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 \(\mu\)g/mL.

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 \(\mu\)g/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 \textit{A. oxyphylla} [7] (15 kg) was partitioned in turn with petroleum ether, ethyl acetate, and \textit{n}-butanol against water. Oxyphyllones 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, [\(\alpha\r{D}\r{22.7}^\circ\)]C 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 \(^1\)H NMR spectrum (Table 1) of 1 exhibited signals for three methyls at \(\delta_H 1.09\) (s, 3H), 2.12 (s, 3H) and 2.40 (s, 3H), one olefinic proton at \(\delta_H 6.49\) (s, 1H) and five methylenes. The \(^1\)H NMR spectrum (DEPT) (Table 1) indicated the presence of five quaternary carbons, including a free ketone carbon at \(\delta_C 208.40\) and conjugated two ketone carbons at \(\delta_C 200.01\) and 205.64. The NMR data of 1 were similar to those of chabrolidione B isolated from Formosan soft coral \textit{Nephthea chabrolii} [9]. Compared with chabrolidione B carefully, a methyl and an oxygenated quaternary carbon signals were absent, whereas a \(\alpha,\beta\)-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-seco-eudesmane skeleton as shown in Fig. 1. This conclusion was supported by \(^1\)H–\(^1\)H COSY, HSQC and HMBC experiments (Fig. 1).

The absolute configuration of 1 ([\(\alpha\r{D}\r{22.7}^\circ\)]C 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 ([\(\alpha\r{D}\) -9.3] [6] and two synthetic enantiomer

\begin{table}[h]
\centering
\begin{tabular}{cccccccc}
\hline
Position & \textbf{1} & & & \textbf{2a/2b} & & & \textbf{Chabrolidione B} \\
& \(\delta_H\) (J in Hz) & \(\delta_c\) & \(\delta_H\) (J in Hz) & \(\delta_c\) & \(\delta_H\) (J in Hz) & \(\delta_c\) & \\
\hline
1 & 1.38 (m); 1.50 (m) & 35.34 (t) & 1.36 (m); 1.52 (m) & 35.30 (t) & 35.94 (t) & 35.8 & \\
2 & 1.50 (m) & 17.97 (t) & 1.52 (m) & 18.08 (t) & 18.11 (t) & 18.3 & 1.51 (m) \\
3 & 2.42 (m) & 43.69 (t) & 2.42 (m) & 43.76 (t) & 43.89 (t) & 44.0 & 2.42 (m) \\
4 & 208.40 (s) & 208.65 (s) & 208.72 (s) & 208.9 & 209.4 & 204.9 & \\
5 & 205.64 (s) & 203.62 (s) & 203.65 (s) & 204.7 & 204.9 & 205.64 (s) & \\
6 & 6.49 (s) & 131.40 (d) & 6.02 (s) & 124.71 (d) & 124.80 (d) & 124.9 & 6.04 (s) & 121.3 \\
7 & 152.68 (s) & 100.1 (s) & 160.91 (s) & 161.00 (s) & 161.00 (s) & 168.2 & \\
8 & 2.51 (m) & 20.40 (s) & 2.29 (m) & 21.53 (s) & 21.56 (t) & 2.42 (m) & 22.5 \\
9 & 1.97 (m) (\(\alpha\)); 1.79 (m) (\(\beta\)) & 32.36 (t) & 1.92 (m) (\(\alpha\)); 1.76 (m) (\(\beta\)) & 32.60 (t) & 32.90 (t) & 33.4 & 1.97 (m) (\(\alpha\)); 1.77 (m) (\(\beta\)) & \\
10 & 44.25 (s) & 43.53 (s) & 43.53 (s) & 33.5 & 33.5 & 1.41 (s) & \\
11 & 200.01 (s) & 56.40 (s) & 56.43 (s) & 72.6 & 72.6 & 72.6 & \\
12 & 2.40 (s) & 26.10 (q) & 1.52 (s) & 19.71 (q) & 19.73 (q) & 28.6 & 1.41 (s) & \\
13 & 2.84 (m) & 54.06 (t) & 54.09 (t) & 14.1 (s) & 14.1 (s) & 14.1 (s) & 28.7 \\
14 & 1.09 (s) & 21.36 (q) & 1.06 (s) & 21.59 (q) & 21.65 (q) & 21.8 & 1.07 (s) & \\
15 & 2.12 (s) & 29.86 (q) & 2.12 (s) & 29.85 (q) & 29.85 (q) & 29.9 & 2.13 (s) & \\
\hline
\end{tabular}
\caption{NMR spectral data of compounds 1\(^a\), 2a/2b\(^a\) and chabrolidione B\(^b\).}
\end{table}

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

\(^b\)\(^1\)H and \(^13\)C NMR spectra were recorded in CDCl₃ at 500 and 125 MHz from reference [9].
compounds, (R)-2-oxo-p-menth-3-ene-1-butyric methyl ester ([α]D -4.6) and (S)-2-oxo-p-menth-3-ene-1-butyric methyl ester ([α]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 10R 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 10R following this similarity.

Oxyphyllone B (2) [11] (isolated as a 1:1 mixture of diasteroisomers) was obtained as a colorless oil, [α]D27.3 -13.64° (c 0.11, CHCl3). Its molecular formula C15H22O3 was provided by HR-ESI-MS (m/z 273.1462 [M+Na]+). The 1H and 13C NMR spectral data of compounds 2a/2b was similar to those of chabrolidione B [9] and oxyphyllone A (1). The main differences in 13C 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

![Fig. 1. 1H–1H COSY and Key HMBC correlations for compounds 1 and 2.](image)

![Fig. 2. Key ROESY correlation of compounds 2a/2b.](image)

![Scheme 1. A possible biogenetic pathway proposed for compounds 1 and 2a/2b.](image)
skeleton was determined by $^1$H–$^1$H COSY, HSQC and HMBC experiments. The relative configuration of 10-CH$_3$ was $\beta$ orientation from the ROESY correlation between H-14 with H-9$\beta$ (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 $R$.

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**References**

[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; $[\alpha]_D^{22.7} = -18.18$ (c 0.55, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 244 (3.83) nm; IR (KBr) $v_{max}$ 2926, 1714, 1678 cm$^{-1}$; HR-ESI-MS [M+Na]$^+$ m/z: 259.1311 (calcd. for C$_{14}$H$_{20}$O$_3$Na$,^+$, 259.1310); $^1$H and $^{13}$C NMR, see Table 1.
[11] Oxyphyllone B (2a/2b): colorless oil; $[\alpha]_D^{27.3} = -13.64$ (c 0.11, CHCl$_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 238 (4.02) nm; IR (KBr) $v_{max}$ 3436, 2959, 2934, 1714, 1668 cm$^{-1}$; HR-ESI-MS [M+Na]$^+$ m/z: 273.1462 (calcd. for C$_{15}$H$_{22}$O$_3$Na$,^+$, 273.1466). $^1$H and $^{13}$C NMR, see Table 1.