

Oxyphyllones A and B, novel sesquiterpenes with an unusual 4,5-secoeudesmane skeleton from *Alpinia oxyphylla*

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Abstract

Two novel 4,5-secoeudesmane sesquiterpenoids, oxyphyllones A (**1**) and B (**2**) were isolated from the fruits of *Alpinia oxyphylla*. Their structures were established by spectroscopic methods including 1D and 2D NMR spectra. These two compounds are the first example of naturally occurring sesquiterpenoids with a 4,5-secoeudesmane skeleton in the family of Zingiberaceae and oxyphyllone A (**1**) is the first 4,5-secoeudesmane type of 13-norsesquiterpenoid. Compounds **1** and **2** exhibited no cytotoxicities against three cancer cell lines at 10 $\mu\text{g}/\text{mL}$.

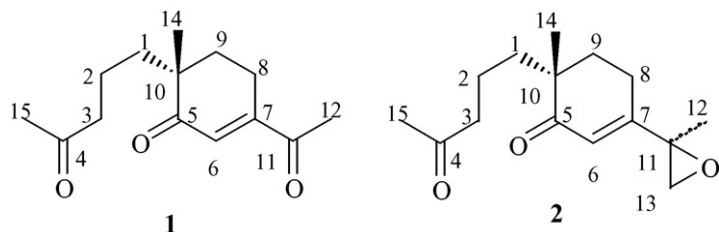
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Keywords: *Alpinia oxyphylla*; Zingiberaceae; 4,5-Secoeudesmane sesquiterpenoid; Oxyphyllone A; Oxyphyllone B

Alpinia oxyphylla Miq. (Zingiberaceae) distributes widely in South China and is used in folk medicine to treat intestinal disorders, urosis, diuresis, ulceration and dementia [1–3]. Sesquiterpenes, diterpenes, flavonoids, diarylheptanoids in *A. oxyphylla* have been reported previously and some of which showed inhibitory effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages [1,2,4,5]. In our study, oxyphyllone A (**1**), a novel 4,5-secoeudesmane 13-norsesquiterpenoid, and a novel 4,5-secoeudesmane sesquiterpenoid oxyphyllone B (**2**) (shown as a 1:1 mixture of diastereoisomers), were found from the fruits of *A. oxyphylla*. This is the first time to find naturally occurring sesquiterpenoids with 4,5-secoeudesmane skeleton in the family of Zingiberaceae. Compound **1** is the first 4,5-secoeudesmane type of 13-norsesquiterpenoid. The cytotoxic activity of compounds **1** and **2** against A549, HT-29 and SGC-7901 cell lines was tested [6]. They showed no cytotoxicities on these cancer cell lines at 10 $\mu\text{g}/\text{mL}$. This paper mainly deals with the isolation and structure elucidation of the novel sesquiterpenoids and a possible biogenetic pathway is also proposed for these compounds.

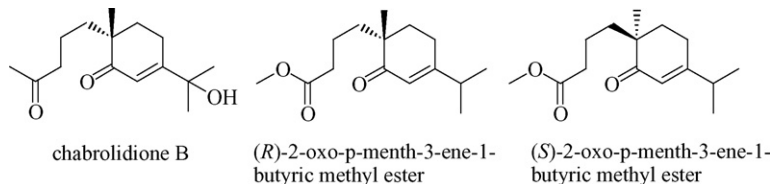
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The Me₂CO/H₂O (70%) extract of the fruits of *A. oxyphylla* [7] (15 kg) was partitioned in turn with petroleum ether, ethyl acetate, and *n*-butanol against water. Oxyphyllone A (**1**) (8 mg) and B (**2**) (8 mg) were isolated from the ethyl acetate fraction (480 g) by normal-phase column chromatography (silica gel, petroleum ether/acetone 9:1–1:1 and CHCl₃/AcOEt 100:1–9:1), and semipreparative HPLC (RP-18, MeOH/H₂O 34:66 and CH₃CN/H₂O 2:8).

Oxyphyllone A (**1**) [8] was obtained as a colorless oil, $[\alpha]_D^{22.7} -18.18$ (c 0.55, CHCl₃). The molecular formula C₁₄H₂₀O₃ was as revealed by HR-ESI-MS (*m/z* 259.1311 [M+Na]⁺). Its IR spectrum displayed the presence of free ketone and conjugated ketone functions (1714, 1678 cm⁻¹). The ¹H NMR spectrum (Table 1) of **1** exhibited signals for three methyls at δ_H 1.09 (s, 3H), 2.12 (s, 3H) and 2.40 (s, 3H), one olefinic proton at δ_H 6.49 (s, 1H) and five methylenes. The ¹³C NMR (DEPT) (Table 1) indicated the presence of five quaternary carbons, including a free ketone carbon at δ_C 208.40 and conjugated two ketone carbons at δ_C 200.01 and 205.64. The NMR data of **1** were similar to those of chabrolidione B isolated from Formosan soft coral *Nephthea chabrolii* [9]. Compared with chabrolidione B carefully, a methyl and an oxygenated quaternary carbon signals were absent, whereas a α,β -conjugated ketone signal was present. Besides, the signals due to C-6 (131.40, d) shifted downfield and C-7 (152.68, s) shifted upfield. The above information suggested that **1** could be a 13-norsesquiterpene with a 4,5-secoeudesmane skeleton as shown in Fig. 1. This conclusion was supported by ¹H–¹H COSY, HSQC and HMBC experiments (Fig. 1).



The absolute configuration of **1** ($[\alpha]_D^{22.7} -18.18$ (c 0.55, CHCl₃)) was established by comparing the analogous compounds with the same chiral carbon at C-10, i.e. chabrolidione B ($[\alpha]_D -9.3$) [6] and two synthetic enantiomer

Table 1
NMR spectral data of compounds **1**^a, **2a/2b**^a and chabrolidione B^b.

Position	1		2a/2b		Chabrolidione B		
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	
1	1.38 (m); 1.50 (m)	35.34 (t)	1.36 (m); 1.52 (m)	35.30 (t)	35.94 (t)	1.38 (m); 1.55 (m)	35.8
2	1.50 (m)	17.97 (t)	1.52 (m)	18.08 (t)	18.11 (t)	1.51 (m)	18.3
3	2.42 (m)	43.69 (t)	2.42 (m)	43.76 (t)	43.89 (t)	2.42 (m)	44.0
4		208.40 (s)		208.65 (s)	208.72 (s)		208.9
5		205.64 (s)		203.62 (s)	203.65 (s)		204.7
6	6.49 (s)	131.40 (d)	6.02 (s)	124.71 (d)	124.80 (d)	6.04 (s)	121.3
7		152.68 (s)		160.91 (s)	161.00 (s)		168.2
8	2.51 (m)	20.40 (t)	2.29 (m)	21.53 (t)	21.56 (t)	2.42 (m)	22.5
9	1.97 (m) (α); 1.79 (m) (β)	32.36 (t)	1.92 (m) (α); 1.76 (m) (β)	32.60 (t)	32.90 (t)	1.97 (m) (α); 1.77 (m) (β)	33.4
10		44.25 (s)		43.53 (s)	43.53 (s)		43.5
11		200.01 (s)		56.40 (s)	56.43 (s)		72.6
12	2.40 (s)	26.10 (q)	1.52 (s)	19.71 (q)	19.73 (q)	1.41 (s)	28.6
13			2.84 (m)	54.06 (t)	54.09 (t)	1.41 (s)	28.7
14	1.09 (s)	21.36 (q)	1.06 (s)	21.59 (q)	21.65 (q)	1.07 (s)	21.8
15	2.12 (s)	29.86 (q)	2.12 (s)	29.85 (q)	29.85 (q)	2.13 (s)	29.9

^a ¹H and ¹³C NMR spectra were recorded in CDCl₃ at 400 and 100 MHz. δ in ppm, J in Hz.

^b ¹H and ¹³C NMR spectra were recorded in CDCl₃ at 500 and 125 MHz from reference [9].

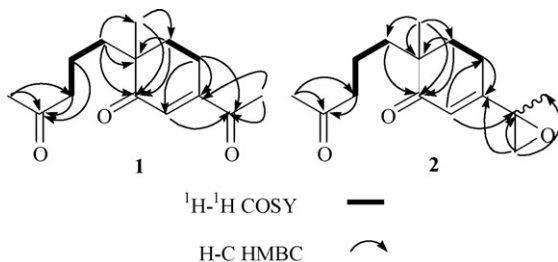


Fig. 1. ^1H - ^1H COSY and Key HMBC correlations for compounds **1** and **2**.

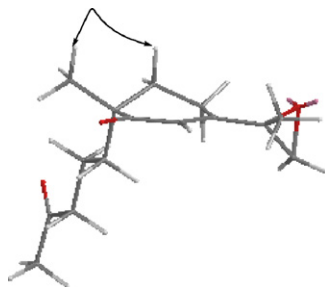
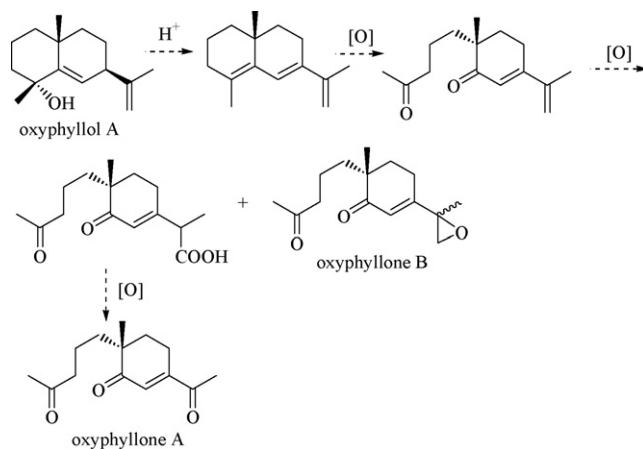


Fig. 2. Key ROESY correlation of compounds **2a/2b**.

compounds, (*R*)-2-oxo-*p*-menth-3-ene-1-butyric methyl ester ($[\alpha]_{\text{D}} -4.6$) and (*S*)-2-oxo-*p*-menth-3-ene-1-butyric methyl ester ($[\alpha]_{\text{D}} +5.5$) arising from β -eudesmol and valeranone, respectively [10]. Hikino et al. (1965) determined the absolute configuration of C-10 in valeranone by comparing the optical rotation value of (*S*) and (*R*)-2-oxo-*p*-menth-3-ene-1-butyric methyl ester [10], and the absolute configuration of chabrolidione B was determined to be 10*R* because of the similar structure and the same sign of optical rotation to (*R*)-2-oxo-*p*-menth-3-ene-1-butyric methyl ester [9]. Thus, compound **1** was inferred to be 10*R* following this similarity.

Oxyphyllone B (**2**) [11] (isolated as a 1:1 mixture of diastereoisomers) was obtained as a colorless oil, $[\alpha]_{\text{D}}^{27.3} -13.64^\circ$ (c 0.11, CHCl_3). Its molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_3$ was provided by HR-ESI-MS (m/z 273.1462 $[\text{M}+\text{Na}]^+$). The ^1H and ^{13}C NMR spectral data of compounds **2a/2b** was similar to those of chabrolidione B [9] and oxyphyllone A (**1**). The main differences in ^{13}C NMR spectrum were that the signals corresponding to C-11 (56.40/56.43, s) shifted upfield, and C-13 (54.06/54.09, t) shifted downfield in compounds **2a/2b** compared with chabrolidione B, which suggest the presence of epoxy ring at C-11 and C-13. The structure of **2** as shown in Fig. 1 with 4,5-secoeudesmane



Scheme 1. A possible biogenetic pathway proposed for compounds **1** and **2a/2b**.

skeleton was determined by ^1H - ^1H COSY, HSQC and HMBC experiments. The relative configuration of 10- CH_3 was β orientation from the ROESY correlation between H-14 with H-9 β (Fig. 2) [9].

Although the absolute stereochemistry of **2** was not established, this compound and **1** were presumed to originate from the same precursor, oxyphyllol A [2] (Scheme 1). Therefore, compounds **2a/2b** are diastereoisomers and the absolute configuration of C-10 could be *R*.

Acknowledgments

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- [6] P. Skehan, R. Storeng, D. Scudiero, et al. *J. Natl. Cancer Inst.* 82 (13) (1990) 1107, A549 = lung cancer cell line, HT-29 = colon cancer cell line, SGC-7901 = gastric cancer cell line.
- [7] The fruit of *Alpinia oxyphylla* were bought from Kunming medicinal market, Kunming, Yunnan Province, People's Republic of China, in August 2006. The sample was identified by Professor Ning-Hua Tan.
- [8] Oxyphyllone A (**1**): colorless oil; $[\alpha]_{\text{D}}^{22.7} -18.18$ (c 0.55, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 244 (3.83) nm; IR (KBr) ν_{max} 2926, 1714, 1678 cm^{-1} ; HR-ESI-MS $[\text{M}+\text{Na}]^+$ m/z : 259.1311 (calcd. for $\text{C}_{14}\text{H}_{20}\text{O}_3\text{Na}^+$, 259.1310); ^1H and ^{13}C NMR, see Table 1.
- [9] J.H. Su, C.F. Dai, H.H. Huang, et al. *Chem. Pharm. Bull.* 55 (4) (2007) 594.
- [10] H. Hikino, Y. Hikino, Y. Takeshita, et al. *Chem. Pharm. Bull.* 13 (12) (1965) 1408.
- [11] Oxyphyllone B (**2a/2b**): colorless oil; $[\alpha]_{\text{D}}^{27.3} -13.64$ (c 0.11, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 238 (4.02) nm; IR (KBr) (ν_{max} 3436, 2959, 2934, 1714, 1668 cm^{-1}); HR-ESI-MS $[\text{M}+\text{Na}]^+$ m/z : 273.1462 (calcd. for $\text{C}_{15}\text{H}_{22}\text{O}_3\text{Na}^+$, 273.1466). ^1H and ^{13}C NMR, see Table 1.