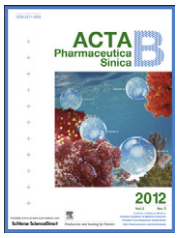
Acta Pharmaceutica Sinica B 2012;2(3):256–259

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

A new pimarane-type diterpenoid from moss *Pseudoleskeella papillosa* (Lindb.) Kindb

Na Liu, Song Wang, Hongxiang Lou*

Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China

Received 8 February 2012; revised 2 March 2012; accepted 7 March 2012

KEY WORDS

Moss;
Pseudoleskeella papillosa (Lindb.) Kindb;
Pimarane-type diterpenoid

Abstract A new pimarane-type diterpenoid, momilactone C (**1**), together with one known compound, momilactone A (**2**), was isolated from moss *Pseudoleskeella papillosa* by column chromatography on silica gel, Sephadex LH-20 and semi-preparative HPLC. Their structures were established on the basis of HR–ESI–MS, 1D and 2D NMR spectroscopic methods. Allelopathic test showed that the two diterpenoids inhibited germination of the *Arabidopsis thaliana* seeds.

*Corresponding author. Tel.: +86 531 88382012; fax: +86 531 88382548.
E-mail address: louhongxiang@sdu.edu.cn (Hongxiang Lou).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
http://dx.doi.org/10.1016/j.apsb.2012.03.003

Production and hosting by Elsevier

1. Introduction

Bryophytes, the second largest group of land plants, are morphologically classified into liverworts (Hepaticae), mosses (Musci), and hornworts (Anthocerotae)[1]. A number of bryophytes, in particular, mosses have been widely used as medicinal plants in China, to cure burns, bruises, external wounds, snake bite, etc[2]. Over the last few decades, investigation of the chemical constituents of bryophytes has focused mainly on liverworts, which contain oil bodies in their cells and produce mono-, sesqui- and di-terpenoids with a variety of carbon skeletons and aromatic compounds as major constituents[3]. On the contrary, most of the review articles on the chemistry of bryophytes emphasized the absence or only scattered occurrence of terpenoid compounds, particularly of sesquiterpenes, in mosses[4]. Previously, Nozaki et al.[5] reported the isolation and structural elucidation of momilactones A and B from the moss *Hypnum plumaeforme*, which showed the inhibitory activities toward the growth of angiosperms, liverworts and mosses. Here we report the isolation of momilactone A (**2**), and the structural elucidation of a new pimarane-type diterpenoid momilactone C (**1**) (Fig. 1) from the moss *Pseudoleskeella papillosa* (Lindb.) Kindb, a species which has not yet been investigated phytochemically[6]. Allelopathic test showed that the two isolates retarded *Arabidopsis thaliana* seeds germination.

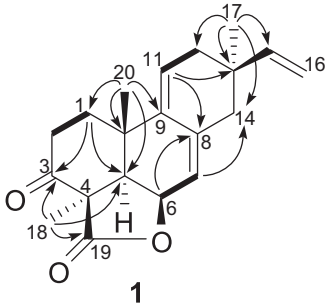
2. Results and discussion

Momilactone C (**1**), colorless needles, presented a molecular formula of $C_{20}H_{24}O_3$ as determined by the HR–ESI–MS at *m*/*z* 330.2065 $[M+NH_4]^+$ (calcd. 330.2064) requiring 9° of unsaturation. The IR spectrum revealed the strong absorption bands at 1766 cm^{-1} (γ-lactone) and 1621 cm^{-1} (carbonyl). The ^1H NMR spectrum (Table 1) displayed signals characteristic for vinylic protons at δ_H 5.86 (dd, J=17.4, 10.7 Hz, H-15), 4.99 (d, J=17.4 Hz, H-16a), and 5.02 (d, J=10.7 Hz, H-16b), one oxygenated methine at δ_H 4.94 (t, J=5.0 Hz, H-6), as well as three tertiary methyls at δ_H 0.96 (s, H-17), 1.54 (s, H-18), and 1.04 (s, H-20). Additionally, resonances for two trisubstituted olefinic protons at δ_H 5.76 (d, J=5.0 Hz, H-7) and 5.64 (s, H-11) were observed. The signals of 3 methyls, 5 methylenes (one olefinic), 5 methines (one oxygenated and two olefinic ones), and 7 quarternary carbons (two olefinic ones and two carbonyls) in the ^{13}C NMR spectrum and 2D NMR data revealed a pimarane-type diterpenoid skeleton with γ-lactone between C-19 and C-6 for **1** (Table 1). The ^1H NMR and ^{13}C NMR spectrum (Table 1) characters were very similar to the known compound **2,** except for the presence of the $\Delta^{9,11}$ double bond in **1**. The spin system of CH(11)−CH_2(12) deduced from the ^1H–^1H COSY spectrum together with the HMBC correlations of H-11/C-8, H-11/C-13 and H_3-20/C-9 (Fig. 2) confirmed the position of the olefinic bond.

Table 1 ^1H and ^{13}C NMR, and HMBC data of **1** in $CDCl_3$[a] (δ in ppm and J in Hz).

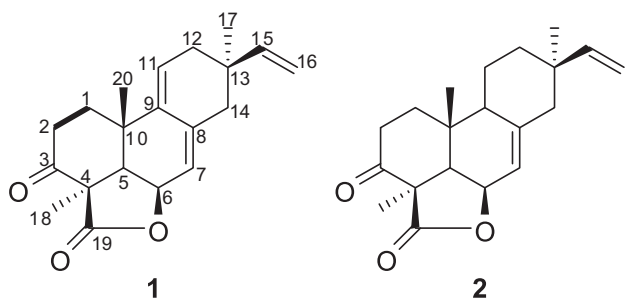
Position	δ_C	δ_H	HMBC (H–C)
1α	31.0	1.95 m	C-2, 3, 5, 9, 10, 20
1β		2.09 m	C-2, 3, 5, 9, 10, 20
2	34.4	2.67 m	C-1, 3, 4, 10
3	205.2		
4	53.2		
5	50.4	2.37 d (5.0)	C-1, 4, 9, 10, 18, 19, 20
6	72.7	4.94 t (5.0)	C-7, 8, 10, 19
7	115.6	5.76 d (5.0)	C-5, 14, 9
8	139.4		
9	139.3		
10	34.1		
11	121.7	5.64 s	C-8, 9, 10, 12, 13
12α	38.1	2.06 m	C-9, 11, 13, 15, 17
12β		2.29 d (18.3)	C-9, 11, 13, 14, 15, 17
13	36.5		
14α	42.2	2.12 d (13.7)	C-7, 8, 9, 13, 15, 17
14β		2.40 d (13.7)	C-7, 8, 9, 12, 13, 15, 17
15	147.6	5.86 dd (10.7, 17.4)	C-12, 13, 14, 17
16a	110.9	4.99 d (17.4)	C-13, 15
16b		5.02 d (10.7)	C-13, 15
17	23.4	0.96 s	C-12, 13, 14, 15
18	21.3	1.54 s	C-3, 4, 5, 19
19	173.9		
20	21.9	1.04 s	C-1, 5, 9, 10

[a]600 and 150 MHz for ^1H and ^{13}C NMR, respectively.



1

Figure 2 Key $^1H-^1H$ COSY (——) and HMBC (H→C) correlations of **1**.



1 **2**

Figure 1 Structures of compounds **1** and **2**.

The relative configuration of **1** was elucidated by NOESY experiments and the coupling constants. In the NOESY spectrum, the observed correlations of H-20/H-1β, H-1α/H-5, H-5/H-6, H-6/H-18, H-17/H-12α and H-17/H-14α, indicated the same stereostructure as **2**. The α-orientation of H-6 was further confirmed by the small vicinal coupling constant (J=5.0 Hz) detected from the H-6 signal at δ_H 4.94 (t, J=5.0 Hz)[7]. Optical rotation studies confirmed that the absolute stereochemistry of **1** was identical to that of **2**[8]. Additionally, from a biosynthetic standpoint, their absolute configurations

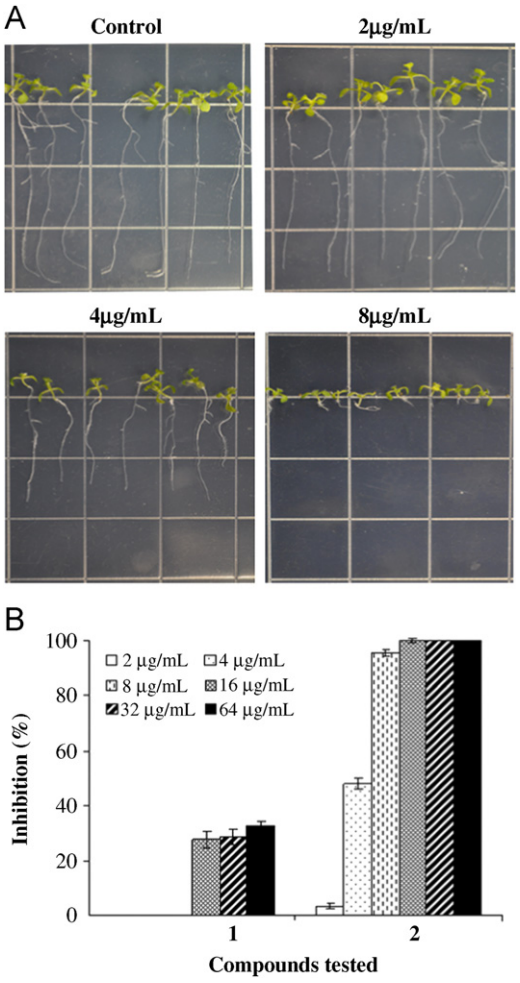


Figure 3 Effects of compounds on seedling growth of *A. thaliana*: (A) The growth of *A. thaliana* on Petri dishes with different concentrations of compound **2**: 0, 2, 4 and 8 μg/mL. The same volume of DMSO was used as blank control. (B) The inhibition of different concentrations of compounds on *A. thaliana* root growth.

will be the same. Thus, the structure of **1** was established as shown. The known compound **2** was identified as Momilactone A on the basis of comparison of its ^{1}H and ^{13}C NMR spectroscopic data with the reported[5].

Allelopathic potential of the compounds from *P. papillosa* was tested with the model of *A. thaliana* germination. The root growth of *A. thaliana* was inhibited after the treatment of the two compounds in a dose dependent manner (Fig. 3A). The inhibition of compounds **1** and **2** in different concentrations as shown in Fig. 3B was calculated by the equation described by Fan et al.[9]. Results showed that compound **2** significantly retarded the *Arabidopsis* seeds germination with IC_{50} (the effective concentration to afford 50% inhibitions) value of 4.3 μg/mL. Compound **1** was found to display weak inhibitory activity.

3. Experimental

3.1. General experimental procedures

Melting points were measured on an X-6 micromelting point apparatus (uncorrected). Optical rotations were performed on a GYROMAT-HP polarimeter. CD spectra were acquired on a Chirascan spectropolarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer, whereas IR spectra were acquired on a Thermo Nicolet NEXUS 470 FT-IR spectrometer with KBr discs. NMR spectra were measured in $CDCl_3$ on a Bruker Avance DRX-600 spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz, respectively, with TMS as internal standard. HR–ESI–MS and ESI–MS were determined using a LTQ-Orbitrap XL and an API 4000 triple-stage quadrupole instrument, respectively. All solvents used were of analytical grade. Column chromatography (CC) was run on Silica gel (200−300 mesh, Haiyang Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 (25–100 μm; Pharmacia Biotek, Denmark), or MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.). Semi-preparative HPLC was performed on an Agilent 1100 G1310A isopump equipped with a G1322A degasser, a G1314A VWD detector (210 nm) and a Phenomenex Luna 5 μm C18(2) column (250 mm × 4.60 mm). Fractions were monitored by TLC, and spots were visualized under UV (254 nm) light and by heating silica gel plates which were sprayed with 10% H_2SO_4 in EtOH.

3.2. Plant materials

P. papillosa (Lindb.) Kindb, collected in July 2006 from Jiaozixueshan of Yunnan Province, China, was authenticated by Prof. Yuanxin Xiong (College of Life Sciences, Guizhou University, China). A voucher specimen (No. 20060734) has been deposited at the Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, China.

3.3. Extraction and isolation

The air-dried plant materials of *P. papillosa* (243 g) was powdered and extracted with 90% EtOH (1L × 3, each for 1 week) at room temperature. The crude extract (21.9 g) was subsequently partitioned with Et_2O (3 × 100 mL) and *n*-BuOH (3 × 100 mL). The Et_2O extract (7.7 g) was fractionated on MCI gel column chromatography (MeOH/H_2O, 4:6–9:1) to give three fractions. Fraction B, eluted with petroleum ether/acetone (20:1), was further purified with Sephadex LH-20 ($CHCl_3$/MeOH) column chromatography and semi-preparative HPLC (70% MeOH/H_2O, 0.8 mL/min) to afford compound **1** (17.5 mg). Fraction C, eluted with petroleum ether/acetone (25:1), was further purified with silica gel column chromatography and semi-preparative HPLC (75% MeOH/H_2O, 0.8 mL/min) to afford compound **2** (2.8 mg).

3.4. Seedling growth test

Seeds of *A. thaliana* were surface sterilized using 5% sodium hypochlorite for 5 min, followed by five washes with sterile distilled water. Compounds **1** and **2** were dissolved with DMSO and diluted with two-fold distilled water to final concentration of 1 mg/mL. Then was added to 25 mL 1/2 MS medium supplemented with 0.8% (*w*/*v*) agar to get plates with different concentrations of compounds (0, 2, 4, 8, 16, 32, 64 μg/mL). To eliminate the effect of DMSO on the growth of *A. thaliana*, plates with DMSO (the concentration of DMSO was equivalent to plates with compounds of 64 μg/mL) were

used as blank control. 15 seeds were distributed on each Petri dishes described before. Three replicates were done for each concentration. The Petri dishes were placed in a growth chamber at 23 ± 1 °C under 18 h of light and 6 h of darkness. The lengths of seedling roots were measured after 7 days. The percentage of growth inhibition of root lengths was calculated from the following equation:

$$I(\%) = [1 - T/C] \times 100\%$$

where T is the average length of treatment (cm) and C is the average length of control (cm).

3.5. Structure elucidation

Momilactone C (**1**): Colorless crystal ($CHCl_3$); $[\alpha]_D^{20}$ −41.07 (*c* 0.144, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193 (+1.85), 241 (−1.40) nm; UV (MeOH) λ_{max} (log ε) 240 (1.51) nm; IR (KBr) ν_{max} 3402, 2961, 1766, 1621, 1457, 1374, 1123 cm^{-1}; $C_{20}H_{24}O_3$; positive ESI–MS *m/z* 330.2 $[M+NH_4]^+$; positive HR–ESI–MS *m/z*: 330.2065 $[M+NH_4]^+$ (calcd. for $C_{20}H_{28}O_3$ *m/z* 330.2064); ^1H and ^{13}C NMR see Table 1.

Momilactone A (**2**): Colorless solid ($CHCl_3$); $C_{20}H_{26}O_3$; ^1H NMR (600 MHz, $CDCl_3$) δ_H: 2.82 (1H, d, *J* = 5.0 Hz, H-5), 4.87 (1H, t, *J* = 5.0 Hz, H-6), 5.73 (1H, d, *J* = 5.0 Hz, H-7), 2.08 and 2.24 (2H, d, *J* = 12.5 Hz, H-14), 5.87 (1H, dd, *J* = 17.4, 10.7 Hz, H-15), 4.97 (1H, d, *J* = 17.4 Hz, H-16), and 5.01 (1H, d, *J* = 10.7 Hz, H-16), 0.92 (3H, s, H-17), 1.55 (3H, s, H-18), 1.02 (3H, s, H-20). ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 34.9 (C-1), 31.2 (C-2), 204.9 (C-3), 53.5 (C-4), 46.5 (C-5), 73.1 (C-6), 114.1 (C-7), 148.0 (C-8), 50.2 (C-9), 32.4 (C-10), 24.0 (C-11), 37.2 (C-12), 40.1 (C-13), 47.5 (C-14), 149.0 (C-15), 110.2 (C-16), 22.0 (C-17), 21.5 (C-18), 174.3 (C-19), 21.8 (C-20); ESI–MS (int. rel) *m/z*: 315.3 $[M+H]^+$, 332.3 $[M+NH_4]^+$.

Acknowledgments

Financial supports from the National Natural Science Foundation of China (nos. 30730109 and 30925038) are gratefully acknowledged.

References

1. Toyota M, Masuda K, Asakawa Y. Triterpenoid constituents of the moss *Floribundaria aurea* subsp. *Nipponica*. *Phytochemistry* 1998;**48**: 297–9.
2. Wang XN, Yu WT, Lou HX. Antifungal constituents from the Chinese moss *Homalia trichomanoides*. *Chem Biodivers* 2005;**2**:139–45.
3. Freitag P, Mues R, Brill-Fess C, Stoll M, Zinsmeister HD, Markham KR. Isoorientin 3′-*O*-sophoroside and 3′-*O*-neohesperidoside from the moss *Plagiomnium affine*. *Phytochemistry* 1986;**25**:669–71.
4. Saritas Y, Sonwa MM, Iznaguen H, König WA, Muhle H, Mues R. Volatile constituents in mosses (Musci). *Phytochemistry* 2001;**57**: 443–57.
5. Nozaki H, Hayashi K, Nishimura N, Kawaide H, Matsuo A, Takaoka D. Momilactone A and B as allelochemicals from moss *Hypnum plumaeforme*: first occurrence in bryophytes. *Biosci Biotechnol Biochem* 2007;**71**:3127–30.
6. Xie CF, Lou HX. Secondary metabolites in bryophytes: an ecological aspect. *Chem Biodivers* 2009;**6**:303–12.
7. Thongnest S, Mahidol C, Sutthivaiyakit S, Ruchirawat S. Oxygenated pimarane diterpenes from *Kaempferia marginata*. *J Nat Prod* 2005;**68**:1632–6.
8. Kato T, Kabuto C, Sasaki N, Tsunagawa M, Aizawa H, Fujita K, et al. Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedron Lett* 1973;**39**:3861–4.
9. Fan PH, Hostettmann K, Lou HX. Allelochemicals of the invasive neophyte *Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae). *Chemoecology* 2010;**20**:223–7.