

Inhibitory activity of eudesmane sesquiterpenes from *Alpinia oxyphylla* on production of nitric oxide

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ABSTRACT

Sixteen eudesmane-type sesquiterpenes including seven new compounds oxyphyllanene A–G (**1–2** and **5–9**) were isolated from the fruits of *Alpinia oxyphylla*. Among them, compounds **1–2** are novel trinoreudesmane sesquiterpenes, and **5** is a noreudesmane one. Their structures were established by spectroscopic analysis, including 2D-NMR techniques. Inhibitory activity of compounds **3–8** and **10–16** were tested against nitric oxide production in LPS and IFN- γ -induced RAW 264.7 murine macrophages, and their IC₅₀ values ranged from 9.85 to 13.95 μ g/ml.

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The genus of *Alpinia* (Zingiberaceae) comprising ca. 250 species, is widely distributed in subtropical parts of many countries, and about 48 species grows in south of China.^{1,2} The plants of this genus are reported to be rich of diarylheptanoids, sesquiterpenes, diterpenes and phenolics.^{3–7} *Alpinia oxyphylla* Miq. is used as a traditional Chinese medicine for curing intestinal disorders, diuresis, uresis, ulceration and dementia,^{8–10} which mainly contains sesquiterpenes, diterpenes, flavonoids and diarylheptanoids.^{8,9,11,12} Some of them showed inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.^{8,9} As a part of our ongoing project towards the discovery of novel biologically active metabolites from traditional Chinese medicines,^{13,14} two new trinoreudesmane sesquiterpenes oxyphyllanene A (**1**, 0.00027%), B (**2**, 0.00019%), a new noreudesmane sesquiterpene oxyphyllanene C (**5**, 0.000053%), and four new eudesmane sesquiterpenes oxyphyllanene D (**6**, 0.00013%), E (**7**, 0.00036%), F (**8**, 0.000033%), G (**9**, 0.000047%), together with nine known eudesmane sesquiterpenes, oxyphyllanone A (**3**),⁸ teuhetenone A (**4**),¹⁵ teucrone (**10**),¹⁶ 7-*epi*-teucrone B (**11**),¹⁵ (4a*S*,7*S*,8*R*)-8-hydroxy-1,4a-dimethyl-7-(prop-1-en-2-yl)-4,4a,5,6,7,8-hexahydronaphthalen-2(3*H*)-one (**12**),¹⁷ (\pm)1 β , 4 β -dihydroxyeudesman-11-ene (**13**),¹⁸ (4a*S*,7*S*)-7-hydroxy-1,4a-dimethyl-7-(prop-1-en-2-yl)-4,4a,5,6,7,8-hexahydronaphthalen-2(3*H*)-one (**14**),¹⁷ ligucyperonol (**15**),^{19,20} 7 α (H), 10 β -eudesm-4-en-3-one-11,12-diol (**16**),^{21,22} were iso-

lated from the fruits of *A. oxyphylla*.²³ Additionally, the inhibitory effects of compounds **3–8** and **10–16** on NO production in LPS and IFN- γ -induced RAW 264.7 murine macrophages were measured. This paper deals with structural elucidation of new compounds and results of inhibitory activity against NO production.

Oxyphyllanene A (**1**)²⁴ was obtained as a colorless oil and exhibited the molecular formula C₁₂H₁₆O₂ with five degrees of unsaturation by HRESIMS (*m/z* 193.1233 [M+H]⁺). The ¹H NMR spectrum exhibited signals due to two tertiary methyls at δ_{H} 1.05 and 1.84, an olefinic proton at δ_{H} 5.88. Analysis of the ¹³C NMR and HSQC data revealed that **1** contains four quaternary carbons including an olefinic and two carbonyl ones, two methines including an olefinic one, four methylenes, and two methyls. These data were consistent with the HRESIMS empirical formula and suggested that **1** was probably an 11, 12, 13 trinoreudesmane-type sesquiterpenoid,⁸ which was further supported by ¹H–¹H COSY correlations between 5-H and 6-H, 8-H and 9-H, and HMBC correlations between 14-H₃ and C-1, C-5, C-9, C-10, 15-H₃ and C-4, C-5, 1-H₂ and C-3, C-5, 6-H₂, 8-H₂ and C-7 (Fig. 2). Since NOE interactions between the proton signals of 1 β -H, 6 β -H, 8 β -H, 9 β -H and 14-H₃, 1 α -H, 9 α -H and 5-H were observed in ROESY spectrum (Fig. 3), the relative configuration of 5-H and methyl group at C-10 should be α - and β -oriented, respectively. Therefore, compound **1** was determined as shown in Fig. 1.

Oxyphyllanene B (**2**),²⁵ a colorless oil, had the molecular formula C₁₂H₁₄O₂ as determined by positive HRESIMS (*m/z* 191.1073 [M+H]⁺). Comparison of the ¹H- and ¹³C NMR spectroscopic data with those of **1** revealed that **2** was also an 11, 12,

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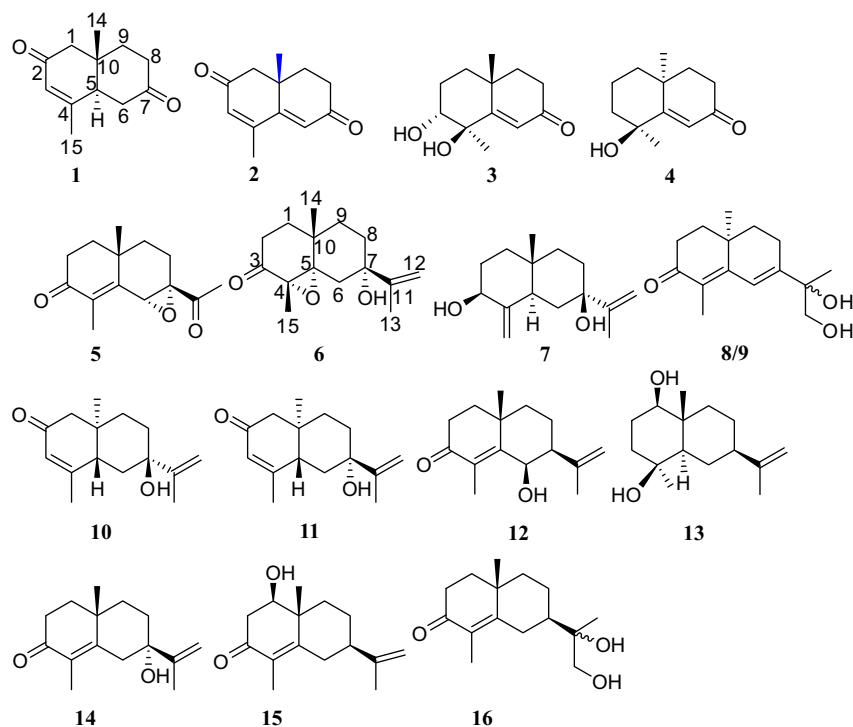


Figure 1. Structures of compounds 1–16 isolated from *A. oxyphylla*.

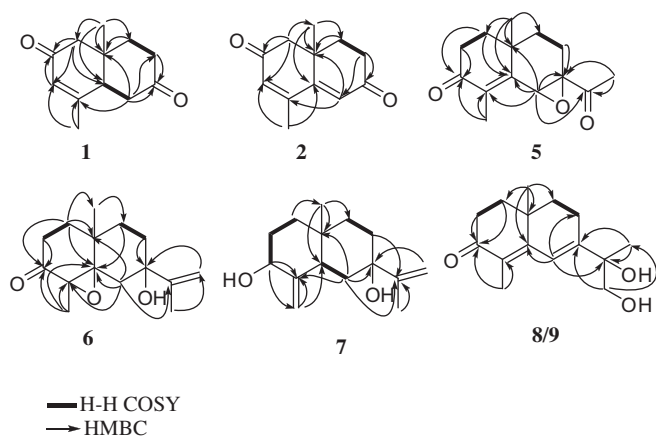


Figure 2. ^1H – ^1H COSY and key HMBC correlations for compounds 1–2 and 5–9.

13-trinoreudesmane-type sesquiterpenoid except for the presence of a double bond [158.7 (s), 126.4 (d)] between C-5 and C-6 in **2**, which was in agreement with HMBC correlations between 6-H and C-4, C-10 (Fig. 2). Small coupling constants ($J = 2.5, 5.1$ Hz) of the proton singlet at $\delta_{\text{H}} 1.95$ indicated that $9\beta\text{-H}$ was equatorially oriented,⁸ and NOE correlations of 14- H_3 with $9\beta\text{-H}$ and $8\beta\text{-H}$ [2.60 (1H, m)] suggested that the methyl group at C-10 was β -orientated (Fig. 3). Here, the structure of **2** was defined (Fig. 1).

Oxyphyllanene C (**5**)²⁶ was obtained as a colorless oil with the formula $\text{C}_{14}\text{H}_{18}\text{O}_3$, in agreement with HRESIMS (m/z 257.1155 $[\text{M}+\text{Na}]^+$). Analysis of the ^{13}C NMR and HSQC data established that **5** contains two carbonyl carbons ($\delta_{\text{C}} 198.4, 206.9$), four quaternary carbons including two olefinic ones ($\delta_{\text{C}} 137.0, 151.1$) and an oxygenated one ($\delta_{\text{C}} 63.0$), an oxygenated methine ($\delta_{\text{C}} 53.7$), four methylenes, three methyls. From ^1H – ^1H COSY spectrum of **5** (Fig. 2), it was possible to establish two proton sequences from 1-H to 2-H

and 8-H to 9-H. The location of epoxide group between C-6 and C-7 was assigned by HMBC correlations from 6-H to C-4, C-5, C-7, C-11, 8- H_2 , 12- H_3 to C-7 (Fig. 2). Moreover, ROESY correlations ($1\beta\text{-H}$, $9\beta\text{-H}$ and 14- H_3 ; $8\beta\text{-H}$ and $9\beta\text{-H}$, 12- H_3 ; 6-H and 15- H_3 , 12- H_3) indicated that the epoxy ring and 10- CH_3 were α - and β -orientated, respectively (Fig. 3). Thus, compound **6** had the structure as shown in Fig. 1.

Oxyphyllanene D (**6**)²⁷ a colorless oil, exhibited a quasi-molecular ion peak at m/z 273.1463 $[\text{M}+\text{Na}]^+$ in its HRESIMS, corresponding to $\text{C}_{15}\text{H}_{22}\text{O}_3$, establishing five degrees of unsaturation. The ^1H NMR spectrum showed signals assignable to three tertiary methyls [$\delta_{\text{H}} 1.82, 1.04, 1.36$ (each s, 13, 14, 15- H_3)], six methylenes including an olefinic methylene [$\delta_{\text{H}} 5.07, 4.86$ (both s, 12- H_2)]. ^{13}C NMR and HSQC data indicated the presence of a carbonyl carbon ($\delta_{\text{C}} 207.6$), five quaternary carbons including an olefinic carbon ($\delta_{\text{C}} 150.4$) and three oxygenated ones ($\delta_{\text{C}} 63.2, 70.8, 74.5$). The ^1H – ^1H COSY experiment revealed two spin systems corresponding to H-1/H-2 and H-8/H-9. In addition, the HMBC experiment showed the correlations between 1- H_2 and C-3, C-5, C-10, C-14, 15- H_3 and C-3, C-4, 6- H_2 and C-4, C-5, C-10, C-11, 8- H_2 and C-7, 9- H_2 and C-5, 14- H_3 and C-9 (Fig. 2). Particularly, the epoxy ring located between C-4 and C-5 was confirmed by the correlations of 15- H_3 , 6- H_2 with C-4 and C-5 (Fig. 2). Hence, the data suggested that **6** was an oxygenated eudesmane-type sesquiterpenoid with an epoxy ring. ROESY correlations (Fig. 3) of $6\beta\text{-H}$ with 13- H_3 , 14- H_3 , 15- H_3 determined 4,10- CH_3 to be β -orientated, and 7-OH to be α -orientated, respectively. Thus, the structure of **6** was established (Fig. 1).

Oxyphyllanene E (**7**)²⁸ was obtained as a colorless oil. A molecular formula of $\text{C}_{15}\text{H}_{24}\text{O}_2$ (m/z 259.1301 $[\text{M}+\text{Na}]^+$) for **7** was established from HRESIMS data, and thus requiring four degrees of unsaturation. By analysis of ^{13}C NMR spectra, the carbon signals were assigned into two methyls, seven methylenes including two olefinic ones ($\delta_{\text{C}} 113.3, 108.9$), two methines including an oxygenated one ($\delta_{\text{C}} 73.0$), and four quaternary carbons including an oxygenated one ($\delta_{\text{C}} 74.8$) and two olefinic ones ($\delta_{\text{C}} 151.3, 146.5$). By

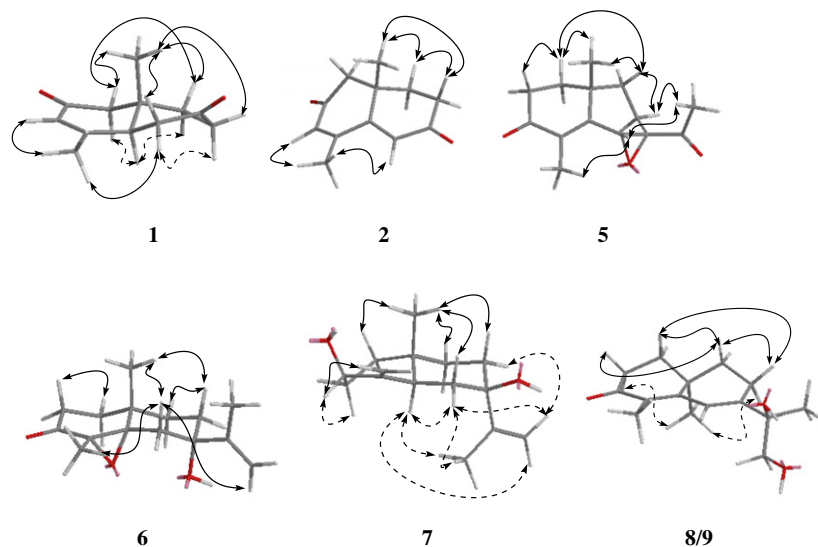


Figure 3. Key ROESY correlations for compounds 1–2 and 5–9.

Table 1
¹³C NMR data for compounds 1–2 and 5–9

Position	1 ^a	2 ^b	5 ^b	6 ^b	7 ^b	8 ^b	9 ^b
C-1	52.7	51.5	35.8	31.5	35.2	36.9	37.1
C-2	197.9	197.5	33.9	33.2	29.5	33.8	33.9
C-3	127.0	131.2	198.4	207.6	73.0	199.6	199.7
C-4	160.0	150.2	137.0	63.2	151.3	128.0	127.8
C-5	46.5	158.7	151.1	70.8	40.4	154.9	155.4
C-6	39.7	126.4	53.7	35.8	33.7	119.8	119.5
C-7	209.2	199.1	63.0	74.5	74.8	151.3	152.3
C-8	37.3	33.7	18.4	30.6	31.9	22.8	22.8
C-9	38.4	36.7	31.0	32.9	38.2	36.2	36.2
C-10	36.8	37.0	32.7	33.4	35.7	32.9	33.0
C-11			206.9	150.4	146.5	75.8	75.9
C-12			24.0	110.0	113.3	23.6	24.0
C-13				18.9	18.7	68.7	68.7
C-14	16.0	24.4	21.2	20.4	15.5	21.0	21.0
C-15	21.4	19.9	10.6	11.2	108.9	10.3	10.3

^a The spectra were taken in CDCl₃ at 125 MHz.

^b The spectra were taken in CDCl₃ at 100 MHz.

the assistance of extensive 2D NMR study (COSY, HSQC, HMBC) (Fig. 2), the eudesmane skeleton of **7** was proposed (Fig. 1). Small coupling constant ($J = 2.7$ Hz) of the proton signal at $\delta_{\text{H}} 4.24$ (1H, t, 3 α -H) revealed that hydroxyl was axially oriented.⁸ (Fig. 3). The large coupling constant ($J = 13.0$ Hz) of the signal at $\delta_{\text{H}} 2.25$ suggested that 5-H was also axially oriented.⁸ In addition, ROESY correlations (14-H₃ and 1 β -H, 9 β -H, 6 β -H; 5-H, 6 α -H, 8 α -H and 13-H₃, 12-H₂) were observed. Thus, the relative configuration of **7** was identified as shown in Fig. 1.

Oxyphyllanene F (**8**)²⁹ ($[\alpha]_{\text{D}}^{25.9} -233.33^\circ$) and G (**9**)³⁰ ($[\alpha]_{\text{D}}^{25.9} -252.38^\circ$) were identified as eudesmene sesquiterpenes with the same planar structure to pyrethroid, by comparison of their NMR data with the previously published values,^{22,31} which was further confirmed by HSQC, ¹H–¹H COSY and HMBC correlations (Fig. 2). The synthetic compound, pyrethroid with two chiral carbons was reported to occur in two isomers ($[\alpha]_{\text{D}}^{25.9} +392.48^\circ$, $+233.88^\circ$) at C-11. The different sign of optical rotation between pyrethroid with **8** and **9** suggested that compounds **8** and **9** are diastereoisomers at C-11, and the methyl group at C-10 was α -orientated.

As previous phytochemical investigation on this plant revealed several sesquiterpenes with inhibitory activity against NO production,^{8,9} compounds **3–8** and **10–16** for their effects on NO

Table 2
 Inhibitory activities of compounds **3–8** and **10–16** on NO production in LPS and IFN- γ -induced RAW 264.7 macrophages

Compounds	IC ₅₀ ($\mu\text{g/ml}$)
3	11.99
4	12.93
5	11.01
6	9.85
7	13.52
8	13.95
10	11.36
11	11.62
12	12.52
13	13.53
14	11.40
15	12.14
16	13.27
MG-132 ^a	0.17

^a Positive control for LPS and IFN- γ stimulated NO production.

production in LPS and IFN- γ -induced RAW 264.7 macrophages were tested according to the method.³² Results showed that these compounds exhibited potent inhibitory activities with IC₅₀ values from 9.85 to 13.95 $\mu\text{g/ml}$ (Table 2).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.114.

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- The fruits of *Alpinia oxyphylla* were bought from Kunming medicinal market, Kunming, Yunnan province, People's Republic of China and identified by Prof. Ning-Hua Tan. Compounds **1–16** were isolated from 70% aqueous acetone extract of the fruits of *Alpinia oxyphylla* by silica gel, MCI, RP-18 column chromatography and HPLC methods.
- Oxyphyllanene A (1)**: Colorless oil; $C_{12}H_{16}O_2$; $[\alpha]_D^{22.9} -56.25^\circ$ (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 239 (4.0); IR (KBr) ν_{max} 2926, 1710, 1657 cm^{-1} ; HRESIMS m/z 193.1233 $[M+H]^+$ (calcd for $C_{12}H_{17}O_2$, 193.1228). 1H NMR [$CDCl_3$, 500 MHz]: δ 2.19 (d, $J = 15.6$ Hz, 1 α -H), 2.34 (d, $J = 15.6$ Hz, 1 β -H), 5.88 (1H, s, 3-H), 2.69 (1H, m, 5-H), 2.64 (1H, dd, $J = 1.7$, 14.5 Hz, 6 α -H), 2.28 (1H, t, $J = 14.5$ Hz, 6 β -H), 2.39 (1H, 8 α -H), 2.47 (1H, m, 8 β -H), 1.72 (1H, m, 9 α -H), 1.79 (1H, m, 9 β -H), 1.05 (3H, s, 14-H₃), 1.84 (3H, s, 15-H₃). ^{13}C NMR data, see Table 1.
- Oxyphyllanene B (2)**: Colorless oil; $C_{12}H_{14}O_2$; $[\alpha]_D^{22.3} +292.00^\circ$ (c 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 288 (4.35); IR (KBr) ν_{max} 3424, 2927, 1669 cm^{-1} ; FABMS m/z 191 (6) $[M+H]^+$, 147 (22), 97 (32), 83 (53), 69 (82), 55 (100); HRESIMS m/z 191.1073 $[M+H]^+$ (calcd for $C_{12}H_{15}O_2$, 191.1072); 1H NMR [$CDCl_3$, 400 MHz]: δ 2.45 (1H, d, $J = 17.0$ Hz, 1 α -H), 2.50 (1H, d, $J = 17.0$ Hz, 1 β -H), 6.15 (1H, s, 3-H), 6.22 (1H, s, 6-H), 2.55 (1H, m, 8 α -H), 2.60 (1H, m, 8 β -H), 2.11 (1H, ddd, $J = 5.5$, 13.5, 14.0 Hz, 9 α -H), 1.95 (1H, ddd, $J = 2.5$, 5.1, 13.5 Hz, 9 β -H), 1.32 (3H, s, 14-H₃), 2.12 (3H, s, 15-H₃). ^{13}C NMR data, see Table 1.
- Oxyphyllanene C (5)**: Colorless oil; $C_{14}H_{18}O_3$; $[\alpha]_D^{27.5} -105.00^\circ$ (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 248 (4.07); IR (KBr) ν_{max} 3442, 2960, 2928, 1710, 1672 cm^{-1} ; HRESIMS m/z 257.1155 $[M+Na]^+$ (calcd for $C_{14}H_{18}O_3Na$, 257.1153); 1H NMR [$CDCl_3$, 400 MHz]: δ 1.80 (1H, dt, $J = 4.9$, 14.3 Hz, 1 α -H), 1.71 (1H, ddd, $J = 2.0$, 5.6, 14.3 Hz, 1 β -H), 2.49 (1H, ddd, $J = 2.2$, 4.9, 17.8 Hz, 2 α -H), 2.65 (1H, m, 2 β -H), 3.78 (1H, s, 6-H), 1.93 (1H, ddd, $J = 2.4$, 4.6, 14.5 Hz, 8 α -H), 2.60 (1H, m, 8 β -H), 1.39 (1H, m, 9 α -H), 1.37 (1H, m, 9 β -H), 2.17 (3H, s, 12-H₃), 1.08 (3H, s, 14-H₃), 1.97 (3H, s, 15-H₃). ^{13}C NMR data, see Table 1.
- Oxyphyllanene D (6)**: Colorless oil; $C_{15}H_{22}O_3$; $[\alpha]_D^{23.2} +27.03^\circ$ (c 0.19, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.18); IR (KBr) ν_{max} 3464, 2937, 2960, 1702 cm^{-1} ; FABMS m/z 251 (15) $[M+H]^+$, 233 (100); HRESIMS m/z 273.1463 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1466); 1H NMR [$CDCl_3$, 400 MHz]: δ 2.04 (1H, m, 1 α -H), 1.29 (1H, dd, $J = 7.8$, 13.5 Hz, 1 β -H), 2.44 (1H, ddd, $J = 1.2$, 8.7, 19.5 Hz, 2 α -H), 2.34 (1H, ddd, $J = 7.8$, 11.6, 19.5 Hz, 2 β -H), 1.43 (1H, br d, $J = 14.8$ Hz, 6 α -H), 2.33 (1H, br d, $J = 14.8$ Hz, 6 β -H), 1.90 (1H, td, $J = 4.0$, 13.9 Hz, 8 β -H), 1.65 (1H, ddd, $J = 1.4$, 6.4, 13.9 Hz, 8 α -H), 1.45 (1H, dt, $J = 1.4$, 13.9 Hz, 9 β -H), 2.02 (1H, m, 9 α -H), 5.07, 4.86 (2H, both s, 12-H₂), 1.82 (3H, s, 13-H₃), 1.04 (3H, s, 14-H₃), 1.36 (3H, s, 15-H₃). ^{13}C NMR data, see Table 1.
- Oxyphyllanene E (7)**: Colorless oil; $C_{15}H_{24}O_2$; $[\alpha]_D^{27.1} +2.15^\circ$ (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (3.28); IR (KBr) ν_{max} 3418, 2958, 1644 cm^{-1} ; positive FABMS m/z 237 (2) $[M+H]^+$, 219 (45), 201 (100); HRESIMS m/z 259.1301 $[M+Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 193.1228); 1H NMR [$CDCl_3$, 400 MHz]: δ 1.58 (1H, td, $J = 5.1$, 13.1 Hz, 1 α -H), 1.21 (1H, m, 1 β -H), 1.76 (2H, m, 2-H₂), 4.24 (1H, t, $J = 2.7$ Hz, 3 α -H), 2.25 (1H, dd, $J = 1.5$, 13.0 Hz, 5 α -H), 1.90 (1H, m, 6 α -H), 1.47 (1H, t, $J = 13.0$ Hz, 6 β -H), 1.99 (ddd, $J = 3.1$, 6.3, 13.6 Hz, 8 α -H), 1.72 (1H, m, 8 β -H), 1.29 (1H, m, 9 α -H), 1.37 (1H, m, 9 β -H), 5.01, 4.99 (2H, both br s, 12-H₂), 1.76 (3H, s, 13-H₃), 0.74 (3H, s, 14-H₃), 4.92, 4.59 (2H, both br s, 15-H₂). ^{13}C NMR data, see Table 1.
- Oxyphyllanene F (8)**: Colorless oil; $C_{15}H_{22}O_3$; $[\alpha]_D^{25.9} -233.33^\circ$ (c 0.36, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 298 (3.74); positive FABMS m/z 251 (100) $[M+H]^+$; 1H NMR [$CDCl_3$, 400 MHz]: δ 1.52 (1H, m, 1 α -H), 1.64 (1H, m, 1 β -H), 2.65 (1H, m, 2 α -H), 2.46 (1H, m, 2 β -H), 6.70 (1H, d, $J = 2.2$ Hz, 6-H), 2.22 (1H, m, 8 β -H), 2.34 (1H, m, 8 α -H), 1.79 (1H, m, 9 β -H), 1.73 (1H, m, 9 α -H), 1.35 (3H, s, 12-H₃), 3.71, 3.54 (each 1H, both d, $J = 11.0$ Hz, 13-H₂), 1.09 (3H, s, 14-H₃), 1.84 (3H, s, 15-H₃).
- Oxyphyllanene G (9)**: Colorless oil; $C_{15}H_{22}O_3$; $[\alpha]_D^{25.9} -252.38^\circ$ (c 0.36, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 298 (4.12); positive FABMS m/z 251 (100) $[M+H]^+$; 1H NMR [$CDCl_3$, 400 MHz]: δ 1.52 (1H, m, 1 α -H), 1.63 (1H, m, 1 β -H), 2.63 (1H, m, 2 α -H), 2.44 (1H, m, 2 β -H), 6.72 (1H, d, $J = 2.2$ Hz, 6-H), 2.23 (1H, m, 8 β -H), 2.33 (1H, m, 8 α -H), 1.78 (1H, m, 9 β -H), 1.72 (1H, m, 9 α -H), 1.34 (3H, s, 12-H₃), 3.73, 3.53 (each 1H, both d, $J = 11.0$ Hz, 13-H₂), 1.07 (3H, s, 14-H₃), 1.82 (3H, s, 15-H₃). ^{13}C NMR data, see Table 1.
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