Biochemical Systematics and Ecology 57 (2014) 238–241

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

A new macrolide and six cycloartane triterpenoids from the tubers of *Bletilla striata*

Lian Yang [a, b], Cheng Peng [a, b, **], Chun-Wang Meng [b], Cheng-Jun He [a, b], Xiao-Hong Li [a, b], Li Guo [a, b], Qin-Mei Zhou [a, b], Liang Xiong [a, b, *]

[a] State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, PR China
[b] School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, PR China

ARTICLE INFO

Article history:
Received 24 June 2014
Accepted 24 August 2014
Available online

Keywords:
Bletilla striata
Orchidaceae
Macrolide
Cycloartane triterpenoids
Chemotaxonomy

ABSTRACT

A new 12-membered macrolide (**1**) along with two known C-31 and four known C-32 cycloartane triterpenoids (**2**–**7**) were isolated from the tubers of *Bletilla striata*. Their structures were determined by spectroscopic analysis, including 1D NMR, 2D NMR, and HRESIMS. The chemotaxonomic significance of these isolates, especially C-31 and C-32 cycloartane triterpenoids, are discussed. To our knowledge, this is the first report on the co-occurrence of the unusual 24-methyl, 24-ethyl, and 24,24-dimethyl cycloartane triterpenoids in the family Orchidaceae.

1. Subject and source

Bletilla striata (Thunb.) Rchb.f. (Orchidaceae) is one of the five species in the genus *Bletilla*. It is mainly distributed in China, especially in the areas near the Yangtze River, such as the Sichuan, Yunnan, Guizhou, Hunan, Hubei, and Anhui province. The tubers of *B. striata* are a valuable Chinese herbal medicine usually used to stop bleeding, relieve swelling, and promote tissue regeneration (Peng, 2011). Although the cultivation techniques have been researched, most of the collected *B. striata* originates from populations growing in the wild (Li et al., 2012). Thus, the resources of *B. striata* are currently limited.

The tubers of *B. striata* collected in Neijiang, Sichuan Province, P. R. China, in October 2012, were identified by Prof. Min Li (Chengdu University of Traditional Chinese Medicine, Sichuan, China). A voucher specimen (No. SBS-121023) was deposited at the School of Pharmacy, Chengdu University of Traditional Chinese Medicine.

2. Previous work

There are only five recognized species in the genus *Bletilla*: *Bletilla chartacea* (King & Pantl.) Tang & F.T.Wang, *Bletilla foliosa* (King & Pantl.) Tang & F.T.Wang, *Bletilla formosana* (Hayata) Schltr., *Bletilla ochracea* Schltr., and *B. striata* (Thunb.) Rchb.f.

* Corresponding author. School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, PR China. Tel.: +86 28 62135056.
** Corresponding author. School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, PR China. Tel.: +86 28 61800018.
E-mail addresses: pengchengchengdu@126.com (C. Peng), xiling0505@126.com (L. Xiong).

http://dx.doi.org/10.1016/j.bse.2014.08.020
0305-1978/

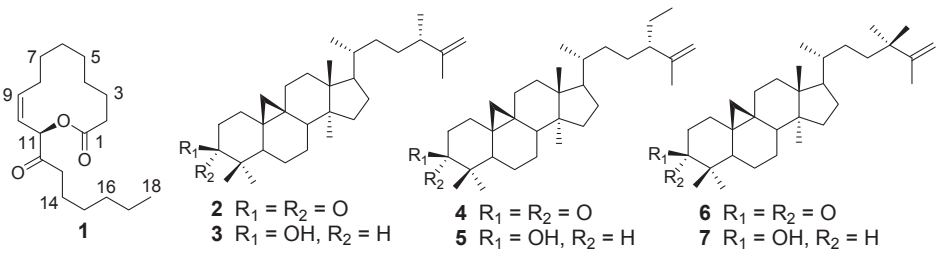

Fig. 1. The structures of the compounds **1**–**7**.

Many studies have investigated the chemical constituents of *B. striata*, resulting in the identification of nearly 80 secondary metabolites. The main types of the compounds include bibenzyls (Takagi et al., 1983; Han et al., 2002), phenanthrenes (Yamaki et al., 1990, 1993), and biphenanthrenes (Yamaki et al., 1989). Moreover, a few triterpenoids, steroidal saponins, lignans, and glucosyloxybenzyl 2-isobutylmalates have been reported for the species (Yamaki et al., 1997; Feng et al., 2008; Park et al., 2014). In addition to *B. striata*, nearly 30 compounds have been isolated from *B. formosana*, most of which belong to phenanthrenes, flavonoids, and glucosyloxybenzyl 2-isobutylmalates (Lin et al., 2005; Wu et al., 2010; Wu and Lay, 2013). Phytochemical analyses of *B. ochracea* identified more than 20 secondary metabolites including phenanthrenes, bibenzyls, glucosyloxybenzyl 2-isobutylmalates, and steroids (Cai et al., 2007; Yang et al., 2012). No phytochemical investigation of *B. chartacea* and *B. foliosa* has been reported.

3. Present study

The air-dried tubers of *Bletilla striata* (3 kg) were extracted with 95% EtOH (3 × 30 L) for 2 h under reflux. The EtOH extract was concentrated *in vacuo* to yield a semi-solid (510 g), which was then suspended in water and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether extract (37 g) was subjected to silica gel chromatography column (CC) using a gradient elution of petroleum ether-$CHCl_3$ (100:1–0:1) to afford thirteen fractions (Fr. 1–Fr. 13). Fr. 9 was further separated by Sephadex LH-20 (petroleum ether-$CHCl_3$-MeOH, 5:5:1) yielding three subfractions (Fr. 9-1–Fr. 9–3). The successive purification of Fr. 9–1 with silica gel CC (petroleum ether-diethyl ether, 2:1), PTLC (petroleum ether-$CHCl_3$, 1:2), and reversed-phase semipreparative HPLC (99.5% MeCN in H_2O) yielded **2** (7.2 mg; Ageta and Arai, 1984), **4** (6.0 mg; Ramírez-Cisneros et al., 2012), and **6** (9.7 mg; Si et al., 2005). Fr. 9–2 was purified via repeated CC (Sephadex LH-20; petroleum ether-$CHCl_3$-MeOH, 5:5:1) followed by PTLC (hexane-CH_2Cl_2, 1:3) to obtain **1** (3.0 mg). Fr. 10 was separated over Sephadex LH-20 (petroleum ether-$CHCl_3$-MeOH, 5:5:1) yielding four subfractions (Fr. 10-1–Fr. 10–4). Compound **7** (160.0 mg, Si et al., 2005) was crystallized from Fr. 10–3 and then recrystallized with Me_2CO. The mother liquor was further purified by reversed-phase semipreparative HPLC (99.5% MeCN in H_2O) to yield **3** (11.4 mg; Ageta and Arai, 1984) and **5** (7.0 mg; Ramírez-Cisneros et al., 2012). Their structures (Fig. 1) were identified by spectroscopic data analysis.

Compound **1** was obtained as a white powder. The molecular formula $C_{18}H_{30}O_3$ of **1**, with four degrees of unsaturation, was indicated by HRESIMS at *m/z* 317.2093 (calculated for $C_{18}H_{30}O_3Na$, 317.2093). The ^{1}H-NMR data (Table 1) showed the presence of a primary methyl [δ_H 0.88 (t, J = 7.2 Hz, H_3-18)], an oxymethine [δ_H 5.78 (d, J = 9.6 Hz, H-11)], a *cis* double bond [$_{\delta H}$ 5.58 (dd, J = 10.8, 9.6 Hz, H-10), 5.83 (ddd, J = 11.4, 10.8, 4.8 Hz, H-9)], and several aliphatic methylenes between δ_H 1.15 and 2.65 ppm. The ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed eighteen carbon resonances, including one CH_3, twelve CH_2, three CH (two olefinic, δ_C 141.7, 122.3; one oxygenated, δ_C 72.9), and two carbonylic quaternary carbons (δ_C 206.2 and 174.1). These spectroscopic data, coupled with the degrees of unsaturation, suggested that **1** was a macrolide (Shimbo et al., 2002; Schulz et al., 2007). In the 2D-NMR spectrum, the HMBC correlations of H_2-2 with C-1, C-3, and C-4; H_2-6 with C-4, C-5, C-7, and C-8; H-9 and H-10 with C-8; and H-11 with C-1, C-9, and C-10, together with the unambiguous homonuclear coupling correlations

Table 1
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in $CDCl_3$ (δ in ppm, J in Hz)[a].

No.	δ_H	δ_C	No.	δ_H	δ_C
1		174.1	10	5.58 dd (10.8, 9.6)	122.3
2	2.61 ddd (14.4, 7.2, 2.4)	35.7	11	5.78 d (9.6)	72.9
	2.21 ddd (14.4, 11.4, 3.0)		12		206.2
3	1.97 m, 1.60 m	21.7	13	2.45 m	39.0
4	1.59 m, 1.27 m	29.7	14	1.59 m, 1.47 m	23.3
5	1.60 m, 1.44 m	24.2	15	1.46 m, 1.26 m	29.0
6	1.16 m	24.9	16	1.28 m	31.7
7	1.53 m, 1.48 m	26.6	17	1.29 m	22.6
8	2.34 m, 2.08 m	25.4	18	0.88 t (7.2)	14.2
9	5.83 ddd (11.4, 10.8, 4.8)	141.7			

[a] The assignments were based on 2D NMR experiments (^{1}H–^{1}H COSY, HSQC, and HMBC).