Fitoterapia 91 (2013) 224–230

Contents lists available at ScienceDirect

Fitoterapia

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

Terpenoids and their anti-HIV-1 activities from *Excoecaria acerifolia*

Sheng-Zhuo Huang [a,d], Xuan Zhang [c], Qing-Yun Ma [a,d], Yong-Tang Zheng [c], Feng-Qing Xu [b], Hua Peng [b], Hao-Fu Dai [a,d], Jun Zhou [b,*], You-Xing Zhao [a,d,**]

[a] Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, People's Republic of China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China
[c] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Science & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, People's Republic of China
[d] Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Haikou 571101, People's Republic of China

ARTICLE INFO

Article history:
Received 6 August 2013
Accepted in revised form 10 September 2013
Available online 21 September 2013

Chemical compounds studied in this article:
Caulophyllogenin (PubChem CID: 104361)

Keywords:
Excoecaria acerifolia
Euphorbiaceae
Anti-HIV
Diterpenoid
Triterpenoid

ABSTRACT

Five new diterpenoids named excocarinols A–E (**1–5**) including three pimaranes, one cleistanthane, and one nor-beyerane, together with nine known compounds, were isolated from the EtOAc extract of the Chinese ethnodrug Gua-jing-ban (*Excoecaria acerifolia* Didr.). Their structures were elucidated by the analysis of spectroscopic data including 1D, 2D NMR and HR-MS. The anti-HIV-1 bioassay on the diterpenoids showed that excocarinol A (**1**) exhibited moderate anti-HIV-1 activity with EC_{50} 5.58 μM and SI (Selection Index) over 112.71.

1. Introduction

Some *Excoecaria* species (Euphorbiaceae), the well-known mangroves-living irritant latex [1], are widely used in Southeast Asia as a uterotonic by the Tai herbalist [2]. *Excoecaria acerifolia* Didr., one of the Euphorbiaceae species, widely distributed in the dry hot valleys in Yunnan and Sichuan provinces, is epibiotic [1]. This feature is unique among these genera. Also *E. acerifolia* is used as an ethnodrug Gua-jing-ban by minority nationalities in Southwest China for antidote, antiphlogistic, laxative, antitussive, anti-malaria, and anti-virus purposes [3]. Previous studies focused on the common species of this genus such as *Excoecaria agallocha* and *Excoecaria oppositifolia*, and reported different types of compounds including phenols [4–6], triterpenoids [7–9], and diterpenoids [2,10,11]. Until now there were few diterpenoids and phenols that had been found in *E. acerifolia* and few anti-virus researches on this species [12,13]. To search for new bioactive compounds, our continuous investigation on chemical compositions of *E. acerifolia* was carried out. Five new diterpenoids named excocarinols A–E (**1–5**), including three pimaranes, one cleistanthane, and one nor-beyerane, were isolated together with nine known compounds agallochin J (**6**) [14], agallochin K (**7**) [14], 14α,18-dihydroxy-7,15-isopimaradiene (**8**) [15],

* Corresponding author. Tel./fax: +86 898 66989095.
** Correspondence to: Y.-X. Zhao, Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, People's Republic of China. Tel./fax: +86 898 66989095.
E-mail addresses: junzhou3264@126.com (J. Zhou), zhaoyouxing@itbb.org.cn (Y.-X. Zhao).

0367-326X/$ – see front matter. Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2013.09.007

7β,12β-dihydroxypimara-8,15-dien-14-one (**9**) [16], ribenone (**10**) [17], 15-*O*-acetylspiraminol (**11**) [18], angustanoic acid B (**12**) [19], caulophyllogenin (**13**) [20], and rubusic acid (**14**) [21]. Additionally, the compounds **1**, **3**, **4**, and **5** have been tested for anti-HIV-1 activities. Excocarinol A (**1**) exhibited moderate anti-HIV-1 activity at the levels of EC_{50} 5.58 μM and SI over 112.71. Herein, the isolation process, the structural elucidation of the new diterpenoids excocarinols A–E (**1**–**5**), and the anti-HIV-1 activities of four new compounds were described.

2. Experimental

2.1. Generals

Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were obtained on a Shimadzu double-beam 210A spectrometer. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400, a DRX-500, or AVANCE III-600 spectrometer with TMS as an internal standard. ESIMS and HRESIMS were recorded with a Bruker HCT/Esquire HPLC-Iron Trap spectrometer and an API QSTAR Pulsar 1 spectrometer. EIMS and HREIMS were recorded with a Waters Autospec Premier. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., People's Republic of China), RP-18 (40–70 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (GE Healthcare, USA) were used for column chromatography (CC). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Fractions were monitored by TLC and spots were visualized by heating after spraying with 5% H_2SO_4 in ethanol (b. p. 77–79 °C).

2.2. Plant

The stems of *E. acerifolia* were collected in Dali, Yunnan Province, People's Republic of China, and identified by Prof. H. Peng, and Dr. Y. Niu (Kunming Institute of Botany, Chinese Academy of Sciences). Voucher specimen (HUANG0006) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

2.3. Extraction and isolation

The dried and powdered stems of *E. acerifolia* (19 kg) were extracted with 95% EtOH under reflux for three times (3 × 30 L). The extract (1.5 kg) was then concentrated and suspended in water followed by successive partition with petroleum ether (3 × 5 L) and EtOAc (3 × 5 L), respectively. The EtOAc extract (305 g) was separated by silica gel CC (ϕ 16 × 160 cm) using a gradient solvent $CHCl_3$/MeOH (9:1, 7:1, 5:1, 3:1, *v*/*v*, each 10 L) to afford fractions A–C. Fraction B (101 g) was separated by silica gel CC (ϕ 16 × 160 cm) using a gradient solvent petroleum ether/EtOAc (10:1, 8:1, 5:1, 3:1, 1:2, *v*/*v*, each 4 L) to afford fractions B1–B7. Fractions B1–B7 were purified by Sephadex LH-20 CC (ϕ 3.6 × 160 cm, $CHCl_3$/MeOH 1:1, *v*/*v*, 1500 mL) to give subfractions B1a–B7a, respectively. Subfraction B1a (3.4 g) was subjected to repeated RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 *v*/*v*, each 1 L) and semi-preparative HPLC (MeOH/H_2O 45:55 or 50:50, *v*/*v*, 2.0 mL/min) to yield **3** (18.9 mg) and **9** (25.4 mg); subfraction B2a (2.1 g) was subjected to repeated RP-18 CC (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 *v*/*v*, each 1 L) and Sephadex LH-20 (ϕ 1.4 × 50 cm, MeOH, 100 mL) to yield **5** (75.6 mg) and **6** (28.6 mg), and **7** (6.5 mg); subfraction B3a (2.6 g) was subjected to repeated RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, *v*/*v*, each 1 L) and silica gel CC (ϕ 2.5 × 30 cm, petroleum ether/EtOAc 3:1, *v*/*v*, 400 mL) to yield **4** (5.3 mg) and **8** (11.5 mg); subfraction B4a (4.7 g) was subjected to repeated RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 *v*/*v*, each 1 L) and semi-preparative HPLC (MeOH/H_2O, 50:50, *v*/*v*, 1.5 mL/min) to yield **1** (6.5 mg) and **10** (7.8 mg); subfraction B5a (1.8 g) was subjected to repeated RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 *v*/*v*, each 1 L) and silica gel CC (ϕ 2.5 × 30 cm, petroleum ether/EtOAc 2:1, *v*/*v*, 700 mL) to yield **2** (17.9 mg), **11** (12.5 mg), and **12** (9.8 mg); subfraction B6a (794 mg) was subjected to repeated RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, *v*/*v*, each 1 L) and semi-preparative HPLC (MeOH/H_2O 45:55, *v*/*v*, 1.7 mL/min) to yield **7** (5.6 mg); and subfraction B7a (530 mg) was subjected to RP-18 (ϕ 3.6 × 40 cm, MeOH/H_2O 3:7, 4:6, 5:5, 6:4, 7:3, *v*/*v*, each 1 L) and silica gel column CC (ϕ 2.5 × 30 cm, $CHCl_3$/MeOH 15:1, *v*/*v*, 500 mL) to yield **13** (28.2 mg) and **14** (19.6 mg).

2.3.1. Excocarinol A (12α,14β-dihydroxyl-3-oxo-pimara-8(9),15-diene) (**1**)

White powder; $[\alpha]_D^{18}$ −50.92 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (logε) 196 (2.31), 215 (2.77), 248 (2.49), 291 (1.919) nm; IR (KBr) ν_{max} 3435, 2966, 2935, 2873, 1704, 1638, 1460, 1383, 1273, 1117, 1017, 1002, 919 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; ESIMS positive *m*/*z* $[M + Na]^+$ 341 (20); HREIMS *m*/*z* $[M + Na]^+$ 341.2101 (calcd. for $C_{20}H_{30}O_3Na$, 341.2092).

2.3.2. Excocarinol B (3α,12α,14β-trihydroxyl-pimara-8(9),15-diene) (**2**)

White powder; $[\alpha]_D^{32}$ −41.2 (*c* 1.13, MeOH); UV (MeOH) λ_{max} (logε) 243 (3.33), 209 (3.85) nm; IR (KBr) ν_{max} 3432, 2936, 2872, 1725, 1633, 1454, 1384, 1288, 1124, 1065, 1021 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; ESIMS positive *m*/*z* $[M + Na]^+$ 343 (10); HRESIMS *m*/*z* $[M + Na]^+$ 343.2246 (calcd. for $C_{20}H_{32}O_2Na$, 343.2249).

2.3.3. Excocarinol C (3α,12α,14α-trihydroxyl-isopimara-8(9),15-diene) (**3**)

White powder; $[\alpha]_D^{32}$ −32.7 (*c* 1.33, MeOH); UV (MeOH) λ_{max} (logε) 242 (3.52), 208 (3.74) nm; IR (KBr) ν_{max} 3431, 2960, 2934, 2872, 1724, 1639, 1458, 1414, 1380, 1287, 1122, 1075, 1031, 1016 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; ESIMS negative *m*/*z* $[M + Cl]^-$ 355 (30); HRESIMS *m*/*z* $[M + Cl]^-$ 355.2042 (calcd. for $C_{20}H_{32}O_3Cl$, 355.2039).

2.3.4. Excocarinol D (cleistantha-8,11,13,15-tetraene-3β-ol) (**4**)

White powder; $[\alpha]_D^{27}$ −24.93 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (logε) 209 (3.66), 245 (3.03) nm; IR (KBr) ν_{max} 3441, 2966, 2939, 2857, 1725, 1630, 1455, 1376, 1287, 1122, 1098, 1072, 1037, 1004, 936, 921, 812 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS *m*/*z* $[M]^+$ 284 (100); HREIMS *m*/*z* $[M]^+$ 284.2140 (calcd. for $C_{20}H_{28}O$, 284.2140).

Table 1
^{1}H NMR data of compounds **1**–**5** (δ in ppm, J in Hz).

Position	**1**[a]	**2**[a]	**3**[b]	**4**	**5**
1α	1.99 m	1.57 m	1.78 m	2.32 ddd (3.5, 3.6, 13.1)	1.41 m
				1.53 ddd (4.1, 11.5, 13.1)	2.08 m
β	1.68 m	1.51 m	1.25 m		
2β	2.56 m	1.96 m	1.75 m	1.82 m	1.56 m
				1.77 m	1.88 m
α	2.52 m	1.65 m	1.62 m		
3		3.47 dd (2.7, 2.9)	3.27 dd (4.5, 11.8)	3.30 dd (4.7, 11.5)	4.35 ddd (2.0, 2.8, 3.4)
4					2.19 m
5	1.76 dd (4.6, 12.4)	1.65 dd (2.0, 11.8)	1.17 dd (1.2, 12.0)	1.29 dd (2.0, 12.5)	1.12 dd (2.1, 11.2)
6α	1.72 m	1.65 m	1.77 m	1.90 m	3.40 ddd (4.0, 11.0, 11.2)
				1.70 m	
β	1.59 m	1.46 m	1.49 m		
7α	2.46 m	1.98 m	2.38 m	2.66 ddd (7.7, 11.2, 17.7)	1.41 dd (4.0, 17.7)
				2.87 ddd (5.3, 6.0, 17.7)	1.12 dd (11.0, 17.7)
β	2.09 m	2.38 m	2.02 m		
9					1.85 dd (4.0, 12.8)
11β	2.36 dd (5.3, 16.5)	2.32 m	2.32 dd (5.6, 16.0)	7.10 d (7.8)	1.94 m
					2.12 m
α	2.07 dd (7.3, 16.5)	2.06 m	2.06 dd (7.4, 16.0)		
12	3.73 dd (5.3, 7.3)	3.67 dd (5.5, 7.8)	3.69 d (5.6, 7.4)	7.03 d (7.8)	1.24 m
					1.39 m
14	3.76 s	3.73 s	3.74 s		1.85 dd (4.0, 12.8)
15	6.05 dd (11.1, 17.7)	6.05 dd (11.1, 17.8)	6.04 dd (11.0, 17.8)	6.60 dd (11.5, 18.0)	5.71 d (5.6)
16	5.42 dd (1.0, 11.1)	5.41 dd (1.1, 11.1)	5.41 dd (1.2, 11.0)	5.51 dd (1.0, 11.5)	5.58 d (5.6)
				5.22 dd (1.0, 18.0)	
	5.33 dd (1.0, 17.7)	5.33 dd (1.1, 17.8)	5.32 dd (1.2, 17.6)		
17	1.16 s	1.17 s	1.14 s	2.26 s	1.06 s
18	1.10 s	1.02 s	1.00 s	0.88 s	1.15 d (7.1)
19	1.12 s	1.01 s	1.02 s	1.07 s	
20	1.08 s	0.91 s	0.83 s	1.20 s	

[a] Recorded in $CDCl_3$ at 400 MHz.
[b] Recorded in $CDCl_3$ at 600 MHz.

*2.3.5. Excocarinol E (6-hydroxyl-15-ene-mononorbeyeran-19(3)-lide) (**5**)*

White powder; $[\alpha]_D^{27} -34.43$ (c 0.07, MeOH); UV (MeOH) λ_{max} (logε) 206(3.38) nm; IR (KBr) ν_{max} 3438, 2957, 2926, 2872, 1729, 1455, 1380, 1366, 1289, 1116, 10, 1098, 1060, 1019, 746 cm^{-1}; ^{1}H and ^{13}C NMR see Tables 1 and 2; ESIMS positive m/z $[M + Na]^+$ 325 (40), 303 (10); HRESIMS m/z $[M + H]^+$ 303.1960 (calcd. for $C_{19}H_{27}O_3$, 303.1958).

2.4. Anti-HIV assays

The anti-HIV test was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) and cytotoxicity assay against C8166 cell line (IC_{50}) as described in the literature [22,23]. AZT (3′-azido-3′-deoxythymidine, Sigma Aldrich, ≥98%) was used as positive control. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC_{50}) was determined from the dose response curve and calculated with Reed and Muench method [24]. The selectivity index (SI) was calculated from the ratio of IC_{50}/EC_{50}.

3. Results and discussion

95% EtOH concentrated extract prepared from the stems of *E. acerifolia* was suspended in water and then partitioned into petroleum ether and EtOAc extracts, respectively. The EtOAc extract was subjected to repeated CC over silica gel, Sephadex LH-20, and RP-18 and purified further by HPLC to give five new diterpenoids excocarinols A–E (**1–5**), along with the nine known compounds (Fig. 1). The new ones were identified by comparing literature and spectroscopic means including 1D and 2D NMR.

Table 2
^{13}C NMR data of compounds **1**–**5** ($CDCl_3$, δ in ppm).

Carbon	**1**[a]	**2**[a]	**3**[c]	**4**[b]	**5**[c]
1	35.1 t	29.4 t	34.8 t	37.3 t	29.2 t
2	34.2 t	25.5 t	27.5 t	28.0 t	20.8 t
3	217.3 s	75.7 d	78.7 d	78.7 d	79.1 d
4	47.1 s	37.5 s	47.2 s	38.9 s	36.5 d
5	50.7 d	44.2 d	50.9 d	49.3 d	55.8 d
6	19.4 t	18.0 t	18.2 t	18.9 t	72.2 d
7	29.0 t	28.6 t	29.1 t	29.7 t	45.5 t
8	129.0 s	127.9 s	128.0 s	133.0 s	49.3 s
9	134.6 s	136.7 s	136.3 s	147.0 s	45.4 d
10	37.1 s	37.2 s	38.8 s	37.6 s	45.2 s
11	30.5 t	30.6 t	30.6 t	123.1 d	20.7 t
12	72.8 d	73.1 d	73.0 d	127.8 d	33.8 t
13	45.9 s	46.1 s	45.9 s	132.6 s	43.6 s
14	77.9 d	78.0 d	77.9 d	137.7 s	61.0 t
15	138.1 d	138.2 d	138.2 d	135.5 d	135.2 d
16	118.8 t	118.8 t	118.7 t	119.1 t	136.1 t
17	20.3 q	20.1 q	20.2 q	20.5 q	19.4 q
18	21.1 q	19.2 q	19.5 q	15.3 q	24.9 q
19	26.7 q	28.0 q	27.8 q	28.1 q	174.9 s
20	19.0 q	22.1 q	15.5 q	24.9 q	

[a] Recorded at 100 MHz.
[b] Recorded at 120 MHz.
[c] Recorded at 150 MHz.

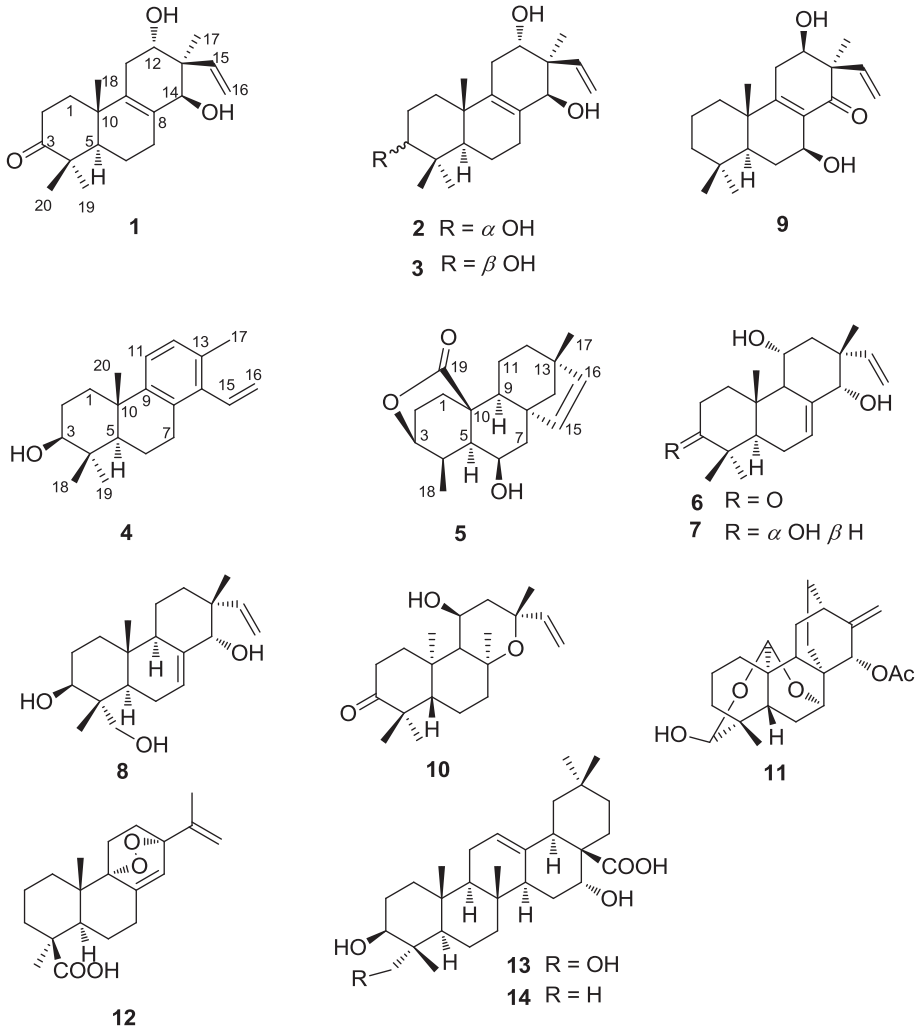

Fig. 1. Structures of **1**–**14**.

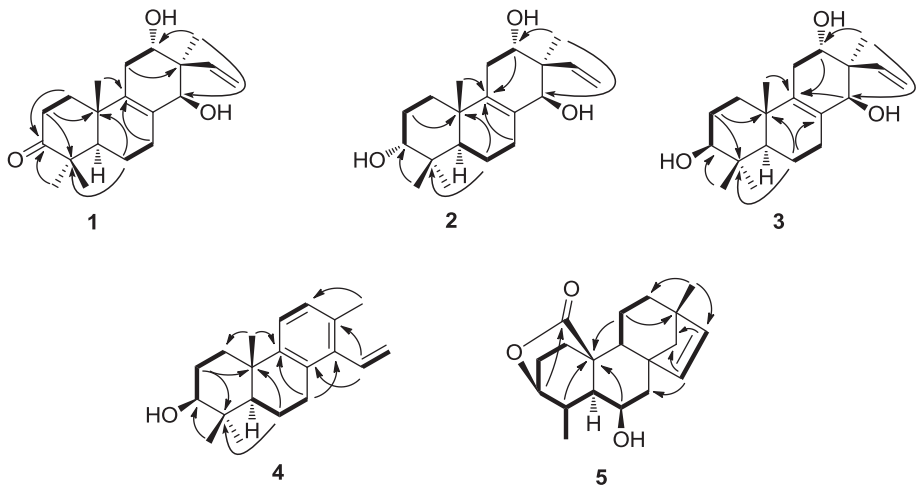

Fig. 2. Key ^{1}H–^{1}H COSY ( ) and HMBC (H → C) correlations of **1**–**5**.

Excocarinol A (**1**) was isolated as white powder and its molecular formula, $C_{20}H_{30}O_3$ requiring six degrees of unsaturation, was determined by the HRESIMS positive (*m/z* 341.2101 $[M + Na]^+$, calcd. for $C_{20}H_{30}O_3Na$, 341.2092) and NMR spectroscopic data (Tables 1 and 2). The IR absorption also indicated the presence of hydroxyls (3435 cm^{-1}), carbonyl (1704 cm^{-1}), and double bonds (1638 cm^{-1}). The analysis of the ^{13}C NMR and DEPT spectra (Table 2) showed 20 carbon resonances, including four methyls, six methylenes (one olefinic), four methines (two oxygenated and one olefinic), and six quaternary carbons (three aliphatic, one carbonyl and two olefinic). These characteristics were reminiscent of the presence of pimarane diterpenoid as 7β,12β-dihydroxypimara-8,15-dien-14-one (**9**) [16]. The differences between **1** and **9** were the positions of the hydroxyl and carbonyl. The location of carbonyl group at C-3 in **1** was determined by the HMBC correlations from H-19 [δ_H 1.12 (3 H, s)] and H-20 [δ_H 1.08 (3 H, s)] to C-3 [δ_C 217.3 (s)]. The linkage of the hydroxyl to C-14 in **1** was elucidated from the HMBC correlations of H-17 [δ_H 1.16 (3H, s)] and H-12 [δ_H 3.73 (1 H, dd, J = 5.3, 7.3 Hz)] with C-14 [δ_C 77.9 (d)]. The other correlations in the HMBC and ^1H–^1H COSY spectrum (Fig. 2) further confirmed the atom connectivities in compound **1**. Compound **1** had the same configurations as **9** with β-orientation of 18-Me and α-orientation of H-5 based on the comparison of their NMR data and the ROESY experiment of **1** (Fig. 3). The β-orientation of 14-OH was determined by NOE of H-14 [δ_H 3.76 (1H, s)]/H-17 [δ_H 1.16 (s)], H-14/H-11α [δ_H 2.07 (1H, dd, J = 7.3, 16.5 Hz)] and H-18 [δ_H 1.10 (3H, s)]/H-11β [δ_H 2.36 (1H, dd, J = 5.3, 16.5 Hz)]. The α-orientation of 12-OH was deduced by NOE of H-12/H-16 [δ_H 5.33 (1H, dd, J = 1.0, 17.7 Hz)]. Thus, compound **1** was assigned as shown with pimarane skeleton, and named excocarinol A.

Excocarinols B (**2**) and C (**3**) were both obtained as white powder. They were respectively assigned the same molecular formula $C_{20}H_{32}O_3$ with five degrees of unsaturation according to the HRESIMS positive *m/z* $[M + Na]^+$ 343.2246 (calcd. for $C_{20}H_{32}O_2Na$, 343.2249) for **2** and negative *m/z* 355.2042 $[M + Cl]^-$ (calcd. for $C_{20}H_{32}O_3Cl$, 355.2039) for **3**. Their ^1H and ^{13}C NMR data (Tables 1 and 2) were similar to those of compound **1**, indicative of the same planar construction of **2** and **3** as **1**. The major difference was the absence of the carbonyl at C-3 (δ_C 217.3) in **1** and the presence of an oxygenated methine signal at δ_C 75.7 (C-3) in **2** and δ_C 78.7 (C-3) in **3**, which was further confirmed by the key HMBC correlation from H-20 [δ_H 0.91, (3 H, s)] to C-3 [δ_C 75.7 (d)] in **2** and from H-20 [δ_H 0.83 (3 H, s)] with C-3 [δ_C 78.7 (d)] in **3**. On the basis of the ROESY correlations (Fig. 3), the relative configuration of **2** was determined to be the same as those of **1** and H-3 was assigned to be β-orientated by the key NOE of H-3 [δ_H 3.47 (1H, dd, J = 2.7, 2.9 Hz)]/H-20. Compound **3** has similar configuration as compound **2** except for the hydroxyl group at C-3. The β-configuration of 3-OH in **3** was established by the key NOE of H-3 [δ_H 3.27 (1H, dd, J = 4.5, 11.8 Hz)]/H-19 [δ_H 1.02 (1H, s)]. Thus, compounds **2** and **3** were assigned as shown, and named excocarinols B and C, respectively.

Excocarinol D (**4**) was given the molecular formula $C_{20}H_{28}O$ with 7° of unsaturation based on its HREIMS *m/z* 284.2140 $[M]^+$ (calcd. 284.2140) and NMR spectroscopic data (Tables 1 and 2). Analysis of its ^{13}C NMR and DEPT spectra (Table 2) showed the presence of 20 carbon resonances, including four methyls, five methylenes (one olefinic), five methines (one oxygenated, two aromatic, and one olefinic), and six quaternary carbons. The NMR data (Tables 1 and 2) of **4** were similar to those of (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol [25], a cleistanthane diterpenoid, except for the additional signal at δ_C 78.7 (d, C-3) in **4** instead of δ_C 65.7 (t, C-19) in (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol, indicating that the hydroxyl group at C-19 in (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol was moved to C-3 in **4**. This was also confirmed by the ^1H–^1H COSY (Fig. 2) correlations of H-2 [δ_H 1.82 (1H, m) and 1.77 (1H, m)] with H-3 [δ_H 3.30 (1H, dd, J = 4.7, 11.5 Hz)] and the HMBC correlations from H-3 to C-4 [δ_C 38.9 (s)] and from H-18 (δ_H 0.88, 3H, s) to C-3. The α-orientation of H-5 and β-orientation of 20-Me in **4** were determined to be the same as those in (4*R*,5*S*,10*S*)-cleistantha-8,11,13-trien-19-ol [25]. The β-orientation of 3-OH was elucidated by the correlations of H-3 with H-5 [δ_H 1.29 (1 H, dd, J = 2.0, 12.5 Hz)] and H-19 [δ_H 1.07

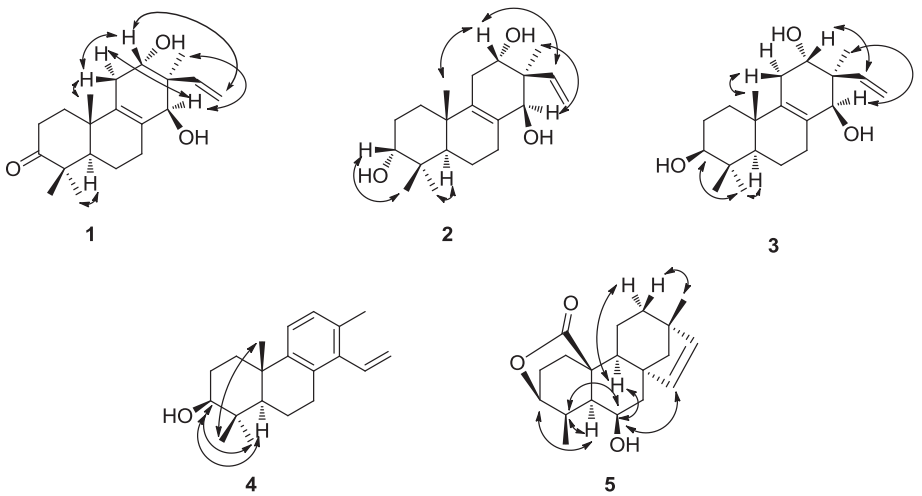

Fig. 3. Key ROESY (↔) correlations of **1**–**5**.

Table 3
Summary of anti-HIV-1 of compounds **1**, **3**, **4**, and **5**.

No.	Cytotoxicity IC_{50} (μM)	Anti-HIV-1activity EC_{50} (μM)	Selectivity index SI (IC_{50}/EC_{50})
1	>628.93	5.58	>112.71
3	163.75	60.13	2.72
4	280.21	57.73	4.85
5	110.66	11.14	9.93
3′-Azido-3′-deoxythymidine	3676.20	0.00951	386561.51

(3H, s)] in the ROESY experiment (Fig. 3). Thus, compound **4** was assigned as shown, and named excocarinol D.

Excocarinol E (**5**) was obtained as white powder as well and its molecular formula $C_{19}H_{26}O_3$ representing 7° of unsaturation was assigned according to the HRESIMS positive *m/z* 303.1960 $[M + H]^+$ (calcd. for $C_{19}H_{27}O_3$, 303.1958) and NMR spectroscopic data (Tables 2 and 3). The ^{13}C NMR and DEPT spectra (Table 2) of **5** showed 19 carbon resonances, including two methyls, six methylenes, seven methines (two oxygenated and two olefinic), and four quaternary carbons (one carbonyl), which were similar to those of agallochin I [26], a nor-beyerane diterpenoid, except for the additional signals at δ_C 79.1 (d, C-3) and 174.9 (s, C-19) in **5** instead of δ_C 98.3 (s, C-3) and 68.5 (t, C-19) in agallochin I. This was reminiscent of the presence of lactone formed between C-19 and C-3 in **5** replaced for the ether structure of C-19-O-C-3 in agallochin I, which was further confirmed by the HMBC correlations from H-3 [δ_H 4.35 (1H, ddd, $J = 2.0, 2.8, 3.4$ Hz)] and H-5 [δ_H 1.12 (1H, dd, $J = 2.1, 11.2$ Hz)] to C-19 [δ_C 174.9 (s)]. The other correlations in the HMBC and ^1H–^1H COSY spectrum (Fig. 2) also supported the atom connectivities in compound **5**. The relative configuration of **5** was established from the ROESY experiment (Fig. 3). The α-orientation of H-5 in **5** was hypothetically assigned to be the same as that of agallochin I. The α-orientations of H-4, H-6, and H-9 were elucidated by NOE of H-5/H-4 [δ_H 2.19 (1H, m)], H-4/H-6 [δ_H 3.40 (1 H, ddd, $J = 4.0, 11.0, 11.2$ Hz)] and H-6/H-9 [δ_H 1.85 (1H, dd, $J = 4.0, 12.8$ Hz)], respectively. The α-orientation of H-3 was deduced by the key NOE of H-5/H-3 [δ_H 4.35 (1H, ddd, $J = 2.0, 2.8, 3.4$ Hz)], which accordingly assigned the β-orientation of C-19. The α-orientations of C-15 and C-16 were determined by the key NOE of H-6/H-15 [δ_H 5.71 (1H, d, $J = 5.6$ Hz)]. Thus, compound **5** was assigned as shown, and named excocarinol E.

Four compounds **1**, **3**, **4**, and **5** isolated here were evaluated for their *in vitro* anti-HIV-1 activity using previously-described method [22,23] (Table 3). Compound **1** exhibited moderate anti-HIV-1 activity at the levels of EC_{50} 5.58 μM and SI over 112.71, though its homologue **3** and other two isolates showed low activity. This may propel the research of pimarane type compounds to inhibit HIV.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Acknowledgments

This work was financially supported by the Special Fund for Agro-scientific Research in the Public Interest (201303117), the National Support Science and Technology Subject (2013BAI11B04), and the Fundamental Scientific Research Funds for CATAS (1630052013001; ITBB110301). The authors thank Dr Y. L. Huang and Prof. Z. H. Jiang for initial proofreading of this paper and the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the spectral measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.09.007.

References

[1] Wu CY, Lu AM, Tang YC, Rui CZ, Zhu LD. The families and genera of angiosperms in China. Beijing: Science Press; 2003 586–8.
[2] Wiriyachitra P, Hajiwangoh H, Boonton P, Adolf W, Opferkuch HJ, Hecker E. Investigations of medicinal plants of *Euphorbiaceae* and *Thymelaeaceae* occurring and used in Thailand; II. Cryptic irritants of the diterpene ester type from three *Excoecaria* species. Planta Med 1985;51:368–71.
[3] Yunnan Istitute of Meteria Medica. The annals of National Medicine in Yunnan. Kunming: The Nationalities Publishing House of Yunnan; 2009 217–8.
[4] Lin JH, Tanaka T, Nonaka G, Nishioka I, Chen IS. Tannins and related compounds. XCVIII. Structures of three new dimeric ellagitannins, excoecarianin and excoecarinins A and B, isolated from the leaves of *Excoecaria kawakamii* Hayata. Chem Pharm Bull 1990;38:2162–71.
[5] Hu J, Zhang L-C, Zhao Q-S. Chemical constituents of *Excoecaria acerifclia*. China J Chin Mater Med 2011;36:1969–74.
[6] LI ZY, Ma C, Huang J. Chemical constituents from *Excoecaria acerifclia* F. Didr. and their antitumor activities. China J Chin Mater Med 2009;44:1294–7.
[7] Kawashima T, Takahashi T, Inoue Y, Kodama M, Ito S. Constituents of *Excoecaria agallocha*. Phytochemistry 1971;10:3308–9.
[8] Li X, Li MY, Zheng YN, Lin WH. Structure elucidation of taraxerone isolated from mangrove *Excoecaria agallocha*. Chin J Magn Reson 2006;23:451–6.
[9] Zou JH, Dai J, Chen X, Yuan JQ. Pentacyclic triterpenoids from leaves of *Excoecaria agallocha*. Chem Pharm Bull 2006;54:920–1.
[10] Liang QL, Dai CC, Jiang JH, Tang YP, Duan JA. A new cytotoxic casbane diterpene from *Euphorbia pekinensis*. Fitoterapia 2009;80:514–6.
[11] Wu XD, Zhang LC, He J, Li GT, Ding LF, Gao X, et al. Two new diterpenoids from *Excoecaria acerifolia*. J Asian Nat Prod Res 2013;15:151–7.
[12] Zhao YL, He QX, Li Y, Wang SF, Liu KC, Yang YP, et al. Chemical constituents of *Excoecaria acerifolia* and their bioactivities. Molecules 2010;15:2178–86.
[13] Huang SZ, Ma QY, Fang WW, Xu FQ, Peng H, Dai HF, et al. Three new isopimarane diterpenoids from *Excoecaria acerifolia*. J Asian Nat Prod Res 2013;15:750–5.
[14] Anjaneyulu ASR, Rao VL, Sreedhar K. Agallochins J-L, new isopimarane diterpenoids from *Excoecaria agallocha* L. Nat Prod Res 2003;17:27–32.
[15] Bruno M, Savona G, Fernandez-Gadea F, Rodriguez B. Diterpenoids from *Salvia greggii*. Phytochemistry 1986;25:475–7.
[16] Thongphasuk P, Suttisri R, Bavovada R, Verpoorte R. Two new pimarane diterpenoids from *Strychnos vanprukii* Craib. Nat Prod Res 2006;20:966–8.

[17] Konishi T, Azuma M, Itoga R, Kiyosawa S, Fujiwara Y, Shimada Y. Three new labdane-type diterpenes from wood, *Excoecaria agallocha*. Chem Pharm Bull 1996;44:229–31.
[18] Zuo GY, He HP, Wang BG, Hong X, Hu YM, Hao XJ. A new diterpene from *Spiraea japonica* var. *ovalifolia*. Chin Chem Lett 2003;14:383–4.
[19] Sy LK, Brown GD. Abietane diterpenes from *Illicium angustisepalum*. J Nat Prod 1998;61:907–12.
[20] Strigina LI, Chetyrina NS, Isakov VV, Dzizenko AK, Elyakov GB. Caulophyllogenin. Novel triterpenoid from roots of *Caulophyllum robustum*. Phytochemistry 1974;13:479–80.
[21] Bhattacharya AK, Dutta HK. Rubusic acid, a new triterpene from *Rubus moluccanus*. J Indian Chem Soc 1969;46:381–2.
[22] Huang SZ, Zhang XJ, Li XY, Jiang HZ, Ma QY, Wang PC, et al. Phenols with Anti-HIV Activity from *Daphne acutiloba*. Planta Med 2012;78:182–5.
[23] Huang SZ, Zhang XJ, Li XY, Kong LM, Jiang HZ, Ma QY, et al. Daphnane-type diterpene esters with cytotoxic and anti-HIV-1 activities from *Daphne acutiloba* Rehd. Phytochemistry 2012;75:99–107.
[24] Reed LJ, Muench H. A simple method for estimating fifty percent endpoints. Am J Hyg 1938;27:493–7.
[25] Gao F, Wang H, Mabry TJ, Abboud KA, Simonsen SH. Sesquiterpene lactones, flavanones and a diterpene acid from *Viguiera laciniata*. Phytochemistry 1989;28:2409–14.
[26] Anjaneyulu ASR, Rao VL, Sreedhar K. Ent-kaurane and beyerane diterpenoids from *Excoecaria agallocha*. J Nat Prod 2002;65:382–5.