealed high concentrations of psilocybin and psilocin and small amounts of baeocystin [11,12]. Recently, we have studied the metabolites of *P. samuiensis* cultured in malt extract broth (MEB) and obtained two novel 2,3-secoaromadendrane-type sesquiterpenoids, named psilosamuiensin A (**1**) and psilosamuiensin B (**2**), from the ethyl acetate extract of culture broth. Herein we describe the isolation and structural elucidation of **1** and **2** (Figure 1).

2. Results and discussion

On the basis of ¹H NMR analysis, the hexane, ethyl acetate, and methanol extracts of *P. samuiensis* mycelia contain mainly fatty acids. The hexane extract of the culture broth was obtained only in a small amount, while the methanol extract was mainly consisted of glucose. The ethyl acetate extract of the

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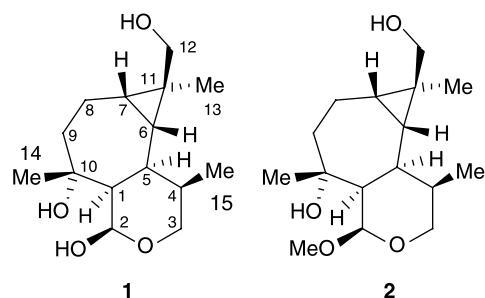


Figure 1. Structures of psilosamuiensin A (**1**) and psilosamuiensin B (**2**).

culture broth was isolated by column chromatography (CC) in stepwise fashion to afford compound **1** as a major metabolite together with compound **2** as a minor product. The molecular formula of **1** was established as $C_{15}H_{26}O_4$ on the basis of HRESIMS which indicated three degrees of unsaturation. IR spectrum was consistent with the presence of hydroxyl group ($3425\text{--}3352\text{ cm}^{-1}$). $^1\text{H-NMR}$ spectrum of **1** showed three signals of methyl groups at δ_{H} 0.83, 1.13 and 1.34 and five signals of the protons attached to carbons bearing oxygen atom at δ_{H} 3.27, 3.55, 3.42, 3.87 and 5.30. The $^{13}\text{C-NMR}$ (Table 1) and HSQC spectra showed 15 carbon signals consisting of three methyls, four methylenes, six methines, and two quaternary sp^3 -carbons. Additionally, the carbon signals at δ_{C} 92.9, 73.4, 73.3, and 61.8 indicated that these carbons were attached with oxygen atom. Since no sp^2 - or sp -carbons were observed in the $^{13}\text{C-NMR}$ spectral data, the accounted three unsaturations implied that **1** should contain three rings. A series of correlation spectroscopy (COSY) and heteronuclear single quantum correlation (HSQC) experiments established partial connectivities and the structure of **1** was established from HMBC and nuclear overhauser enhancement spectroscopy (NOESY) experiments as shown in Figure 2. The relative stereochemistry of **1** was determined by a combination of coupling constant (J) and NOESY experiment (Figure 2). The large coupling constant of $H_{\text{ax}}\text{-3}$ with H-4 ($J = 11.6\text{ Hz}$) and the observed NOEs between $H_{\text{ax}}\text{-3}$ and H-6 and

between $H_{\text{ax}}\text{-3}$ and methyl protons of C-15 in the NOESY experiment suggested that H-3, H-4, and the methine carbon (C-6) were axially oriented. A single crystal X-ray analysis of **1** (Figure 3) was carried out and the relative stereochemistry was found to be completely consistent with the assignments based on NMR spectral data as described above. A literature search revealed that compound **1** is a new 2,3-secoaromadendrane-type sesquiterpenoid and this compound has not yet been reported as the metabolite of the genus *Psilocybes*.

Compound **2** was determined as $C_{16}H_{28}O_4$ on the basis of HRESIMS which indicated three degrees of unsaturation. ^1H and ^{13}C NMR spectral data of **2**, compared with those of **1**, suggested that compound **2** was a derivative of **1**. The HMBC and NOESY experiments (Table 1) with the assistance of COSY and HSQC established the structure of **2**. The observed NOE between $H_{\text{ax}}\text{-3}$ and the methoxy protons suggested that the methoxy group of **2** was axially oriented. Due to a presence of the hemiacetal of **1**, compound **2** was expected to be a product arisen from the reaction of **1** with methanol during isolation process. To prove this doubt, compound **1** was treated with excess methanol in the presence or absence of catalytic amount of *p*-toluenesulfonic acid and the reactions were monitored by thin layer chromatography (TLC). The result showed that the reaction under acid condition was completed within 3 h, while the reaction under non-acid condition was very slowly. After evaporation of the solvent and purification by silica gel CC, ^1H and ^{13}C NMR spectra indicated that the product from the reaction was mainly compound **2**. This result suggested that compound **2** was a product arisen during the process of extraction and isolation and crystallization of **1**.

3. Experimental

3.1 General experimental procedures

Melting points were examined using a Fisher–John melting point apparatus and are uncorrected. Optical rotations were measured on

Table 1. 1D and 2D NMR spectral data of **1** and **2**^a (CDCl₃).

	1				2			
	δ_{H} (J, Hz)	δ_{C}	HMBC	NOESY	δ_{H} (J, Hz)	δ_{C}	HMBC	NOESY
1	1.94 <i>br s</i>	56.6	C-2,5,6,9,10	H-2,14	1.89 <i>br s</i>	56.6	C-2,6,9,10	H-2,14
2	5.30 <i>br s</i>	92.9	C-3,5	H-1,14	4.70 <i>d</i> (2.4)	99.6	C-3,5	H-1,14
3eq	3.42 <i>dd</i> (11.6, 4.4)	61.8	C-2,4,5		3.32 <i>dd</i> (11.6, 4.8)	61.8	C-2,4,5	
3ax	3.87 <i>t</i> (11.6)		C-2,4,5,15	H-6,14	3.64 <i>t</i> (11.6)		C-2,4,5,15	H-6,OMe
4	2.01 <i>m</i>	35.6	C-3,6,15	H-15	1.96 <i>m</i>	35.6	C-3,5,6,15	H-15
5	2.09 <i>m</i>	30.4	C-1,6	H-13,8a	2.00 <i>m</i>	30.8	C-6	H-13,8a
6	0.93 <i>m</i>	21.5	C-7	H-3ax,9b,12a	0.84 <i>m</i>	21.9	C-4,7,12	H-3ax,9b
7	0.91 <i>m</i>	24.2	C-6	H-9b,12b,12a	0.82 <i>m</i>	24.3	C-6,12	H-9b,12b,12a
8a	1.53 <i>m</i>	18.4	C-7,10	H-13	1.47 <i>m</i>	18.5	C-7,9,10	H-5
8b	1.69 <i>m</i>		C-6,7,9,10		1.64 <i>m</i>		C-6,7,9,10	H-9a
9a	1.55 <i>dd</i> (6.8, 14.4)	38.6	C-1,7,8,10,14		1.47 <i>m</i>	38.6	C-1,7,8,10,14	H-8b
9b	2.11 <i>dd</i> (12.4, 14.4)		C-7,8	H-6,14	2.03 <i>m</i>		C-7,8	H-6
10	-	73.3	-	-	-	73.4	-	-
11	-	26.5	-	-	-	26.3	-	-
12a	3.55 <i>d</i> (10.4)	73.4	C-6,7,11,13	H-6	3.51 <i>d</i> (11.2)	73.9	C-6,7,11,13	-
12b	3.27 <i>d</i> (10.4)		C-6,7,11,13	H-6,7	3.21 <i>d</i> (11.2)		C-6,7,11,13	H-7
13	1.13 <i>s</i> ^b	11.4	C-6,7,11,12	H-5,8a	1.08 <i>s</i> ^b	11.5	C-6,7,11,12	H-5
14	1.34 <i>s</i> ^b	31.6	C-1,9,10	H-1,2,9b	1.24 <i>s</i> ^b	31.7	C-1,9,10	H-2
15	0.83 <i>d</i> (7.2) ^b	14.5	C-3,4,5	H-4	0.77 <i>d</i> (6.8) ^b	14.4	C-3,4,5	H-4
OMe					3.34 <i>s</i> ^b	55.3	C-2	H-2,3ax

^a Data were obtained at 400 MHz for ¹H and 100 MHz for ¹³C NMR with chemical shifts (δ) in ppm and were referenced to residual solvent signals with resonances at δ_{H} 7.26 and at δ_{C} 77.0.

^b Intensity of three protons.

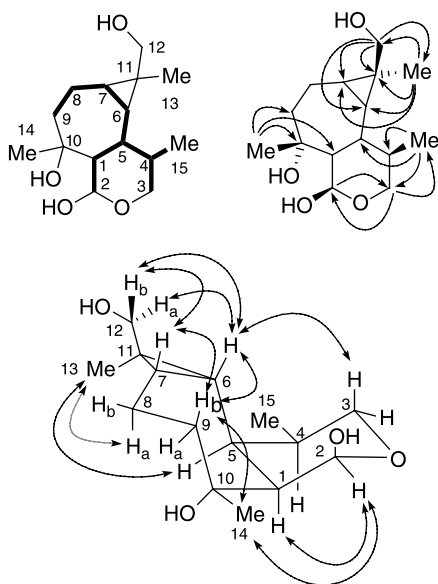


Figure 2. Main ^1H – ^1H COSY (—), HMBC (↷), and NOESY (↔) correlations of **1**.

a Perkin-Elmer 341 polarimeter, using a sodium lamp at wavelength 589 nm. FT-IR spectra were recorded on a Perkin-Elmer Model 1760X Fourier Transform Infrared Spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury

+400 MHz NMR spectrometer (^1H at 400 MHz and ^{13}C at 100 MHz). Chloroform-*d* (CDCl_3) was used in NMR experiments and chemical shifts (δ) were referenced to the signals of residual solvents at δ 7.26 ppm (^1H) and 77.0 ppm (^{13}C). HRESIMS spectra were recorded on Micromass LCT (LC/MS).

All solvents used for CC were of commercial grade and distilled prior to use. TLC were carried out on precoated silica gel 60 (Merck's TLC aluminum sheet, silica gel 60 F₂₅₄ Art. 1.05554.0001, Darmstadt, Germany) and spots were detected under UV (254 and 365 nm) before and after spraying with a vanillin/sulfuric acid solution followed by heating the plate. Isolations were carried out using CC [silica gel 60 (Merck Art. 1.09385.9025, 0.040–0.063 mm, Darmstadt, Germany)]. Malt extract for culture of the fungus was purchased from Himedia, Mumbai, India.

3.2 Plant material

Spores print dry specimens of *P. samuiensis* from Koh Samui, Surat Thani Province, Thailand were received from John W. Allen in July 2004. The spores print was used for cultivation. The molecular identification of *P. samuiensis* was carried out based on the DNA sequence of the internal transcribed spacer (ITS) region of ribosomal RNA gene and the nucleotide sequence data have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB257586.

3.3 Cultivation, extraction and isolation

The spores of *P. samuiensis* from spore print were streaked in Petri dishes containing potato dextrose agar (PDA). The Petri dishes were incubated at room temperature (25–30°C) and examined for fungal mycelium from spores. Outgrowing mycelia were purified and transferred into other Petri dishes containing PDA. Stock culture of *P. samuiensis* was grown on malt extract agar (MEA) at room temperature for 2 weeks. The agar was then cut with

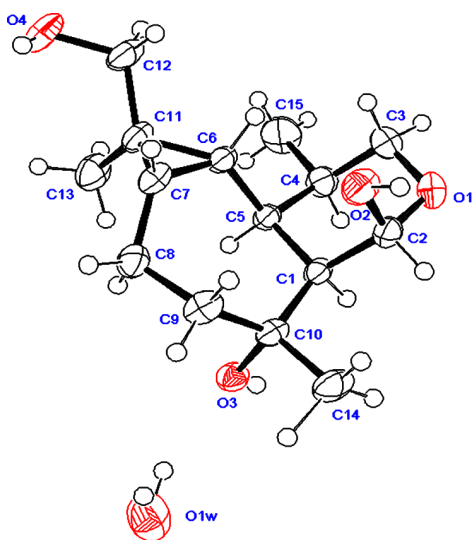


Figure 3. RTEP view for compound **1**.

a flamed 8 mm diameter cork borer. Five pieces of agar cultures were inoculated into 250 ml Erlenmeyer flasks containing 100 ml of malt extract broth (MEB) ($\times 110$), and then statically cultured at ambient temperature for 11 weeks. The culture was filtered through filter paper (Whatman No. 93) and the broth was then concentrated under reduced pressure at 35°C into 500 ml. The concentrated broth was extracted with 200 ml of hexane ($\times 5$), 200 ml of ethyl acetate ($\times 5$) and 200 ml of methanol ($\times 5$). The hexane extract of broth was obtained in a small amount. The combined ethyl acetate layers were dried over anhydrous sodium sulfate and then evaporated under reduced pressure at 35°C to yield the ethyl acetate extract (5.88 g) as yellowish brown viscous liquid. The ethyl acetate extract (4.97 g) was chromatographed on silica gel column eluted with CH_2Cl_2 –MeOH gradient in a stepwise fashion. Fractions with similar components were combined together according to the TLC profile.

Compound **1** was obtained from elution of 3% MeOH in CH_2Cl_2 . The solvent was removed by rotary evaporation and the residue was obtained as white solid mixed with yellow viscous liquid (960 mg). The mixture was purified by crystallization from CH_2Cl_2 –MeOH to give compound **1** as colorless crystals. The filtrate was further purified by silica gel CC and crystallization from CH_2Cl_2 –MeOH to give compound **1** as colorless crystals. Both crystals of **1** were combined and total amount of **1** was 736 mg (67 mg/l of broth). After evaporation of the filtrate, a yellow viscous liquid (179 mg) was obtained. The residue was purified by TLC to give compound **2** as colorless oil (19 mg).

3.3.1 *Psilosamuiensin A (1)*

Colorless crystals; m.p. 91–92°C; $[\alpha]_D^{20} - 48$ (MeOH, c 0.25); IR ν_{max} (KBr) 3425–3352, 2960, 2927, 2872, 1140, 1102, 1043 and 1013 cm^{-1} ; ^1H NMR and ^{13}C NMR (CDCl_3) spectral data: see Table 1; ESITOFMS m/z 293.1732 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{15}\text{H}_{26}\text{O}_4\text{Na}$, 293.1729).

3.3.2 *Psilosamuiensin B (2)*

$[\alpha]_D^{20} - 51$ (CHCl_3 , c 0.07); IR ν_{max} (film) 3397, 2967, 2931, 2878, 1129, 1103, and 1045 cm^{-1} ; ^1H NMR and ^{13}C NMR (CDCl_3) spectral data: see Table 1; ESITOFMS m/z 307.1890 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{16}\text{H}_{28}\text{O}_4\text{Na}$, 307.1885)

4. X-ray crystallography of **1**

A colorless needle crystal suitable for X-ray analysis was recrystallized from CH_2Cl_2 –MeOH. The X-ray data of **1** were collected at 293 (2) K with Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) on a BRUKER SMART CCD diffractometer. Crystal data: $\text{C}_{15}\text{H}_{26}\text{O}_4 \cdot \text{H}_2\text{O}$, orthorhombic, space group $P2_12_12_1$, $a = 6.43650(10) \text{ \AA}$, $b = 13.76020(10) \text{ \AA}$, $c = 17.9071(2) \text{ \AA}$, $V = 1585.99(3) \text{ \AA}^3$, $Z = 4$, $D_{\text{calc}} = 1.208 \text{ g/cm}^3$, $F(000) = 632$, $\mu = 0.089 \text{ mm}^{-1}$. A total of 11,463 reflections were collected, 4,579 were unique ($R_{\text{int}} = 0.0182$). The structure was refined by full-matrix least-squares on F^2 using SHELXL-97 package software [13]. The final reliability factors are: $R = 0.0355$, $wR = 0.1174$ [$I > 2\sigma(I)$] and the goodness of fit on F^2 was equal to 1.040. The X-ray crystallographic data can be found in supplementary publication CCDC 609474, available from the Cambridge Crystallographic Data Centre. Copies of the information may be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336 033. Email: deposit@ccdc.cam.ac.uk or <http://www.ccdc.cam.ac.uk>).

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