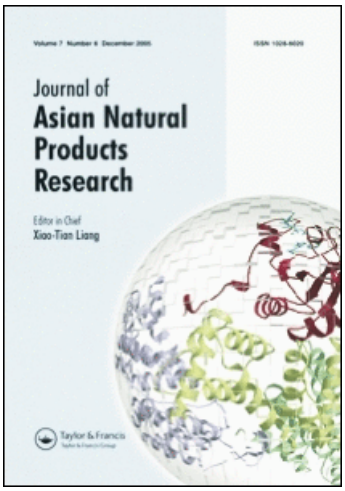

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new sesquiterpenoid metabolite from Psilocybe samuiensis

Surachai Pornpakakul [a]; Sunisa Suwancharoen [a]; Amorn Petsom [a]; Sophon Roengsumran [a]; Nongnuj Muangsin [a]; Narongsak Chaichit [b]; Jittra Piapukiew [c]; Prakistin Sihanonth [d]; John W. Allen
[a] Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand [b] Department of Physics, Faculty of Science and Technology, Thammasat University, Pathumthani, Thailand [c] Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand [d] Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Online Publication Date: 01 January 2009

To cite this Article Pornpakakul, Surachai, Suwancharoen, Sunisa, Petsom, Amorn, Roengsumran, Sophon, Muangsin, Nongnuj, Chaichit, Narongsak, Piapukiew, Jittra, Sihanonth, Prakistin and Allen, John W.(2009)'A new sesquiterpenoid metabolite from Psilocybe samuiensis',Journal of Asian Natural Products Research,11:1,12 — 17

To link to this Article: DOI: 10.1080/10286020802435794

URL: http://dx.doi.org/10.1080/10286020802435794

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 12–17

A new sesquiterpenoid metabolite from *Psilocybe samuiensis*

Surachai Pornpakakul[a]*, Sunisa Suwancharoen[a], Amorn Petsom[a], Sophon Roengsumran[a], Nongnuj Muangsin[a], Narongsak Chaichit[b], Jittra Piapukiew[c], Prakistin Sihanonth[d] and John W. Allen[e]

[a]*Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand;* [b]*Department of Physics, Faculty of Science and Technology, Thammasat University, Pathumthani, Thailand;* [c]*Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand;* [d]*Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand;* [e]*P.O. Box 45164 Seattle, Washington, DC, USA*

(*Received 29 February 2008; final version received 24 July 2008*)

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 ^{1}H 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

*Corresponding author. Email: psuracha@chula.ac.th

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802435794
http://www.informaworld.com

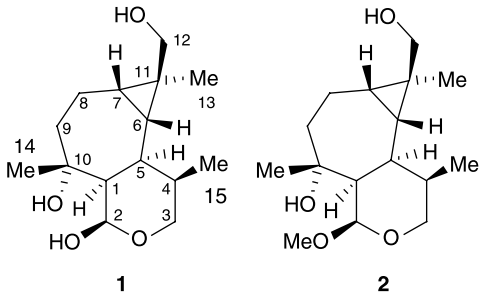

Figure 1. Structures of psilosamuiensin A (**1**) and psilosamuiensin B (**2**).

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 (3425–3352 cm^{-1}). ^{1}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}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}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_{ax}-3 with H-4 ($J = 11.6$ Hz) and the observed NOEs between H_{ax}-3 and H-6 and between H_{ax}-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. ^{1}H 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_{ax}-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, ^{1}H 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] ($CDCl_3$).

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

[a] Data were obtained at 400 MHz for ^1H and 100 MHz for ^{13}C 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.

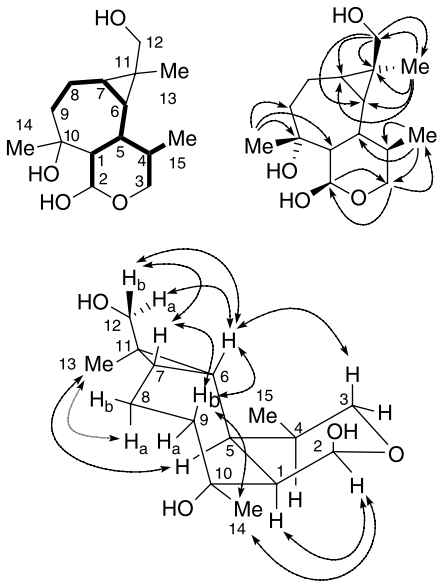

Figure 2. Main $^{1}H—^{1}H$ COSY (▬), HMBC (⟶), and NOESY (⟷) correlations of **1**.

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. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury +400 MHz NMR spectrometer (^{1}H 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 (^{1}H) 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 F_{254} 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.

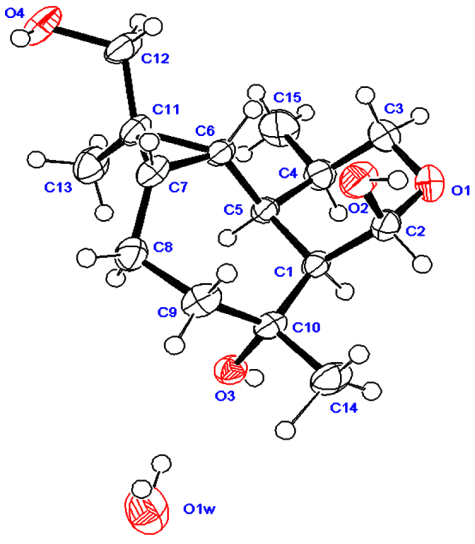

Figure 3. RTEP view for compound **1**.

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

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) (× 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 (× 5), 200 ml of ethyl acetate (× 5) and 200 ml of methanol (× 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 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_4Na$, 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 $[M + Na]^+$ (calcd for $C_{16}H_{28}O_4Na$, 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 Kα radiation ($\lambda = 0.71073$ Å) on a BRUKER SMART CCD diffractometer. Crystal data: $C_{15}H_{26}O_4 \cdot H_2O$, orthorhombic, space group $P2_12_12_1$, $a = 6.43650(10)$ Å, $b = 13.76020(10)$ Å, $c = 17.9071(2)$ Å, $V = 1585.99(3)$ Å^3, $Z = 4$, $D_{calc} = 1.208$ g/cm^3, $F(000) = 632$, $\mu = 0.089$ mm^{-1}. A total of 11,463 reflections were collected, 4,579 were unique ($R_{int} = 0.0182$). 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).

Acknowledgements

We thank Higher Education Commission Grants for Graduate Dissertation for financial support. Department of Chemistry, Faculty of Science, Chulalongkorn University, the Rachadapiseksompoj Endowment, Chulalongkorn University, National Center of Excellence for Petroleum, Petrochemicals and Advanced Materials (NCE-PPAM) and the Thailand Research Fund (RTA4880008) are also gratefully acknowledged.

References

[1] N. Seivewright and O. Lagundoye, *Adv. Psychiatr. Treat.* **6**, 344 (2000).

[2] P. Stamets, *An Identification Guide: Psilocybin Mushrooms of the World* (Ten Speed Press, California, 1996).

[3] G.R. Wasson, *Life* **42**, 100 (1957).

[4] R. Heim, and G.R. Wasson, Les Champignons Hallucinogenes du Mexique. Etudes Ethnologiques, Taxinomiques, Biologiques, Physiologiques et Chimiques. *Archives du Museum National d'Histoire Naturelle*, Series 7, Vol. VI (Paris, France, 1958).

[5] A. Hofmann, A. Frey, H. Ott, T. Petrzilka, and F. Troxler, *Experientia* **14**, 397 (1958).

[6] A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, T. Petrzilka, and F. Troxler, *Helv. Chim. Acta.* **42**, 1557 (1959).

[7] Y.A. Leung and G.A. Paul, *J. Pharm. Sci.* **57**, 1667 (1968).

[8] N. Jensen, J. Gartz, and H. Laatsch, *Planta Med.* **72**, 665 (2006).

[9] Y. Koike, K. Wada, G. Kusano, S. Nozoe, and K. Yokoyama, *J. Nat. Prod.* **44**, 362 (1981).

[10] W.J. Allen and D.M. Merlin, *J. Ethnopharmacol.* **35**, 205 (1992).

[11] J. Gartz, W.J. Allen, and D.M. Merlin, *J. Ethnopharmacol.* **43**, 73 (1994).

[12] G. Guzmán, M.V. Bandala, and W.J. Allen, *Mycotaxon* **46**, 155 (1993).

[13] M.G. Sheldrick, *SHELXL-97 Crystal Structure Refinement Program* (University of Göttingen, Germany, 1997).