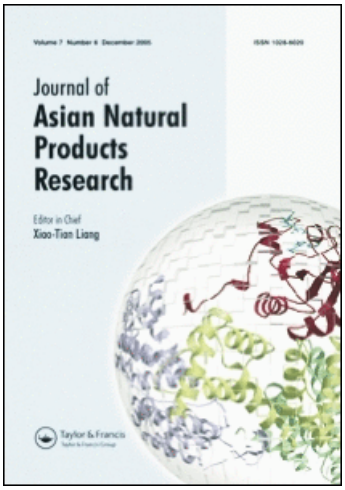

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ORIGINAL ARTICLE

Two new sesquiterpenoids from basidiomycete *Agrocybe salicacola*

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Two new aromadendrane-type sesquiterpenoids (**1** and **2**), together with a known aromadendrane sesquiterpenoid (**3**), were isolated from the culture of basidiomycete *Agrocybe salicacola.* The structures and relative configurations of the new compounds were determined by spectroscopic methods and single-crystal X-ray crystallographic analysis.

Keywords: *Agrocybe salicacola*; basidiomycete; aromadendrane sesquiterpenoid

1. Introduction

Mushrooms, due to their unique flavor, taste, and potential health benefits, are an attractive delicacy and are extensively used as dietary supplements and nutraceuticals along with various combinations of other herbal preparations to treat a number of medical conditions in traditional Chinese medicine [1]. Mushroom, *Agrocybe salicacola* Zhu L. Yang, M. Zang, and X.X. Liu [2], is an edible basidiomycete belonging to the order Agaricales. The genus *Agrocybe* is reported to contain several bioactive metabolites, such as ceramide with inhibitory activity against COX-1,2 [1], indole alkaloids with free radical scavenging ability [3], agrocybin, a peptide with anti-fungal activity [4], polysaccharides with hypoglycemic activity [5], a lectin with mitogenic activity [6], and antiproliferative and differentiating effects [7]. In continuation of our previous chemical and biological investigations of secondary metabolites derived from higher fungi [7–13], the presence of sesquiterpenoids was discerned in the fermentation broth of the basidiomycete *A. salicacola*. The two new sesquiterpenoids 2,3-secoaromadendrane-type sesquiterpenoid (**1**) and aromadendrane-type derivative (**2**), together with a known aromadendrane sesquiterpenoid (**3**), were isolated (Figure 1). Herein, we report on the isolation, structural determination, and relative stereochemistry assignment of **1** and **2**.

2. Results and discussion

Compound **1** was isolated as a colorless crystal (from aqueous acetone), $[\alpha]_D^{16.7}$ -43.9 ($c = 0.22$, $CHCl_3$) from the culture of *A. salicacola*. The culture broth was successively extracted with EtOAc. By using this procedure, 2.8 g of the crude extract was obtained from 20 liters of the culture. Next, the extract was successively purified by several chromatographic steps

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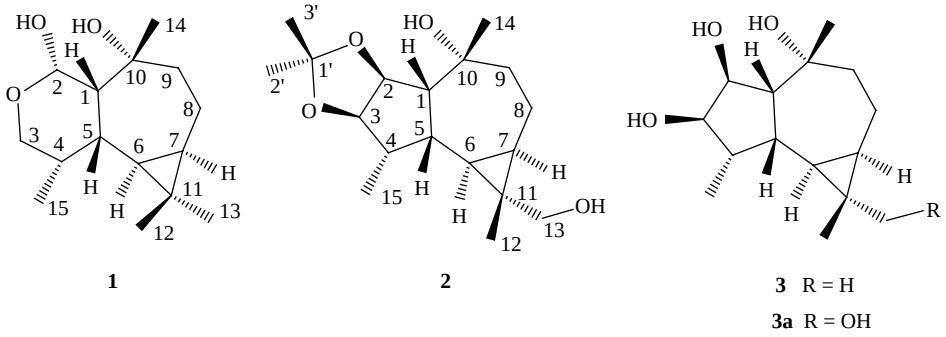


1 **2** **3** R = H

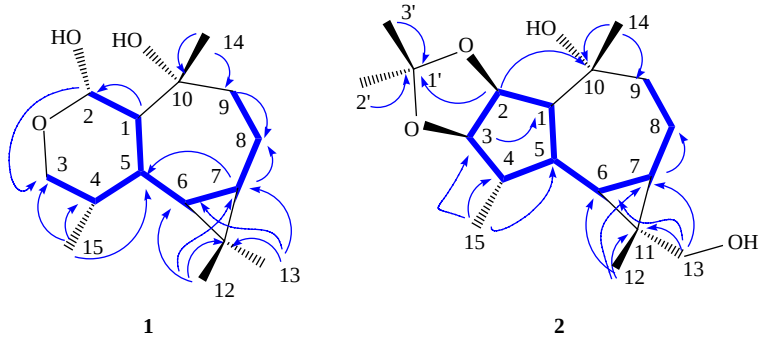
3a R = OH

Figure 1. Structures of compounds **1**–**3**.

to yield 48.5 mg of **1**. The HR-ESI-MS showed a molecular ion at *m/z* 254.1881, corresponding to the formula $C_{15}H_{26}O_3$. Subsequently, the molecular formula $C_{30}H_{36}O_5$ was confirmed after a structural analysis based on the NMR spectroscopy.

Investigation of the ^{1}H and ^{13}C NMR (DEPT) and HSQC experiments revealed the existence of four methyl groups, three aliphatic methylene units (including one oxymethylene group), and six methine units (including one oxymethine group). Additionally, two quaternary carbon centers (including one oxyquaternary carbon) were identified from both the ^{13}C NMR and HMBC spectra. The presence of a cyclopropane ring (H6–H7, 0.66 and 0.75 ppm) is immediately apparent from the ^{1}H NMR spectrum of **1**. The other resonances can be assigned on the basis of the ^{1}H–^{1}H COSY experiment (Figure 2). By the comparison of its spectral data with those of psilosamuiensin A, which was isolated from *Psilocybe samuiensis* by Pornpakakul *et al.* [14], the planar structure of **1** was elucidated, which was a 2,3-secoaromadendrane-type sesquiterpene. This was further confirmed using ^{1}H and ^{13}C long-range correlations of the HMBC experiment as illustrated in Figure 2.

HMBC correlations of H-12 and H-13 with C-6 and C-7, H-14 with C-1, C-9, and C-10, H-15 with C-3, C-4, and C-5, H-1 with C-2, C-4, C-6, and C-9, H-2 with C-3, C-5, and C-10, H-3 with C-2, C-5, and C-15 were observed. Other HMBC correlations were observed between H-4 and C-1 and C-6; between H-5 and C-2, C-3, C-7, and C-10; between H-6 and C-1, C-4, C-7, and C-8; between H-7 and C-5 and C-9; between H-8 and C-6, C-10, and C-11; between H-9 and C-1, C-7, and C-14.



1 **2**

Figure 2. Selected ^{1}H–^{1}H COSY (▬) and HMBC (⌒) correlations of compounds **1** and **2**.

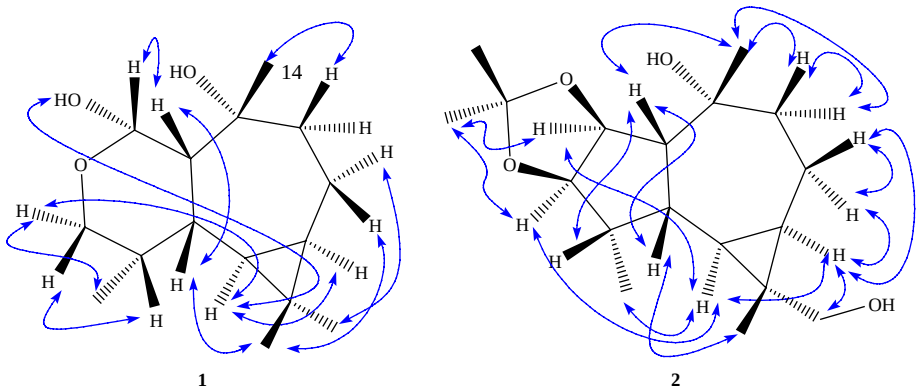


Figure 3. Selected ROESY (↷) correlations of compounds **1** and **2**.

The relative stereochemistry of **1** was suggested by a combination of coupling constant and ROESY experiments (Figure 3). The *cis*-fusion of the seven- and five- membered rings is based on the coupling constant $J_{\text{H-1/H-5}} = 3.7$ Hz, which is characteristic of 2,3-secoaromadendrane [14]. The large coupling constant of Hα-3 with H-4 ($J = 11.7$ Hz), and the observed ROESY correlations between Hα-3 and H-6; between Hα-3 and H-15; between H-1 and H-2, and H-5, between H-6 and H-7 in the ROESY experiments suggested that 2-OH, H-6, H-7, and H-15 are at the α position, while H-1 and H-5 are at the β position. A single-crystal X-ray analysis of **1** (Figure 4) was carried out and the relative stereochemistry was found to be consistent with the assignments based on NMR spectral data as described

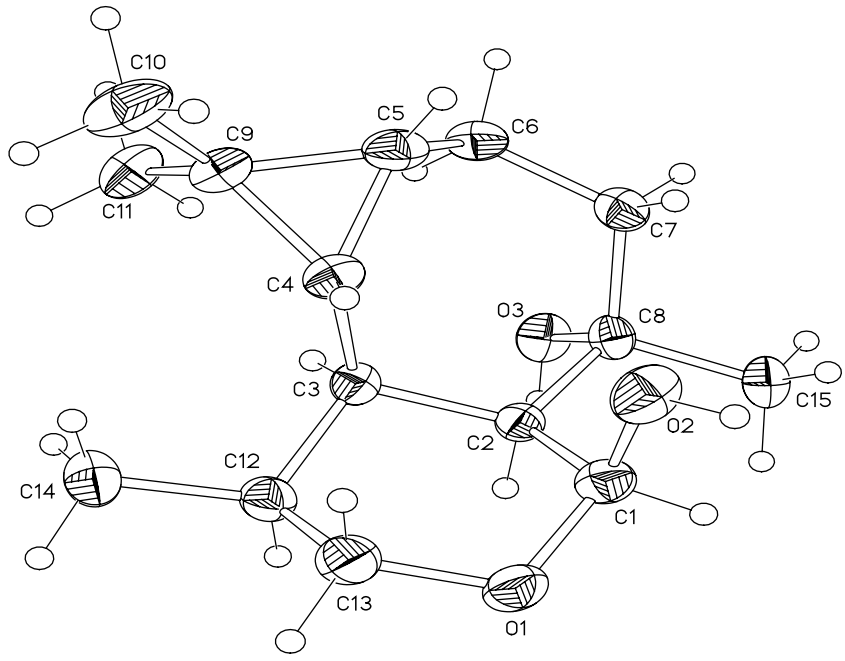


Figure 4. X-ray crystal structure of compound **1**.

above. A literature search revealed that compound **1** is a new sesquiterpene with a 2,3-secoaromadendrane-type structure.

Compound **2** was purified as a colorless powder with a molecular formula of $C_{18}H_{30}O_4$, based on the positive-ion HR-ESI-MS at *m/z* 333.2034 $[M+Na]^+$ and the ^{13}C NMR (DEPT) spectrum. The presence of an isopropyl group and a primary alcohol (H-13) is apparent from the ^1H and ^{13}C NMR spectra of **2**. Compound **2** might not be a natural product. This acetonide is likely to be produced by the reaction of the corresponding diol with the solvent during the isolation. By comparison of its spectroscopic data with those of **3** [15] isolated from the same fungus in our experiment, it suggested that **2** was an acetonide of **3a** (Figure 1). Compound **3a** is a derivative of **3** and has not yet been reported in the literature. The HMBC and ROESY experiments (Figure 2) with the assistance of COSY and HSQC spectra established the structure of **2**.

The relative configurations of the eight successive chiral centers at C-1, C-2, C-3, C-4, C-5, C-6, C-7, and C-10 in **2** were suggested by the following NOE analysis. As shown in Figure 3, H-6 showed correlations with H-2, H-3, H-7, and H-15, exhibiting these protons to orient in the same direction. On the other hand, H-1 correlations with H-4, H-5, and H-14 indicated that these protons oriented in the opposite direction.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 spectropolarimeter. The IR spectrum was obtained with a Bruker Tensor 27 spectrometer, with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers, in CD_3OD or $CDCl_3$, δ in ppm, *J* in Hz. EI-MS was recorded with a VG Autospec-3000 spectrometer, HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Pre-coated silica gel GF254 plates (Qingdao Marine Chemical Inc.) were used for TLC. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2 Mushroom material and culture

The fungus *A. salicacola* was collected at the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, China, in September 2008, and identified by Dr Zang Mu, Kunming Institute of Botany. The voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium consisted of potato (peeled, 200 g), glucose (20 g), KH_2PO_4 (3 g), $MgSO_4$ (1.5 g), citric acid (0.1 g), and thiamine hydrochloride (10 mg), in 1 liter of sterilized H_2O. Reagent bottles were used as a flask (size: 500 ml; volume of media: 350 ml). The pH value was adjusted to 6.5 before autoclaving. Fermentation was carried out on a shaker at 22°C and 150 rpm for 20 days.

3.3 Extraction and isolation

The whole culture broth of *A. salicacola* (20 liters) was initially filtered, and the fitrate was extracted three times with EtOAc. The organic layer was concentrated under reduced pressure to give a crude extract (2.8 g), and this residue was subjected to column chromatography over silica gel (200–300 mesh, 3 × 45 cm), eluting with a petroleum ether–acetone gradient, to afford fractions A–I. Fraction H eluted with petroleum ether–acetone (1:2) was further purified on a silica gel column (petroleum ether–acetone, 50:1 to 1:1) to give five subfractions, H1–H5. Each subfraction was further separated by repeated

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compound **1** in $CDCl_3$ and compound **2** in CD_3OD.

	1		**2**	
	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1	1.93 (br s)	56.5	2.04 (m)	63.4
2	5.25 (dd, $J = 2.6$, 6.0)	93.1	4.54 (dd, $J = 7.9$, 9.2)	82.8
3	3.82 (t, $J = 11.7$), 3.41 (dd, $J = 4.5$, 11.7)	61.7	4.39 (t, $J = 7.1$)	87.6
4	1.96 (m)	35.2	2.01 (m)	45.7
5	1.99 (dm, $J = 3.7$)	31.3	2.21 (m)	43.1
6	0.66 (t, $J = 9.9$)	24.5	0.22 (t, $J = 4.8$)	22.3
7	0.75 (m)	27.4	0.85 (m)	27.1
8a	1.66 (m)	18.7	1.60 (m)	19.3
8b	1.46 (m)		1.52 (m)	
9a	2.05 (m)	38.9	1.78 (m)	39.4
9b	1.54 (m)		1.65 (m)	
10		73.3		74.5
11		19.9		26.9
12	0.98 (s)	15.5	1.04 (s)	11.8
13	1.04 (s)	28.6	3.47 (d, $J = 11.1$), 3.08 (d, $J = 11.1$)	72.7
14	1.31 (s)	31.5	1.24 (s)	32.3
15	0.75 (d, $J = 7.0$)	14.4	1.08 (d, $J = 6.9$)	14.9
1′				114.6
2′			1.30 (s)	25.1
3′			1.46 (s)	28.0

reversed-phased C_{18} (MeOH–H_2O, 3:2 and 4:1) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) column chromatography. Subsequently, **1** (48.5 mg) was obtained from subfraction H3, **3** (64 mg) from H4, respectively. Fraction G, eluted with petroleum ether–acetone (1:1), was again purified by repeated reversed-phased C_{18} (MeOH–H_2O) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) column chromatography to give pure **2** (6.0 mg).

Compound **1**, colorless crystals, mp 145–148°C, $[\alpha]_D^{16.7}$ −43.9 ($c = 0.22$, $CHCl_3$), IR (KBr) ν_{max}: 3416, 2949, 2926, 2866, 1141, 1106, 1043, 1012 cm^{-1}, ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data: see Table 1; EI-MS *m/z*: 254 $[M]^+$, HR-EI-MS *m/z*: 254.1881 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

Compound **2**, a colorless powder, $[\alpha]_D^{15.2}$ −23.0 ($c = 0.35$ CH_3OH); IR (KBr) ν_{max}: 3432, 2923, 1637, 1629 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD) spectral data: see Table 1; FAB-MS *m/z*: 311 $[M - H]^-$; HR-ESI-MS *m/z*: 333.2034 $[M+Na]^+$ (calcd for $C_{18}H_{30}O_4Na$, 333.2041).

Acknowledgements

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