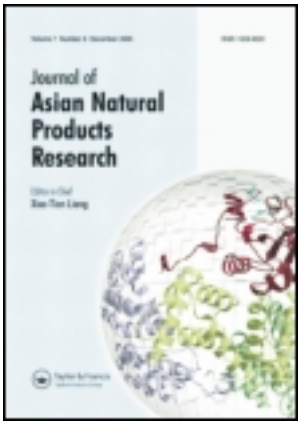

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Three new isopimarane diterpenoids from *Excoecaria acerifolia*

Sheng-Zhuo Huang[a], Qing-Yun Ma[a], Wei-Wei Fang[b], Feng-Qing Xu[b], Hua Peng[b], Hao-Fu Dai[a], Jun Zhou[b]* and You-Xing Zhao[a]*

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Three new isopimarane diterpenoids named excoecarins F–H (**1**–**3**) 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 bioactivity test of **1** and **2** showed weak activity.

Keywords: *Excoecaria acerifolia*; isopimarane; diterpenoid

1. Introduction

Excoecaria acerifolia Didr. (Euphorbiaceae family) is an epibiotic, widely distributed in the dry hot valleys in Yunnan and Sichuan provinces [1]. Its fruits and leaves are used as an ethnodrug Gua-jing-ban by minority nationalities in Southwest China for laxative, antidote, anti-malaria, and anti-virus purposes [2]. Previously, only three diterpenoids and phenols were found in *E. acerifolia*, and there was no anti-virus research on this species [3–6]. To search for new bioactive compounds, we studied the chemical composition of *E. acerifolia*. Three new isopimarane diterpenoids named excoecarins F–H (**1**–**3**) were isolated. Two compounds **1** and **2** were tested for anti-HIV-1 activities, which exhibited weak activity. Herein, the isolation process and the structural elucidation of the new diterpenoids excoecarins F–H (**1**–**3**) (Figure 1) and the anti-HIV-1 activities of **1** and **2** are described.

2. Results and discussion

Excoecarin F (**1**) was obtained as colorless needle crystals. Its molecular formula, $C_{20}H_{30}O_3$ with six degrees of unsaturation, was assigned from its positive HR-ESI-MS (*m/z* 341.2093 $[M + Na]^+$, calcd for $C_{20}H_{30}O_3Na$, 341.2092) and NMR spectroscopic data (Tables 1 and 2). The IR spectrum displayed the presence of hydroxyls (3529, 3443 cm^{-1}), carbonyl (1728 cm^{-1}), and double bonds (1686, 1457 cm^{-1}). The analysis of the ^{13}C NMR and DEPT spectroscopic data (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 isopimarane diterpenoid as agallochin J [7]. The differences between **1** and agallochin J were the additional signals at δ_C 137.7 (s, C-9) and 29.4 (t, C-7) in **1** and the absence of signals at δ_C 54.3 (d, C-9) and 126.1 (d, C-7) in agallochin J, indicating

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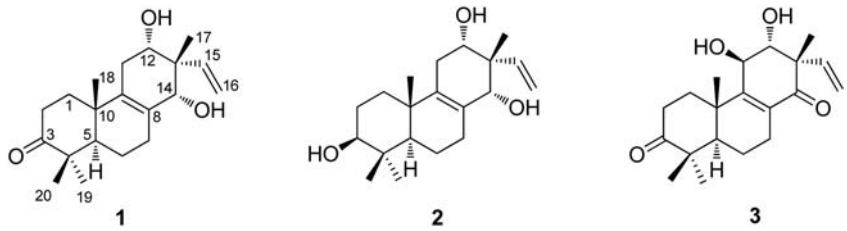

Figure 1. Structures of **1**–**3**.

that the double bond at C-7/C-8 in agallochin J moved to C-8-C-9 in **1**. The ^{1}H–^{1}H COSY (Figure 2) cross-peaks of H-6 [δ_H 1.64 (1H, m) and 1.79 (1H, m)]/H-7 [1.97 (1H, m) and 2.59 (1H, m)] and the HMBC correlation from H-7 to C-9 [δ_C 137.7 (s)] confirmed the hypothesis. The other difference lied in the hydroxyl group linked to C-12 in **1** instead of C-11 in agallochin J, which was determined by the HMBC (Figure 2) correlations from H-11 [δ_H 1.87 (1H, dd, $J = 16.4$, 9.7 Hz) and 2.40 (1H, dd, $J = 16.4$, 5.7 Hz)] to C-8 [δ_C 128.3 (s)], from H-12 [δ_H 3.82 (1H, dd, $J = 9.7$, 5.7 Hz)] to C-14 [δ_C 76.5 (d)], and from H-17 [δ_H 1.23 (3 H, s) to C-12 [δ_C 70.2 (d)]. The other correlations in the HMBC spectrum further confirmed the atom connectivities in compound **1**. The configuration of isopimarane diterpene skeleton in compound **1** was determined to be α-orientations of H-5 and 14-OH, and (-orientations of 17-Me and 18-Me by ROESY experiment (Figure 3). The α-orientation of 12-OH was determined by the key NOE of H-12/H-17 (Figure 3). Thus, compound **1** was assigned as shown and named excoecarin F.

Excoecarin G (**2**) was isolated as colorless powder. The molecular formula $C_{20}H_{32}O_3$ was defined based on its positive HR-ESI-MS at *m/z* 343.2248

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** ($CDCl_3$, δ in ppm, J in Hz, 400 MHz).

C	1	2	3
1α	1.99 m	1.74 m	2.73 ddd (16.0, 5.5, 4.6)
β	1.68 m	1.17 m	2.48 ddd (16.0, 6.5, 3.6)
2β	2.62 m	1.69 m	2.84 m
α	2.48 m	1.61 m	1.80 m
3		3.23 dd (11.5, 4.5)	
5	1.67 dd (12.3, 5.5)	1.09 dd (11.2, 1.3)	1.67 dd (12.4, 2.5)
6α	1.79 m	1.75 m	1.73 m
β	1.64 m	1.49 m	1.59 m
7α	2.59 m	2.48 m	2.64 m
β	1.97 m	1.91 m	2.09 m
11β	2.40 dd (16.4, 5.7)	2.34 m	4.43 d (8.4)
α	1.87 dd (16.4, 9.7)	1.81 m	
12	3.82 dd (9.7, 5.7)	3.77 dd (9.7, 5.8)	3.63 d (8.4)
14	3.61 s	3.65 s	
15	5.77 dd (16.5, 11.1)	5.75 dd (16.4, 11.1)	5.91 dd (17.6, 10.8)
16	5.20 dd (11.1, 1.0)	5.17 dd (11.1, 1.0)	5.20 dd (10.8, 1.1)
	5.15 dd (16.5, 1.0)	5.12 dd (16.4, 1.0)	4.96 dd (17.6, 1.1)
17	1.23 s	1.19 s	1.34 s
18	1.12 s	0.98 s	1.45 s
19	1.11 s	1.00 s	1.14 s
20	1.07 s	0.79 s	1.14 s

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** ($CDCl_3$, δ in ppm, 100 MHz).

C	1	2	3	C	1	2	3
1	34.6 t	34.3 t	34.4 t	11	31.3 t	31.3 t	72.0 d
2	34.2 t	27.4 t	33.7 t	12	70.2 d	70.3 d	79.8 d
3	217.2 s	78.7 d	216.9 s	13	45.2 s	45.1 s	55.0 s
4	47.2 s	38.8 s	47.8 s	14	76.5 d	76.8 d	199.4 s
5	50.9 d	50.3 d	51.8 d	15	138.7 d	138.9 d	135.2 d
6	19.8 t	18.6 t	18.9 t	16	116.7 t	116.7 t	117.9 t
7	29.4 t	29.8 t	25.1 t	17	18.2 q	18.2 q	19.1 q
8	128.3 s	127.3 s	132.0 s	18	19.8 q	19.6 q	19.7 q
9	137.7 s	139.4 s	157.8 s	19	26.5 q	28.0 q	27.8 q
10	37.0 s	37.3 s	39.0 s	20	21.2 q	15.5 q	21.9 q

$[M + Na]^+$. Compound **2** had similar structure as **1** based on the analysis of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2), and the major difference was the presence of an oxygenated methine signal at δ_C 78.7 (C-3) in **2** instead of the carbonyl C-3 (δ_C 217.2) in **1**, which was further confirmed by the key HMBC correlation from H-20 (δ_H 0.79, s) to C-3. Compound **2** had the same configuration as **1** in view of their similar ROESY correlations. The β-configuration of 3-OH in **2** was established by NOE of H-3 [δ_H 3.23 (1H, dd, $J = 11.5$, 4.5 Hz)]/H-5 [δ_H 1.09 (1H, dd, $J = 11.2$, 1.3 Hz)]. Thus, compound **2** was assigned as shown and named excoecarin G.

Excoecarin H (**3**) was isolated as colorless powder too. Its molecular formula $C_{20}H_{28}O_4$ was determined by the HR-EI-MS at *m/z* 332.1911 $[M]^+$. Its ^{13}C NMR spectroscopic data (Table 2) were similar to those of **1**, suggesting that **3** had the isopimarane diterpene skeleton as **1**. The differences were that the hydroxylated methine of C-14 in **1** was oxidized to a carbonyl (δ_C 199.4) in **3** and the methylene (CH_2) unit of C-11 in **1** was hydroxylated to a methine (δ_C 72.0) in **3**. This was confirmed by the HMBC correlations (Figure 2) from H-15 [δ_H 5.91 (1H, dd, $J = 17.6$, 10.8 Hz)], H-17 [δ_H 1.34 (s)], and H-7 [δ_H 2.64 (m) and 2.09 (m)] to C-14 and from H-11 [δ_H 4.43 (1H, d, $J = 8.4$ Hz)] to C-12 [δ_C 79.8 (d)] and C-8 [δ_C 132.0 (s)]. The relative configuration of **3** was the same as in **1** based on the ROESY experiment. The β-configuration of 11-OH was determined by the key NOE of H-11/H-15 (Figure 3). Thus, compound **3** was assigned as shown and named excoecarin H.

Two new compounds **1** and **2** were evaluated for their *in vitro* anti-HIV-1 activity using previously described method [8,9]. Compounds **1** and **2** showed weak activities with IC_{50} (cytotoxity) >628.93 and 92.31 μM, EC_{50} (anti-HIV-1

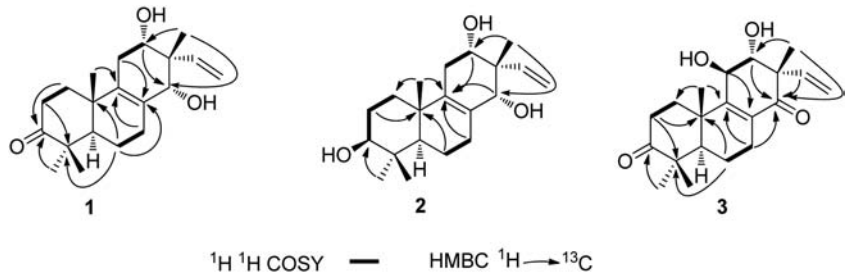

Figure 2. Key ^1H–^1H COSY and HMBC correlations of **1**–**3**.

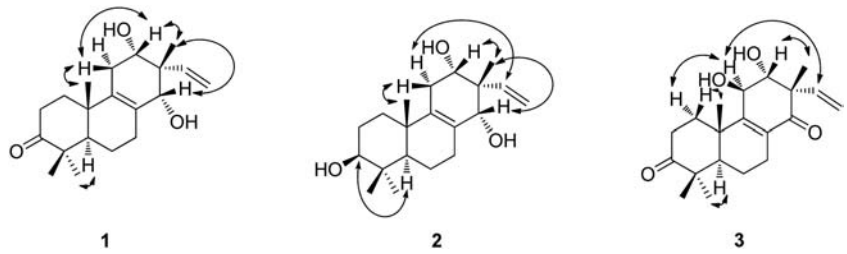

Figure 3. Key ROESY (↔) correlations of **1**–**3**.

activity) 169.64 and 21.97 μM, [IC_{50}/EC_{50}, selectivity index (SI)] > 3.71 and 4.20, respectively, whereas the positive standard 3′-azido-3′-deoxythymidine (AZT) showed IC_{50} 3676.20 μM, EC_{50} 0.00951 μM, and SI 386561.51.

3. Experimental

3.1 General experimental procedures

Melting points were recorded on a WRX-4 (Shanghai YCYQ, Shanghai, China). Optical rotations were measured on a P-1020 polarimeter (Jasco, Easton, MD, USA). UV spectra were obtained on a double-beam 210A spectrometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Tensor 27 spectrometer (Bruker, Billerica, Germany) with KBr pellets. NMR spectra were recorded on a AV-400 spectrometer (Bruker) with tetramethylsilane as an internal standard. ESI-MS and HR-ESI-MS were recorded with HCT/Esquire HPLC-Iron Trap spectrometer and API QSTAR Pulsar 1 spectrometer (Bruker). EI-MS and HR-EI-MS were recorded with a Autospec Premier (Waters, Milford, MA, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), RP-18 (40–70 μm, Fuji Silysia Chemical Ltd, Kasugai, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used for column chromatography. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatography with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Fractions were monitored by thin-layer chromatography, and spots were visualized by heating after spraying with 5% H_2SO_4 in ethanol (b.p. 77–79°C).

3.2 Plant material

The stems of *E. acerifolia* were collected from Dali (Yunnan Province, 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, China.

3.3 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 liters). The extract was then concentrated and suspended in water, followed by successive partition with petroleum ether (3 × 5 liters) and EtOAc (3 × 5 liters), respectively. The EtOAc extract (305 g) was separated by silica gel column using a gradient solvent $CHCl_3$/-MeOH (9:1–3:1 × 10 liters) to afford fractions A–C. Fraction B (101 g) was separated by silica gel column (ϕ16 × 160 cm) using a gradient solvent petroleum ether/EtOAc (10:1–1:2) to afford fractions B1–B4. B1–B4 were depigmented on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to give fractions B1a–B4a, respectively. Fraction B2a

(4.7 g) was subjected to repeated RP-18 (MeOH/H_2O, 3:7–8:1) and semi-preparative HPLC (MeOH/H_2O, 50:50, 1.0 ml/min, 230 nm, $t = 12.1$ min) to yield **1** (14.6 mg); fraction B3a (1.8 g) was subjected to repeated RP-18 (MeOH/H_2O, 3:7–9:1) and silica gel column (petroleum ether/EtOAc, 2:1) to yield **2** (10.2 mg); and fraction B4a (794 mg) was subjected to repeated RP-18 (MeOH/H_2O, 3:7–7:3) and semi-preparative HPLC (MeOH/H_2O, 45:55, 1.3 ml/min, 230 nm, $t = 14.2$ min) to yield **3** (11.5 mg).

*3.3.1 12α,14α-Dihydroxyl-3-oxo-isopimara-8(9),15-diene (excoecarin F) (**1**)*

Colorless needle crystal; mp. 190–193°C; $[\alpha]_D^{18}$ − 77.03 ($c = 1.30$, MeOH); UV (MeOH) λ_{max} (log ε) 197 (2.49), 206 (2.49), 214 (2.60), 252 (2.25), 290 (1.99) nm; IR (KBr) ν_{max} 3529, 3443, 2979, 2955, 2935, 2869, 1728, 1686, 1637, 1457, 1382, 1289, 1273, 1206, 1117, 1076, 1007, 910 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; positive ESI-MS: *m/z* 319 (10) $[M + H]^+$; HR-EI-MS: *m/z* 341.2093 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2092).

*3.3.2. 3β,12α,14α-Trihydroxyl-isopimara-8(9),15-diene (excoecarin G) (**2**)*

Colorless amorphous powder; $[\alpha]_D^{12}$ − 41.5 ($c = 3.96$, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.65) nm; IR (KBr) ν_{max} 3433, 2963, 2933, 2871, 1724, 1633, 1456, 1379, 1287, 1127, 1091, 1032, 1008 cm^{-1}; ^1H and ^{13}C NMR spectral data see Tables 1 and 2; ESI-MS positive *m/z* 343 (20) $[M + Na]^+$; HR-ESI-MS: *m/z* 343.2248 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2249).

*3.3.3. 13β,12α-Dihydroxyl-3,14-dioxo-isopimara-8(9),15-diene (excoecarin H) (**3**)*

Colorless amorphous powder; $[\alpha]_D^{32}$ − 43.33 ($c = 1.20$, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.66), 205 (3.56) nm; IR (KBr) ν_{max} 3443, 2958, 2933, 2873, 1725, 1706, 1667, 1632, 1446, 1415, 1287, 1118, 1074 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; negative ESI-MS: *m/z* 367 (100) $[M + Cl]^-$; HR-EI-MS: *m/z* 332.1911 $[M]^+$ (calcd for $C_{20}H_{28}O_4$, 332.1988).

3.4 Anti-HIV assays

The anti-HIV activity 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 [8,9]. AZT 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 using Reed and Muench method [10]. The SI was calculated from the ratio of IC_{50}/EC_{50}.

Acknowledgments

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References

[1] C.Y. Wu, A.M. Lu, Y.C. Tang, C.Z. Rui, and L.D. Zhu, *The Families and Genera of Angiosperms in China* (Science Press, Beijing, 2003), p. 586.

[2] Yunnan Istitute of Meteria Medica, *The Annals of National Medicine in Yunnan* (The Nationalities Publishing House of Yunnan, Kunming, 2009), p. 217.

[3] Z.Y. Li, C. Ma, and J. Huang, *Chin. Pharm. J.* **44**, 1294 (2009).

[4] Y.L. Zhao, Q.X. He, Y. Li, S.F. Wang, K.C. Liu, Y.P. Yang, and X.L. Li, *Molecules* **15**, 2178 (2010).

[5] Z.Y. Li, C. Ma, and J. Huang, *Chin. J. Chin. Mat. Med.* **35**, 1145 (2010).
[6] J. Kang, R.Y. Chen, and D.Q. Yu, *J. Asian Nat. Prod. Res.* **7**, 729 (2005).
[7] A.S.R. Anjaneyulu, V.L. Rao, and K. Sreedhar, *Nat. Prod. Res.* **17**, 27 (2003).
[8] S.Z. Huang, X.J. Zhang, X.Y. Li, H.Z. Jiang, Q.Y. Ma, P.C. Wang, Y.Q. Liu, J.M. Hu, Y.T. Zheng, J. Zhou, and Y.X. Zhao, *Planta Med.* **78**, 182 (2012).
[9] S.Z. Huang, X.J. Zhang, X.Y. Li, L.M. Kong, H.Z. Jiang, Q.Y. Ma, Y.Q. Liu, J.M. Hu, Y.T. Zheng, Y. Li, J. Zhou, and Y.X. Zhao, *Phytochemistry* **75**, 99 (2012).
[10] L.J. Reed and H. Muench, *Am. J. Hyg.* **27**, 493 (1938).