3,4-Secocycloartane Triterpenoids from the Cones of *Pseudolarix amabilis*

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Abstract

Four new 3,4-secocycloartane triterpenoids, pseudolactones A–D (**1–4**), were isolated from the ethanol extract of the cones of *Pseudolarix amabilis*. Their structures were established by extensive 1D- and 2D-NMR experiments. The cones of *P. arixamabilis* are enriched in the ring-expanded or cleaved cycloartane triterpenoids. This work provides new insight into cycloartane triterpenoids from the cones of *P. arixamabilis*.

Graphic Abstract

Keywords *Pseudolarix amabilis* · Triterpenoids · 3,4-Secocycloartane · Pseudolactones A–D

1 Introduction

Pseudolarix amabilis is a plant indigenous to the south-east of China [1]. The root and trunk barks of it, known as ‘Tu Jin Pi’ in traditional Chinese medicine, have been used to treat skin diseases caused by fungal infection [2, 3]. Previous phytochemical studies on the root barks and seeds of *P. amabilis* revealed a variety of bioactive compounds with novel structures, with the main chemical constituents being pseudolaric acid analogous and triterpenoids [4–9]. Some

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of them, such as pseudolaric acids A and B [10], have shown potent antimicrobial and cytotoxic activities. Pseudolarolide B, a triterpene lactone, has shown potent cytotoxic activity [8]. Novel nortriterpenoid lactone, pseudolarenone [11], as well as triterpenoid–diterpenoid dimers [12], have also been reported from the cones of this plant. In this paper, we describe the isolation and structure elucidation of four new 3,4-secocycloartane triterpenoids from the cones of *P. amabilis*.

2 Results and Discussion

The dried cones of *P. amabilis*, collected in Jiujiang, Jiangxi province, P. R. China, were extracted with 80% EtOH for three times at room temperature. The extract was separated by chromatography techniques to yield four new triterpenes, pseudolactones A–D (1–4) (Fig. 1). The structures of four new triterpenoids were determined by analysis of HRESIMS and NMR spectroscopic data.

Compound 1 corresponds to the molecular formula $C_{32}H_{50}O_6$ as established by the hydrogen adduct ion peak at m/z 531.3678 $[M+H]^+$ (calcd. 531.3680 for $C_{32}H_{51}O_6^+$) in HR-ESI–MS spectrum, indicative of 8 degrees of unsaturation.

The 1H NMR spectrum (Table 1) of 1 showed four tertiary (δ_H 1.00 (s), 1.17 (s), 1.18 (s), 1.08 (s)) and two secondary (δ_H 0.86 (d, $J=6.4$ Hz), 1.19 (overlap)) methyls. Moreover, an oxygenated proton signal was observed at δ_H 4.05. In ^{13}C NMR spectrum (Table 2), there existed 32 carbon resonances, which were sorted into seven methyls, eleven methylenes, six methines (including one oxygenated methine at δ_C 77.4), and eight quaternary carbons (including two ester

carbonyls at δ_C 174.6 and 179.7, an oxygenated quaternary carbon at δ_C 76.1, and a ketal carbon at δ_C 107.3), by DEPT NMR spectrum. The above evidences, combined with the characteristic methylene protons at δ_H 0.51 (d, $J=4.4$ Hz) and 0.70 (d, $J=4.4$ Hz) as well as the carbon resonances at δ_C 32.4 (t), 21.8 (s), and 27.6 (s), revealed a cyclopropyl motif. The diagnostic chemical shifts for two ester carbonyls at δ_C 174.6 (C-3) and 179.7 (C-26), one oxygenated methine at δ_C 77.4, and the ketal carbon at δ_C 107.3, implied that compound 1 might be a 3,4-secocycloartane triterpenoid with a unique 16,23-epoxy-23,26-spirolactone side chain.

Comparison of the NMR spectroscopic data of compound 1 with those of known pseudolarolide C [8] indicated that they were structurally quite similar except that an additional ethoxy in compound 1 (δ_H 1.19 (3H), 4.05 (2H); δ_C 14.1, 60.2) replaced the methoxyl in pseudolarolide C, which was confirmed by key 1H – 1H COSY correlations (Fig. 2) of H_2 – $1'/H_3$ – $2'$ and HMBC correlation from H_2 – $1'$ (δ_H 4.05) to carboxylic carbon C-3 (δ_C 174.6 ppm). The structure of 1 was further evidenced by key 1H – 1H COSY correlations of H_2 – $2/H_2$ – 1 , H – $5/H_2$ – $6/H_2$ – $7/H$ – 8 , H_2 – $11/H_2$ – 12 , H_2 – $15/H$ – $16/H$ – $17/H$ – 20 (H_3 – 21)/ H_2 – 22 together with key HMBC correlations (Fig. 2) from H_2 – 2 , H_2 – 19 , H_2 – 6 to C-10 (δ_C 27.6), from H_2 – 11 , H – 15 to C-13 (δ_C 43.5), from H_2 – 12 , H – 16 to C-14 (δ_C 47.3), from H_2 – 22 , H – 16 , H_2 – 24 to C-23 (δ_C 107.3), and H_3 – 27 to C-26 (δ_C 179.7). The relative configurations of 1 were elucidated to be identical with those of pseudolarolide C on the basis of the REOSY correlations (Fig. 2) of H – 19 with H – 8 , H – 8 with CH_3 – 18 , CH_3 – 18 with H – 16 , H – 16 with H – 20 , H – 17 with CH_3 – 28 and CH_3 – 21 , H – 22 with H – 24 , and H – 24 with CH_3 – 27 . In cycloartane triterpenoids, the C-20 position of the 17-side chain is usually *R*-configuration. From the biogenetic point of view,

Fig. 1 Chemical structures of 1–4

Table 1 ^1H NMR (400 MHz) spectroscopic data for compounds **1–4** in CDCl_3 (J in Hz)

No.	δ_{H} (δ in ppm, J in Hz)			
	1	2	3	4
1	2.17 m 2.59 ddd (6.0, 10.0, 16.0)	1.35 overlap, 2.03 dd (2.8, 6.0, 16.0)	1.34 m 2.02 dd (6.0, 16.0)	2.29 ddd (4.0, 9.2, 15.6) 2.60 ddd (5.6, 10.0, 15.6)
2	1.31 overlap 2.64 ddd (6.0, 10.8, 16.4)	2.22 m 2.49 ddd (6.0, 12.0, 15.6)	2.21 m 2.48 ddd (6.0, 12.0, 15.6)	1.36 m 2.15 m
3				
4				
5	1.27 m	2.41 overlap	2.38 overlap	1.85 m
6	0.63 m 1.69 m	1.08 m 1.51 m	1.05 overlap 1.48 m	0.69 m 1.73 m
7	0.96 m 1.23 m	1.10 m 1.26 overlap	1.05 overlap 1.23 m	1.23 m 1.28 m
8	1.82 m	1.53 m	1.50 m	1.30 m
9				
10				
11	2.10 m 1.20 overlap	2.17 m 1.26 overlap	2.13 m 1.26 m	1.20 m 1.87 m
12	1.40 m 1.14 m	1.68 m 1.58 overlap	1.57 m 1.66 m	1.64 overlap 1.40 overlap
13				
14				
15	1.21 overlap 1.72 m	1.23 overlap 1.79 dd (11.2, 14.0)	1.20 m 1.75 dd (11.2, 12.4)	1.33 m 1.62 overlap
16	4.05 overlap	4.09 td (5.6, 11.2)	4.06 m	1.39 overlap 2.64 ddd (4.0, 8.8, 15.6)
17	1.43 m	1.47 m	1.45 m	1.63 overlap
18	1.00 s	1.04 s	1.01 s	1.00 s
19	0.51 d (4.4) 0.70 d (4.4)	0.42 d (4.8) 0.78 d (4.8)	0.39 d (4.8) 0.76 d (4.8)	0.57 d (4.4) 0.68 d (4.4)
20	2.03 m	2.10 m	2.05 m	2.02 m
21	0.86 d (6.4)	0.89 d (6.8)	0.87 d (6.4)	0.86 d (6.0)
22	1.35 m 1.86 m	1.40 m 1.90 dd (4.0, 14.0)	1.38 dd (2.4, 14.0) 1.87 dd (4.0, 14.0)	2.18 m 2.51 m
23				
24	1.66 m 2.38 dd (8.4, 12.8)	1.70 m 2.40 overlap	1.69 d (1.2) 2.38 overlap	2.47 dd (4.8, 17.2) 2.93 dd (12.4, 17.2)
25	2.87 m	2.93 m	2.91 m	2.96 m
26				
27	1.19 overlap	1.25 d (7.2)	1.22 d (7.2)	1.22 d (9.6)
28	1.17 s	1.12 s	1.09 s	0.92 s
29	1.18 s	4.73 dd (1.6, 2.0) 4.80 d (2.0)	4.71 dd (2.0, 1.6) 4.77 d (2.0)	1.25 s
30	1.08 s	1.67 s	1.65 s	1.21 s
1'	4.05 q (6.8)	4.03 t (6.8)	3.61 s	4.11 q (6.8)
2'	1.19 overlap	1.59 overlap		1.25 t (6.8)
3'		1.37 overlap		
4'		0.93 t (7.6)		

Table 2 ^{13}C (100 MHz) NMR spectroscopic data of compounds **1–4** in CDCl_3

No.	δ_{C} (δ in ppm)			
	1	2	3	4
1	32.2 (t)	28.6 (t)	28.6 (t)	32.3 (t)
2	29.5 (t)	31.6 (t)	31.4 (t)	26.4 (t)
3	174.6 (s)	174.0 (s)	174.3 (s)	174.8 (s)
4	76.1 (s)	149.2 (s)	149.2 (s)	76.3 (s)
5	48.1 (d)	46.1 (d)	46.1 (d)	45.2 (d)
6	25.0 (t)	27.5 (t)	27.5 (t)	25.4 (t)
7	26.0 (t)	25.5 (t)	25.5 (t)	25.7 (t)
8	45.4 (d)	47.9 (d)	47.8 (d)	48.6 (d)
9	21.8 (s)	20.8 (s)	20.8 (s)	22.6 (s)
10	27.6 (s)	27.7 (s)	27.6 (s)	26.5 (s)
11	26.7 (t)	26.9 (t)	26.9 (t)	28.3 (t)
12	31.1 (t)	31.0 (t)	31.0 (t)	33.0 (t)
13	43.5 (s)	43.8 (s)	43.8 (s)	44.9 (s)
14	47.3 (s)	47.4 (s)	47.4 (s)	49.0 (s)
15	41.1 (t)	40.8 (t)	40.8 (t)	35.9 (t)
16	77.4 (d)	77.3 (d)	77.2 (d)	30.2 (t)
17	54.8 (d)	54.8 (d)	54.8 (d)	52.3 (d)
18	19.4 (q)	19.3 (q)	19.3 (q)	18.5 (q)
19	32.4 (t)	31.6 (t)	31.6 (t)	30.9 (t)
20	29.9 (d)	30.0 (d)	30.0 (d)	33.0 (d)
21	19.1 (q)	19.2 (q)	19.2 (q)	19.2 (q)
22	44.1 (t)	44.2 (t)	44.1 (t)	50.2 (t)
23	107.3 (s)	107.3 (s)	107.3 (s)	209.3 (s)
24	42.6 (t)	42.7 (t)	42.7 (t)	46.4 (t)
25	34.1 (d)	34.2 (d)	34.1 (d)	34.4 (d)
26	179.7 (s)	179.8 (s)	179.7 (s)	180.8 (s)
27	14.9 (q)	14.9 (q)	14.9 (q)	16.9 (q)
28	31.7 (q)	23.1 (q)	23.0 (q)	19.5 (q)
29	25.8 (q)	111.6 (t)	111.6 (t)	31.6 (q)
30	23.1 (q)	19.7 (q)	19.7 (q)	26.2 (q)
1'	60.2 (t)	64.2 (t)	51.5 (q)	60.3 (t)
2'	14.1 (q)	30.6 (t)		14.2 (q)
3'		19.1 (t)		
4'		13.7 (q)		

the C-20 position of **1** should be *R*-configuration, too. Zhao et al. reported a series of similar 16,23-epoxy-26(23)-olide-3,4-secocycloartan-3-oic acid esters, and determined their absolute configurations by single crystal X-ray diffraction [13]. By comparing the Cotton effect (-0.7 at 223 nm) of **1** in ECD spectrum with those known compounds [13], in combination with analysis of chemical shifts, the absolute configurations of C-23 and C-25 positions of **1** were proposed to be *S*- and *R*-configuration, respectively. Thus, the structure of **1** was elucidated as (20*R*,23*S*,25*R*)-4-hydroxy-16,23-epoxy-26(23)-olide-3,4-secocycloartan-3-oic acid ethyl ester, and named pseudolactone A.

Compound **2** was obtained as colorless oil. Its molecular formula was determined as $\text{C}_{34}\text{H}_{54}\text{O}_6$, by HR-ESI-MS spectrum. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **2** showed typical signals similar to those of **1**, including two ester carbonyls (δ_{C} 174.0, 179.8), a ketal carbon at δ_{C} 107.3 (C-23), an oxygenated methine at δ_{C} 77.3 (C-16), a cyclopropyl at δ_{H} 0.42 (d, $J=4.8$ Hz), 0.78 (d, $J=4.8$ Hz) and δ_{C} 31.6 (t), one oxygen-bearing proton at δ_{H} 4.09 (td, $J=5.6, 11.2$ Hz) and δ_{C} 77.3 (d), one oxygenated methylene at δ_{H} 4.03 (t, $J=6.8$ Hz) and δ_{C} 64.2 (t), three singlet methyls at δ_{H} 1.04 (s), 1.12 (s), and 1.67 (s), two doublet methyls at δ_{H} 0.89 (d, $J=6.8$ Hz) and 1.25 (d, $J=7.2$ Hz), one triplet methyl at δ_{H} 0.93 (t, $J=7.6$ Hz). These information revealed that compound **2** possessed the similar 16,23-epoxy-26(23)-olide-3,4-secocycloartane skeleton to that of **1**. The main differences between compounds **2** and **1** are that the resonance signals for one terminal double bond (δ_{H} 4.73 (dd, $J=1.6, 2.0$ Hz), 4.80 (d, $J=2.0$ Hz); δ_{C} 149.2 (s), 111.6 (t)), and for one butoxy (δ_{H} 4.03, 1.59, 1.37, 0.93; δ_{C} 64.2 (t), 30.6 (t), 19.1 (t), 13.7 (q)) in **2**, took place of the signals for the oxygenated quaternary carbon at δ_{C} 76.1 (s, C-4) and the methyl at δ_{H} 1.17 (s) and δ_{C} 25.8 (q), as well as for the ethoxy at δ_{H} 4.05, 1.19 and δ_{C} 60.2 (t), 14.1 (q).

In ^1H - ^1H COSY spectrum (Fig. 3), a butoxy was determined on the basis of the correlations of $\text{H}_2\text{-1'}/\text{H}_2\text{-2'}/\text{H}_2\text{-3'}/\text{H}_3\text{-4'}$. The butoxy was linked to C-3 through key HMBC cross peak (Fig. 3) from $\text{H}_2\text{-1'}$ (δ_{H} 4.03) to the ester carbonyl at δ_{C} 174.0. The terminal double bond was evidenced to be

Fig. 2 Key ^1H - ^1H COSY, HMBC, and NOESY correlations of compound **1**

Fig. 3 Key ^1H - ^1H COSY, HMBC, and NOESY correlations of compound **2**

placed between C-4 and C-29 as shown by key HMBC correlations (Fig. 3) from H-5 (δ_{H} 2.41), H_2 -29 (δ_{H} 4.73 and 4.80), CH_3 -30 (δ_{H} 1.67) to C-4 (δ_{C} 149.2). In REOSY spectrum of **2**, the observation of key NOE correlations (Fig. 3) of H-19 with CH_3 -30, H-8 (δ_{H} 1.53), of CH_3 -18 (δ_{H} 1.04) with H-8, H-16 (δ_{H} 4.09) and H-20 (δ_{H} 2.10), of H-17 (δ_{H} 1.47) with CH_3 -28 (1.12) and CH_3 -21 (δ_{H} 0.89), suggested that the relative configuration of **2** was identical to that of compound **1**. From structure, compound **2** could be considered as a dehydrated analogue of **1**, implying that two compounds may share same configuration. Also, compound **2** had a negative Cotton effect (-1.5) at 201 nm. Consequently, the structure of **2** was identified as (20*R*,23*S*,25*R*)-16,23-epoxy-26(23)-olide-3,4-secocycloartan-4(29)-en-3-oic acid *n*-butyl ester, and named pseudolactone B.

The molecular formula of compound **3** was assigned as $\text{C}_{31}\text{H}_{46}\text{O}_5$ with nine degrees of unsaturation, based on the hydrogen adduct ion $[\text{M} + \text{H}]^+$ at m/z 499.3414 (calcd. 499.3418 for $\text{C}_{31}\text{H}_{47}\text{O}_5^+$). Its molecular weight is 42 Da less than that of **2**. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were quite close to those of **2**. The only difference was that a methoxyl at δ_{H} 3.61 and δ_{C} 51.5 was observed in **3**, instead of those signals for the butoxy (δ_{H} 4.03, 1.59, 1.39, 0.93; δ_{C} 64.2 (t), 30.6 (t), 19.1 (t), 13.7 (q)) in **2**. Key HMBC correlation from the methoxyl proton at δ_{H} 3.61 to the ester carbonyl at δ_{C} 174.3 further confirmed the above deduction, and established the structure of **3** as (20*R*,23*S*,5*R*)-16,23-epoxy-26(23)-olide-3,4-secocycloartan-4(29)-en-3-oic acid methyl ester, and given the name pseudolactone C.

Compound **4** had a molecular formula $\text{C}_{32}\text{H}_{52}\text{O}_6$ with seven degrees of unsaturation, as evidenced by positive HR-ESI-MS at m/z 555.3674 $[\text{M} + \text{Na}]^+$. The ^1H NMR spectroscopic data (Table 1) of **4** exhibited four singlet methyls

at δ_{H} 1.00 (s), 0.92 (s), 1.25 (s), and 1.21 (s), two doublet methyls at δ_{H} 0.86 (d, $J=6.0$ Hz) and 1.22 (d, $J=9.6$ Hz), one triplet methyl at δ_{H} 1.25 (t, $J=6.8$ Hz), and an oxygenated methylene at δ_{H} 4.11 (2H, q, $J=6.8$ Hz). In addition, a typical AB coupling system was observed at δ_{H} 0.57 (1H, d, $J=4.4$ Hz) and 0.68 (1H, d, $J=4.4$ Hz). In ^{13}C NMR spectrum (Table 2), thirty-two carbon resonances were observed, including 7 methyls (δ_{C} 18.5, 19.2, 16.9, 19.5, 31.6, 26.2, 14.2), 12 methylenes (including one oxygenated methylene at δ_{C} 60.3), 5 methines, and 8 quaternary carbons (including one ester carbonyl at δ_{C} 174.8, one carboxyl at δ_{C} 180.8, one ketone carbonyl at δ_{C} 209.3, and one oxygenated quaternary carbon at δ_{C} 76.3). Deducting three degrees of unsaturation accounted for one ketone carbonyl and two carboxylic carbon, the remaining four degrees of unsaturation were indicative of the tetracyclic ring system of **4**. The NMR spectroscopic data (Tables 1 and 2) of **4** quite resemble those of known compound pseudolarnoid G ((25*S*)-4-hydroxy-3,4-seco-cycloartan-23-one-3,26-dioic acid methyl), previously reported from the seeds of the tilted plant [13]. The main differences are that compound **4** had an ethoxy ester and one carboxyl functionalities, while pseudolarnoid G had two methyl ester functionalities.

The diagnostic chemical shifts at δ_{C} 174.8 (ester carbonyl) and δ_{C} 76.3 (s) were indicative of oxidative cleavage of ring A between C-3 and C-4, and formed an ethyl ester at C-3 and an oxygenated isopropyl moiety at C-4. The deduction was evidenced by key ^1H - ^1H COSY correlation (Fig. 4) between H_2 -1' (δ_{H} 4.11) and H_2 -2' (δ_{H} 1.25), and between H_2 -1 and H_2 -2, together with HMBC correlations (Fig. 4) of H_2 -1 and H_2 -1' with C-3 (δ_{C} 174.8), of CH_3 -29 (δ_{H} 1.25), CH_3 -30 (δ_{H} 1.21), H-5 (δ_{H} 1.85), H_2 -6 (δ_{H} 0.69, 1.73) with the oxygenated quaternary carbon at δ_{C} 76.3 (C-4). Also,

Fig. 4 Key ^1H - ^1H COSY, HMBC, and NOESY correlations of compound **4**

the ketone carbonyl at δ_{C} 209.3 was assigned to be C-23 on the basis of HMBC correlations from H_2 -22 and H_2 -24 to the ketone carbonyl. Key HMBC cross-peaks of H_2 -24 and CH_3 -27 (δ_{H} 1.22 (d, $J=9.6$ Hz) supported the attribution of C-26 carboxyl. In the ROESY spectrum, key NOE correlations (Fig. 4) of H-20 with CH_3 -18, of H-8 (δ_{H} 1.30) with CH_3 -18 and H-19, and of CH_3 -29 with H-19 indicated that H-8, CH_3 -18, CH_2 -19, H-20, and 4-hydroxyl isopropyl are β -oriented, whereas H-17 and CH_3 -28 were α -oriented based on the NOE cross-peak of H-17 with CH_3 -28. With regard to the absolute configurations of two chiral centers (C-20 and C-25) at C-17 side chain, Zhao et al. had ever determined the absolute configurations of C-20 and C-25 of similar compound pseudolarnoid G as *R* and *S*, respectively, by single crystal X-ray diffraction. The ECD spectrum of compound **4** showed a negative Cotton effect -1.5 at 285 nm, in accordance with that of pseudolarnoid G (-1.23 (283 nm)), revealed that compound **4** had same absolute configuration as that of pseudolarnoid G. Therefore, the structure of **4** was identified as (20*R*,25*S*)-4-hydroxy-23-oxo-3,4-secocycloartan-26-oic acid-3-ethyl ester, and named pseudolactone D.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded with a Bruker Vector-22 Spectrophotometer with KBr discs. NMR spectra were recorded with Bruker DRX-400 spectrometer (400 MHz). The chemical shifts (δ) are given in ppm with TMS as internal standard and coupling constants (J) are given in Hz. MS spectra were recorded with a Agilent MSD-Trap-XCT (for ESI) and Waters Micro-mass Q-TOF mass spectrometer (for HR-ESI-MS), in m/z . Column chromatographic separations were carried out by using silica gel

(200–300 mesh; Marine Chemical Factory, Qingdao, P. R. China), Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, NJ, USA) as packing material. TLC was carried out on precoated silica gel GF 254 plates (Yantai Chemical Industrials) and the TLC spots were viewed at 254 nm and visualized by using 10% sulfuric acid in alcohol containing 10 mg/mL vanillin.

3.2 Plant Material

The cones of *P. amabilis* were collected in Jiu Jiang, Jiangxi province, P. R. China, in October 2010, and authenticated by Prof. Han-Ming Zhang of Second Military Medical University. A voucher specimen (No. 20101015) is deposited in School of Pharmacy, Second Military Medical University.

3.3 Extraction and Isolation

The air-dried cones (12.0 kg) of *P. amabilis* were ground into powder and extracted with 80% EtOH for four times at room temperature to give a crude extract, which was further partitioned with petroleum ether (60–90 °C) (PE), CHCl_3 , and EtOAc, successively. The CHCl_3 -soluble extract was subjected to a silica gel column chromatography (CC) eluting with a gradient PE/EtOAc (from 30:0 to 0:1) to obtain eight fractions 1–8. Fraction 2 (58 g) was chromatographed over RP-18 column eluting with MeOH/ H_2O (from 3:7 to 10:0) to afford five subfractions. Subfraction 2 was further chromatographed on a silica gel column (CH_2Cl_2 /PE, from 0:20 to 1:0, and MeOH) and purified by preparative TLC (cyclohexane/ CH_2Cl_2 /EtOAc, 20:1:1) to afford **2** (20 mg) and **3** (20 mg). Subfraction 4 was separated by repeated column chromatography on Sephadex LH-20 (CHCl_3 /MeOH, 1:1, and MeOH), and then purified by preparative TLC (cyclohexane/ CH_2Cl_2 /EtOAc, 15:1:1) to yield **1** (10 mg). Fraction 7 (70 g) was separated by RP-18 CC (MeCN/ H_2O , from 2:8 to 10:0) to afford 6 subfractions. Subfraction 3

was further chromatographed on a silica gel column ($\text{CHCl}_3/\text{MeOH}$ from 50:1 to 0:1) and purified by preparative TLC ($\text{PE}/\text{EtOAc}/\text{MeOH}$, 20:1:0.1) to afford **4** (8 mg).

3.3.1 Pseudolactone A (1)

Colorless oil, $[\alpha]_D^{25}$ 45.1 ($c = 0.37$, CH_2Cl_2). CD ($c = 2.83$ mmol/L, CH_3CN , 20 °C) nm ($\Delta\epsilon$) 223 (−0.7). IR (KBr) ν_{max} 1731, 1778, 2964 cm^{-1} . For ^1H and ^{13}C NMR data (400 MHz, CDCl_3), see Tables 1 and 2. ESI–MS: m/z 553.5 $[\text{M} + \text{Na}]^+$, 529.3 $[\text{M} - \text{H}]^-$. HR-ESI–MS: m/z 531.3678 $[\text{M} + \text{H}]^+$ (calcd $\text{C}_{32}\text{H}_{51}\text{O}_6^+$, 531.3680).

3.3.2 Pseudolactone B (2)

Colorless oil, $[\alpha]_D^{25}$ 51.6 ($c = 0.24$, CH_2Cl_2). CD ($c = 2.11$ mmol/L, CH_3CN , 20 °C) nm ($\Delta\epsilon$) 201 (−1.5). IR (KBr) ν_{max} 1727, 1770, 2974 cm^{-1} . For ^1H (400 MHz, CDCl_3) and ^{13}C NMR data (100 MHz, CDCl_3), see Tables 1 and 2. ESI–MS: m/z 563.2 $[\text{M} + \text{Na}]^+$, 539.1 $[\text{M} - \text{H}]^-$. HRESIMS: m/z 541.3902 $[\text{M} + \text{H}]^+$ (calcd $\text{C}_{34}\text{H}_{53}\text{O}_5^+$, 541.3888).

3.3.3 Pseudolactone C (3)

Colorless oil, $[\alpha]_D^{25}$ 51.1 ($c = 0.23$, CH_2Cl_2). CD ($c = 2.61$ mmol/L, CH_3CN , 20 °C) nm ($\Delta\epsilon$) 201 (−1.6). IR (KBr) ν_{max} 1737, 1778, 2964 cm^{-1} . For ^1H (400 MHz, CDCl_3) and ^{13}C NMR data (100 MHz, CDCl_3), see Tables 1 and 2. ESIMS: m/z 521.2 $[\text{M} + \text{Na}]^+$. HR-ESI–MS: m/z 499.3414 $[\text{M} + \text{H}]^+$ (calcd $\text{C}_{31}\text{H}_{47}\text{O}_5^+$, 499.3418).

3.3.4 Pseudolactone D (4)

White amorphous powder, $[\alpha]_D^{25}$ 51.6 ($c = 0.23$, CH_2Cl_2). CD ($c = 1.88$ mmol/L, CH_3CN , 20 °C) nm ($\Delta\epsilon$) 210 (−1.9), 285 (−1.5). IR (KBr) ν_{max} 1712, 1735, 2873, 2925, 2962, 3440 cm^{-1} . For ^1H (400 MHz, CDCl_3) and ^{13}C NMR data (100 MHz, CDCl_3), see Tables 1 and 2. ESI–MS: m/z 531.4 $[\text{M} - \text{H}]^-$. HR-ESI–MS: m/z 555.3674 $[\text{M} + \text{Na}]^+$ (calcd $\text{C}_{32}\text{H}_{52}\text{O}_6\text{Na}^+$, 555.3656).

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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References

1. Editor committee, *Flora of China*, vol. 7 (Science Press, Beijing, 1978), pp. 196–200
2. G.P. Ni, *Zhongguo Zhongyao Zazhi* **3**, 156–158 (1957)
3. P. Chiu, L.T. Leung, B.C.B. Ko, *Nat. Prod. Rep.* **27**, 1066–1083 (2010)
4. Z.L. Li, K. Chen, D.J. Pan, G.G. Xu, *Acta. Chim. Sin.* **47**, 258–261 (1989)
5. S.P. Yang, Y. Wu, J.M. Yue, *J. Nat. Prod.* **65**, 1041–1044 (2002)
6. Z.L. Li, D.J. Pan, C.Q. Hu, Q.L. Wu, S.S. Yang, G.Q. Xu, *Acta. Chim. Sin.* **40**, 447–457 (1982)
7. Z.L. Li, K. Chen, D.J. Pan, G.Q. Xu, *Acta. Chim. Sin.* **43**, 786–788 (1985)
8. G.F. Chen, Z.L. Li, D.J. Pan, *J. Nat. Prod.* **56**, 1114–1122 (1993)
9. G.F. Chen, Z.L. Li, C.M. Tang, X. He, K. Chen, D.J. Pan, C.Q. Hu, D.R. McPhail, A.T. McPhail, K.H. Lee, *Heterocycles* **31**, 1903–1906 (1990)
10. E. Li, A.M. Clark, C.D. Hufford, *J. Nat. Prod.* **58**, 57–67 (1995)
11. B. Li, D.Y. Kong, Y.H. Shen, K.L. Fu, R.C. Yue, Z.Z. Han, H. Yuan, Q.X. Liu, L. Shan, H.L. Li, X.W. Yang, W.D. Zhang, *Chem. Commun.* **49**, 1187–1189 (2013)
12. B. Li, D.Y. Kong, Y.H. Shen, H. Yuan, R.C. Yue, Y.R. He, L. Lu, L. Shan, H.L. Li, J. Ye, X.W. Yang, J. Su, R.H. Liu, W.D. Zhang, *Org. Lett.* **14**, 5432–5435 (2012)
13. X.T. Zhao, M.H. Yu, S.Y. Shi, C. Lei, A.J. Hou, *Phytochemistry* **171**, 112229 (2020)

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