Chemical Constituents from the Stems of *Excoecaria acertiflia*

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Six new compounds, including two diterpenoids excocarinols F and G (**1** and **2**, resp.), two carotane (daucane) sesquiterpenoids excoecafolinols A and B (**3** and **4**, resp.), one lignanoid compound, excoecanol A (**5**), and one simple phenol, excoecanol B (**6**), together with 17 known compounds, were isolated from the BuOH extract of *Excoecaria acerifolia* DIDR. stems. Their structures were elucidated through the analysis of the spectroscopic data. The AChE-inhibitory activities of 17 compounds were evaluated and revealed that four of them possessed moderate inhibitory activities against AChE.

Introduction. – As terminator of synaptic transmission, AChE is found at main neuromuscular junctions and cholinergic nervous synapses [1]. It provided a good approach to treat and relieve *Alzheimer*'s disease (AD) [2]. Nowadays, two types of natural AChE inhibitors, galantamine and huperzine, display a commercial potential in the treatment of AD [3], suggesting that natural products may present a promising source for finding effective AChE inhibitors [4][5]. *Excoecaria acerifolia* DIDR. (Euphorbaceae), distributed in hot valleys of Southwest China, is used as a folk medicine due to its antiphlogistic, antidote, antitussive, laxative, antivirus, and resuscitation-inducing properties by minority inhabitants in Yunnan Province [6]. Previously, constituents of the AcOEt extract of *E. acerifolia* were studied, and mainly terpenoids and phenols were isolated [7–10]; however, investigation of the BuOH extract from this plant was rarely carried out. In our continuing study of the BuOH extract, six new compounds, together with 17 known ones, were isolated. Herein, the isolation and structure elucidation of the new compounds are described, as well as the AChE-inhibitory activity of some isolates.

Results and Discussion. – Searching for new bioactive compounds, the BuOH extract of *E. acerifolia* stems was investigated. Six new compounds (*Fig. 1*), including the two diterpenoids excocarinols F and G (**1** and **2**, resp.), the two carotane (daucane) sesquiterpenoids excoecafolinols A and B (**3** and **4**, resp.), the lignanoid compound excoecanol A (**5**), and one simple phenol, excoecanol B (**6**), together with 17 known compounds, 1,4-bis(4-hydroxy-3-methoxyphenyl)-2,3-bis(hydroxymethyl)butane-1,4-diol [11], (−)-silandrin B [12], (−)-(7*S*,8*R*,8′*R*)-7-hydroxysecoisolariciresinol [13],

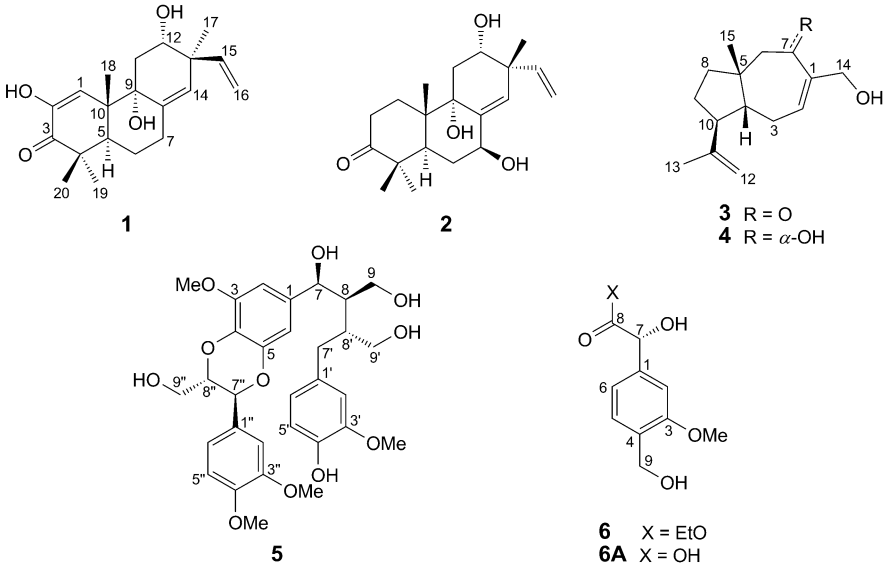

Fig. 1. *Structures of compounds* **1**–**6**, *and* **6A**

(+)-cyclolariciresinol [13], secoisolariciresinol [14], isolariciresinol-4′-ether [14], (7*S*,8*S*)-nitidanin [15], (7*S*,8*S*)-5-hydroxynitidanin [15], nocomtol [16], americanin A [17], integracin B [18], (*R*)-4,7-dihyroxy-3-methoxybenzeneacetic acid ethyl ester [19], (*S*)-4,7-dihydroxy-3-methoxybenzeneacetic acid ethyl ester [19], 1,2-bis(4-hydroxy-3-methoxyphenyl)propane-1,3-diol [20], cinchonain Ib [21], ethyl (+)-3-hydroxycyanidane-8-carboxylate [22], and linarigenin [23].

Excocarinol F (**1**), isolated as colorless powder, had the molecular formula $C_{20}H_{28}O_4$, with seven degrees of unsaturation, as deduced from its HR-ESI-MS (positive-ion mode; *m*/*z* 355.1877 ($[M+Na]^+$, $C_{20}H_{28}NaO_4^+$; calc. 355.1885)) and NMR data (*Table 1*). The IR spectrum displayed OH (3418 cm^{-1}), C=O (1711 cm^{-1}), and C=C (1671 and 1649 cm^{-1}) absorptions. The ^{13}C-NMR and DEPT spectra (*Table 1*) showed 20 C-atom resonances, including those of four Me, four CH_2 (one olefinic), five CH groups (one O-bearing and three olefinic), and seven C-atoms (one C=O and two olefinic). A detailed analysis of its 2D-NMR data indicated that **1** had a pimarane skeleton, like excocarinol A [8]. Comparing their ^{13}C-NMR data, the major differences consisted in the position of the C-atom signals at δ(C) 123.2 (*d*, C(1)) and 145.0 (*s*, C(2)) of **1** instead of the signals at δ(C) 35.1 (*t*, C(1)) and 34.2 (*t*, C(2)) in excocarinol A, indicating that **1** had a hydroxylated C(1)=C(2) bond. This was confirmed by the HMBCs (*Fig. 2*) from H–C(1) (δ(H) 6.31 (*s*)) and Me(19) (δ(H) 1.26 (*s*)) to C(3) (δ(C) 200.6 (*s*)), and from Me(18) (δ(H) 1.09 (*s*)) to C(1). The other difference was that the signals at δ(C) 78.0 (*d*, C(14)) and 136.7 (*s*, C(9)) in excocarinol A shifted to δ(C) 126.3 (*d*, C(14)) and 73.8 (*s*, C(9)) in **1**, evidencing that the OH group at C(14) in excocarinol A was moved to C(9) in **1**, and the (*E*)–C(8)=C(9) bond was shifted to C(8)=C(14) in **1**. This was confirmed by the key HMBCs from Me(18) and

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	6.31 (*s*)	123.2 (*d*)	2.58–2.63 (*m*, H_α), 2.40–2.44 (*m*, H_β)	34.6 (*t*)
2		145.0 (*s*)	2.32–2.35 (*m*, H_β), 2.11–2.15 (*m*, H_α)	31.1 (*t*)
3		200.6 (*s*)		216.5 (*s*)
4		42.9 (*s*)		47.4 (*s*)
5	2.76 (*dd*, J=3.0, 12.4, 1 H)	42.1 (*d*)	2.59 (*dd*, J=5.5, 12.3, 1 H)	39.4 (*d*)
6	1.57–1.62 (*m*, H_α), 1.51–1.58 (*m*, H_β)	21.5 (*t*)	1.87–1.89 (*m*, H_α), 1.68–1.72 (*m*, H_β)	31.0 (*t*)
7	2.66 (*ddd*, J=4.1, 5.9, 14.1, H_α), 2.26 (*ddd*, J=1.8, 4.0, 14.1, H_β)	31.8 (*t*)	4.31 (*dd*, J=2.9, 5.8)	73.7 (*d*)
8		138.4 (*s*)		138.7 (*s*)
9		73.8 (*s*)		75.5 (*s*)
10		43.6 (*s*)		37.0 (*s*)
11	2.19–2.23 (*m*, H_β), 2.17–2.20 (*m*, H_α)	31.0 (*t*)	2.12 (*dd*, J=1.9, 16.3, H_β), 1.99 (*dd*, J=3.8, 16.3, H_α)	30.6 (*t*)
12	3.84 (*dd*, J=2.1, 3.7)	71.7 (*d*)	3.83 (*dd*, J=1.9, 3.8, 1 H)	71.7 (*d*)
13		43.5 (*s*)		43.7 (*s*)
14	5.36 (*s*)	126.3 (*d*)	7.73 (*s*)	143.3 (*d*)
15	6.81 (*dd*, J=11.1, 17.7)	143.6 (*d*)	5.88 (*dd*, J=11.3, 16.7)	131.2 (*d*)
16	5.33 (*dd*, J=1.0, 11.1, 1 H), 5.18 (*dd*, J=1.0, 17.7, 1 H)	117.1 (*t*)	5.37 (*dd*, J=1.0, 11.3, 1 H), 5.30 (*dd*, J=1.0, 16.7, 1 H)	116.7 (*t*)
17	1.06 (*s*, 3 H)	25.3 (*q*)	1.11 (*s*, 3 H)	25.0 (*q*)
18	1.09 (*s*, 3 H)	19.2 (*q*)	1.08 (*s*, 3 H)	17.4 (*q*)
19	1.26 (*s*, 3 H)	26.3 (*q*)	1.18 (*s*, 3 H)	26.1 (*q*)
20	1.10 (*s*, 3 H)	22.8 (*q*)	1.13 (*s*, 3 H)	19.7 (*q*)
2-OH	6.08 (br. *s*)			

[a]) ^{1}H- and ^{13}C-NMR data recorded at 400 and 100 MHz, respectively. [b]) ^{1}H- and ^{13}C-NMR data recorded at 400 and 150 MHz, respectively.

H–C(12) (δ(H) 3.84 (*dd*, J=2.1, 3.7, 1 H)) to C(9), and from H–C(15) (δ(H) 6.81 (*dd*, J=11.1, 17.7, 1 H)) to C(14). The configuration of pimarane diterpene skeleton in compound **1**, elucidated by the ROESY experiment (*Fig. 3*), was as in excocarinol A with α-orientations of H–C(5) and Me(17), and β-orientation of Me(18). The α-configurations of HO–C(12) and Me(17) were determined by NOEs H–C(15)/H–C(12) and H–C(15)/H_β–C(11) (δ(H) 2.19–2.23 (*m*, 1 H)). The α-orientation of OH at C(9) was deduced from the key NOE Me(18)/H_β–C(11), combined with a molecular model of the pimarane diterpene skeleton. Thus, the structure of compound **1** was assigned as depicted in *Fig. 1*, and named excocarinol F.

Excocarinol G (**2**) was obtained as colorless powder and had the molecular formula $C_{20}H_{30}O_4$ based on its HR-EI-MS (*m*/*z* 334.2134 (M^+, $C_{20}H_{30}O_4^+$; calc. 334.2144). Comparison of the NMR data (*Table 1*) of **2** with those of excoecarin F [7] suggested that **2** had an isopimarane skeleton. The difference was that **2** has an O-bearing

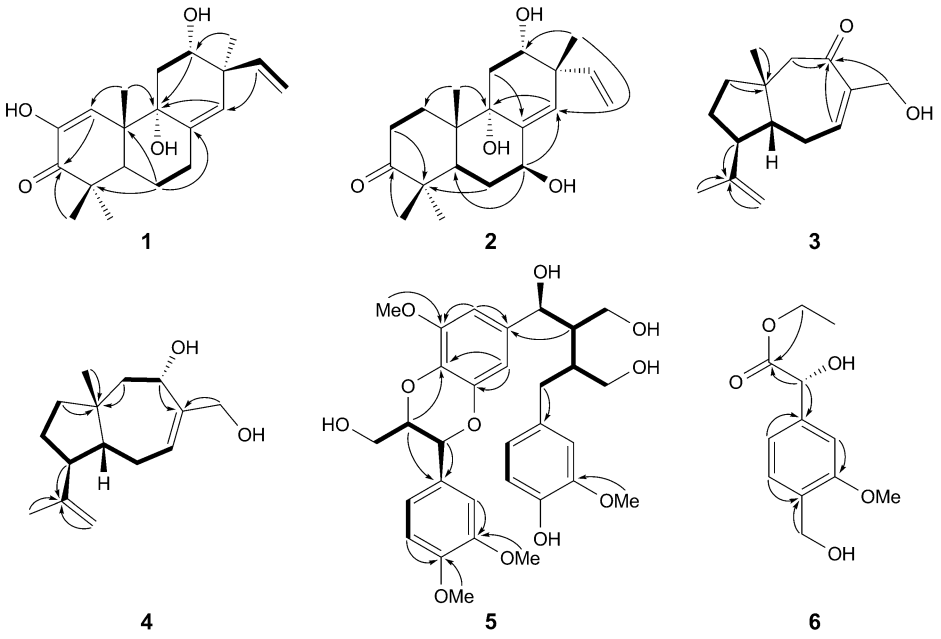

Fig. 2. *Key* $^{1}H,^{1}H$*-COSY correlations* (—) *and HMBCs* (H→C) *of* **1**

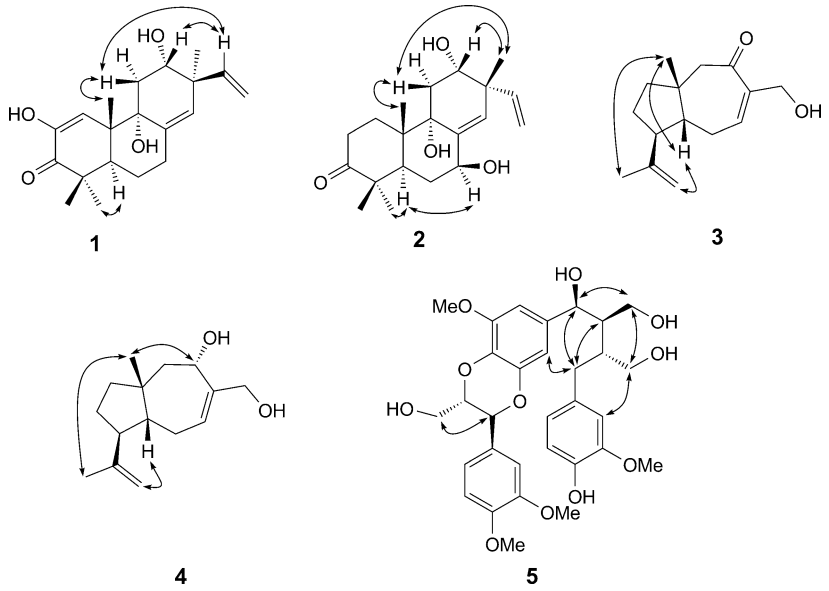

Fig. 3. *Key ROESY correlations* (H→H) *of* **1**–**5**

quaternary C-atom (δ(C) 75.5, C(9)) and one olefinic CH (δ(C)) 143.3 (*d*, C(14)) instead of the O-bearing CH group (δ(C) 76.5, C(14)) and an olefinic quaternary C-

atom (δ(C) 137.7 (*d*, C(9))) in excoecarin F. This was consistent with the OH group at C(9) and the presence of the C(8)=C(14) bond as in compound **1**. This was further confirmed by the HMBCs (*Fig. 2*) from Me(18) (δ(H) 1.08 (*s*)) to C(9), and from Me(17) (δ(H) 1.11 (*s*)) to C(14). The other CH group bearing a OH group was assigned to C(7) through the HMBCs from H–C(7) (δ(H) 4.31 (*dd*, J = 2.9, 5.8, 1 H)) to C(14) and C(5) (δ(C) 39.4). Further correlations in the HMBC and ^{1}H,^{1}H-COSY spectrum (*Fig. 2*) confirmed the atom connectivity in compound **2**. The relative configuration of the isopimarane diterpene skeleton in compound **2**, determined by the ROESY experiments (*Fig. 3*), was similar to that of excoecarin F with α-orientation of H–C(5), and β-orientations of Me(17) and Me(18). The β-orientation of OH at C(7) was determined by NOE H–C(7)/H–C(5) (δ(H) 2.59 (*dd*, J = 5.5, 12.3, 1 H)), and the α-orientation of HO–C(12) was deduced from NOE of Me(17)/H–C(12) (δ(H) 3.83 (*dd*, J = 1.9, 3.8, 1 H)). The α-configuration of OH at C(9) was deduced from the NOE Me(18)/H$_\beta$–C(11) (δ(H) 2.12 (*dd*, J = 1.9, 16.3, 1 H)) and a molecular model of the isopimarane diterpene skeleton. Thus, the structure of compound **2**, named excocarinol G, was assigned as depicted in *Fig. 1*.

Compound **3** was obtained as a colorless amorphous powder, and its molecular formula was determined as $C_{15}H_{22}O_2$ based on its HR-ESI-MS (positive-ion mode) (*m*/*z* 257.1514 ([M + Na]$^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.1517) and NMR data (*Table 2*). The IR spectrum revealed the presence of OH (3440 cm^{-1}), C=O (1724 cm^{-1}), and C=C (1646 cm^{-1}) moieties. Its ^{13}C-NMR and DEPT spectra exhibited 15 C-atom resonances, including those of two Me, six CH_2 (one O-bearing and one olefinic), and three CH groups, and four quaternary C-atoms (one C=O and two olefinic), which were similar to those of schisanwilsonene A [24], a carotane-type sesquiterpenoid. The differences were the C-atoms signals at (δ(C) 204.2 (*s*, C(7)), 146.0 (*s*, C(11)), 113.7 (*t*, C(12))) in **3** instead of the signals ats δ(C) 26.0 (*t*, C(7)), 74.4 (*d*, C(11)), 27.1 (*q*, C(12)) in schisanwilsonene A, indicating that **3** was formally derived from schisanwilsonene A by oxidation of C(7) to a C=O group, and formation of a C(11)=C(12) bond. The HMBCs (*Fig. 2*) of compound **3** from CH_2(6) (δ(H) 2.78 (*d*, J = 15.9, 1 H), 2.51 (*d*, J = 15.9, 1 H)), H–C(2) (δ(H) 6.57 (*dd*, J = 2.4, 7.2, 1 H)), CH_2(14) (δ(H) 4.22 (*d*, J = 16.9, 1 H), 4.14 (*d*, J = 16.9, 1 H)) to C(7), and from H–C(10) (δ(H) 2.97–3.02 (*m*, 1 H)), Me(13) (δ(H) 1.77 (*s*)) to C(11) and C(12) verified this hypothesis. Other HMBCs and ^{1}H,^{1}H-COSY correlations further confirmed the atom connectivities in compound **3**. The relative configuration of the carotane skeleton in compound **3** was determined to possess β-orientations of H–C(4) and Me(15), and α-orientation of H–C(10) through a ROESY experiment (*Fig. 3*). Thus, the structure of compound **3**, named excoecafolinol A, was assigned as shown in *Fig. 1*.

Compound **4** had the molecular formula $C_{15}H_{24}O_2$ based on its HR-ESI-MS (positive-ion mode) (*m*/*z* 259.1667 ([M + Na]$^+$, $C_{15}H_{24}NaO_2^+$; calc. 259.1673) and NMR data (*Table 2*). The highly similar ^{13}C-NMR and DEPT (*Table 2*) data of compound **4** to those of compound **3** suggested that **4** had the same skeleton and a similar structure as **3**. The only difference was that the C(7)=O group in **3** was replaced by a CH–O group in **4**, in accordance with the appearance of signals at δ(C) 70.2 (*d*, C(7))) and δ(H) 4.59 (*dd*, J = 1.7, 9.9, H–C(7)) in the spectra of compound **4**. The assignment of the structure of compound **4** was further confirmed by HMBCs and ^{1}H,^{1}H-COSY correlations (*Fig. 2*). The configurations at C(4), C(5), and C(10) in

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **3**, **4**, *and* **6** (in $CDCl_3$). Atom numbering as indicated in *Fig. 1.*

Position	**3**[a])		**4**[b])		**6**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		139.8 (*s*)		142.3 (*s*)		145.2 (*s*)
2	6.57 (*dd*, *J*=2.4, 7.2, 1 H)	144.4 (*d*)	5.76 (*dd*, *J*=1.5, 10.0, 1 H)	129.0 (*d*)	6.81 (*d*, *J*=2.1, 1 H)	110.4 (*d*)
3	2.43–2.49 (*m*, H_α), 2.08–2.14 (*m*, H_β)	30.4 (*t*)	2.12 (*dd*, *J*=1.5, 15.7, H_α), 1.91 (*dd*, *J*=10.0, 15.7, H_β)	26.6 (*t*)		146.6 (*s*)
4	1.83–1.89 (*m*, 1 H)	48.7 (*d*)	1.83–1.89 (*m*, 1 H)	49.9 (*d*)		145.2 (*s*)
5	–	40.7 (*s*)	–	41.9 (*s*)	6.78 (*d*, *J*=8.1, 1 H)	114.6 (*d*)
6	2.78 (*d*, *J*=15.9, H_β), 2.51 (*d*, *J*=15.9, H_α)	59.1 (*t*)	2.07 (*dd*, *J*=1.7, 13.7, H_β), 1.48 (*dd*, *J*=9.9, 13.7, H_α)	51.0 (*t*)	6.77 (*dd*, *J*=2.1, 8.1, 1 H)	121.1 (*d*)
7		204.2 (*s*)	4.59 (*dd*, *J*=1.7, 9.9, 1 H)	70.2 (*d*)	3.75 (*s*, 1 H)	53.6 (*d*)
8	1.62–1.64 (*m*, H_β), 1.47–1.51 (*m*, H_α)	42.1 (*t*)	1.55–1.59 (*m*, H_β), 1.40–1.44 (*m*, H_α)	42.3 (*t*)		173.3 (*s*)
9	1.80–1.86 (*m*, 2 H)	28.9 (*t*)	1.72–1.76 (*m*, 2 H)	27.9 (*t*)	4.09 (*d*, *J*=13.4, 1 H), 3.77 (*d*, *J*=13.4, 1 H)	64.8 (*t*)
10 or EtO	2.97–3.02 (*m*, 1 H)	50.7 (*d*)	2.88–2.96 (*m*, 1 H)	49.5 (*d*)	4.17 (*q*, 7.8, 2 H), 1.23 (*t*, 7.8, 3 H)	61.1 (*t*), 14.1 (*q*)
11 or 3-MeO		146.0 (*s*)		147.0 (*s*)	3.89 (*s*, 3 H)	55.9 (*q*)
12	4.95 (*d*, *J*=1.8, 1 H), 4.80 (*d*, *J*=1.8, 1 H)	113.7 (*t*)	4.83 (*d*, *J*=1.8, 1 H), 4.72 (*d*, *J*=1.8, 1 H)	113.2 (*t*)		
13	1.77 (*s*, 3 H)	24.1 (*q*)	1.70 (*s*, 3 H)	22.8 (*q*)		
14	4.22 (*d*, *J*=16.9, 1 H), 4.14 (*d*, *J*=16.9, 1 H)	67.8 (*t*)	4.17–4.21 (*m*, 2 H)	69.9 (*t*)		
15	0.96 (*s*, 3 H)	19.5 (*q*)	0.92 (*s*, 3 H)	18.4 (*q*)		

[a]) ^{1}H- and ^{13}C-NMR data recorded at 400 and 150 MHz, respectively. [b]) ^{1}H- and ^{13}C-NMR data recorded at 400 and 100 MHz, respectively.

Table 3. *^{1}H- and ^{13}C-NMR Data of Compound* **5**[a]). δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1		141.0 (*s*)	7′	2.90 (*dd*, *J* = 5.0, 13.1, 1 H), 2.50 (*dd*, *J* = 11.3, 13.1, 1 H)	29.5 (*t*)
2	6.66 (*d*, *J* = 1.4, 1 H)	104.0 (*d*)	8′	2.70–2.72 (*m*, 1 H)	43.8 (*d*)
3		154.4 (*s*)	9′	4.01 (*dd*, *J* = 8.0, 8.6, 1 H), 3.73–3.79 (*m*, 1 H)	73.7 (*t*)
4		135.8 (*s*)	3′-MeO	3.81 (*s*, 3 H)	56.3 (*q*)
5		154.4 (*s*)	1″		133.8 (*s*)
6	6.65 (*d*, *J* = 1.4, 1 H)	104.0 (*d*)	2″	6.98 (*d*, *J* = 1.7, 1 H)	111.3 (*d*)
7	4.84 (*d*, *J* = 6.4, 1 H)	84.0 (*d*)	3″		148.7 (*s*)
8	2.32–2.37 (*m*, 1 H)	54.1 (*d*)	4″		146.8 (*s*)
9	3.83–2.87 (*m*, 1 H), 3.67–3.71 (*m*, 1 H)	60.6 (*t*)	5″	6.73 (*d*, *J* = 7.2, 1 H)	115.7 (*d*)
3-MeO	3.82 (*s*, 3 H)	56.6 (*q*)	6″	6.76 (*dd*, *J* = 1.7, 7.2, 1 H)	120.6 (*d*)
1′	–	133.5 (*s*)	7″	4.90 (*d*, *J* = 6.7, 1 H)	74.0 (*d*)
2′	6.98 (*d*, *J* = 1.8, 1 H)	113.3 (*d*)	8″	4.22 (*ddd*, *J* = 3.2, 6.7, 7.9, 1 H)	87.4 (*d*)
3′		149.0 (*s*)	9″	3.90 (*dd*, *J* = 7.9, 11.9, 1 H), 3.56 (*dd*, *J* = 3.2, 11.9, 1 H)	61.5 (*t*)
4′		145.8 (*s*)	3″-MeO	3.81 (*s*, 3 H)	56.3 (*q*)
5′	6.71 (*d*, *J* = 8.0, 1 H)	116.2 (*d*)	4″-MeO	3.81 (*s*, 3 H)	56.3 (*q*)
6′	6.77 (*dd*, *J* = 1.8, 8.0, 1 H)	122.1 (*d*)			

[a]) ^{1}H- and ^{13}C-NMR recorded in $CDCl_3$ at 400 and 150 MHz, respectively.

compound **4** were proposed to be same as in **3** based on their similar NMR data and ROESY correlations (*Fig. 3*). The α-orientation of HO–C(7) was established by the key NOE H–C(7)/Me(15) (δ(H) 0.92 (*s*)). Thus, the structure of compound **4**, excoecafolinol B, was determined as depicted in *Fig. 1*.

Compound **5** was obtained as a colorless oil, and its molecular formula was determined as $C_{31}H_{38}O_{11}$, with 13 degrees of unsaturation, from its HR-ESI-MS (positive-ion mode; *m*/*z* 609.2330 ($[M+Na]^+$, $C_{31}H_{38}NaO_{11}^+$; calc. 609.2335) and NMR data (*Table 3*) analysis. The ^{13}C-NMR spectrum of compound **5** exhibited 31 C-atom resonances, comprising those of four MeO groups, 18 aromatic C-atoms, and nine saturated C-atoms, which were reminiscent of a dimer of two lignanoid moieties and a phenylpropanoid in **5**. The lignanoid moiety in compound **5** was proposed to be like (−)-(7*S*,8*R*,8′*R*)-7-hydroxysecoisolariciresinol [13] based on its ^{13}C-NMR data. The differences of ^{13}C-NMR data were the signals at δ(C) 135.8 (*s*, C(4)) and 154.4 (*s*, C(5)) of **5** instead of the signals at δ(C) 116.2 (*t*, C(5)) and 146.3 (*d*, C(4)) of (−)-(7*S*,8*R*,8′*R*)-7-hydroxysecoisolariciresinol. The phenylpropanoid moiety was determined as 3,4-dimethoxyphenylpropane-1,2,3-triol [16] on the basis of their similar ^{13}C-NMR data. Compound **5** was constructed by connection of above two moieties between C(4) and C(8″), and C(5) and C(7″) *via* two O-atoms, which was confirmed by the HMBCs of H–C(8″) (δ(H) 4.22 (*ddd*, *J* = 3.2, 6.7, 7.9, 1 H)) and H–C(6) (δ(H) 6.65 (*d*, *J* = 1.4, 1 H)) to C(4); of H–C(7″) (δ(H) 4.90 (*d*, *J* = 6.7, 1 H)) and H–C(8″) to C(1″) (*Fig. 2*), and comparison of the NMR data of the 1,4-dioxane unit in nocomtol [16]. The

configuration of the dioxane skeleton in **5** was determined by NOE correlations in a ROESY experiment (*Fig. 3*). The β-orientation of H–C(8″) and α-orientation of H–C(7″) were elucidated by the key NOE H–C(9″)/H–C(7″). The configurations at C(7), C(8), and C(8′) in compound **5** were proposed to be same as those of (−)-(7*S*,8*R*,8′*R*)-7-hydroxysecoisolariciresinol, based on the similarity of their NMR data. Thus, the structure compound **5**, named excoecanol A, was elucidated as shown in *Fig. 1*.

Obtained as colorless amorphous powder, compound **6** had the molecular formula $C_{12}H_{16}O_5$ based on its HR-ESI-MS (positive-ion mode; *m/z* 263.0901 ($[M+Na]^+$, $C_{12}H_{16}NaO_5^+$; calc. 263.0895) and NMR data (*Table 2*). The ^{1}H- and ^{13}C-NMR data of compound **6** were similar to those of ethyl (*R*)-4,7-dihyroxy-3-methoxybenzeneacetate [19], except for the additional signals at δ(C) 64.8 (*t*, C(9)), and δ(H) 4.09 (*d*, *J* = 13.4, 1 H of CH_2(9)) and 3.77 (*d*, *J* = 13.4, 1 H of CH_2(9)) attributed to a HO–CH_2 group in **6**. Compound **6** was derived from ethyl (*R*)-4,7-dihydroxy-3-methoxybenzeneacetate by the replacement of the OH groups at C(4) with a CH_2OH group, which was verified by the HMBCs (*Fig. 2*) from H–C(9) and H–C(5) (δ(H) 6.78 (*d*, *J* = 8.1, 1 H)) to C(4), and from MeO (δ(H) 3.89 (*s*)) to C(3) (δ(C)) 146.6 (*s*)). Other HMBCs and ^{1}H,^{1}H-COSY correlations (*Fig. 2*) further confirmed the assignment of compound **6**. The configuration at the only stereogenic center C(7) in compound **6** was determined as (*R*), based on the comparison of the $[\alpha]_D^{21} = -12.62$ (*c* = 1.50, MeOH) of **6** with $[\alpha]_D = -88.3$ (*c* = 1.50, MeOH) of an analogous homolog [19]. Thus, compound **6**, named excoecanol B, was assigned as shown in *Fig. 1*. According the isolation processes, compound **6** was most likely an outcome of the isolation process, *i.e.*, esterification of the original carboxylic acid **6A** with EtOH.

The AchE-inhibitory activities of the new compounds and eleven known compounds were evaluated. Four compounds, excocarinol F (**1**), (7*S*,8*S*)-nitidanin, (7*S*,8*S*)-5-hydroxynitidanin, and 1,2-bis(4-hydroxy-3-methoxyphenyl)propane-1,3-diol showed inhibitory activities with inhibitory index 9.4, 12.2, 27.4, and 59.0% at a concentration of 50 μM, respectively. Among these active compounds, 1,2-bis(4-hydroxy-3-methoxyphenyl)propane-1,3-diol exhibited strong inhibitory activity close to the level of the positive control tacrine (57.7%; 0.333 μM).

Conclusions. – Although highly oxygenated diterpenoids are numerous in the genus *Excoecaria*, the (iso)pimarane diterpenoids with a C(9)–OH group and carotane sesquiterpenoids were isolated for the first time from this genus. This genus is also rich on flavones, flavans, and ordinary lignanoids (dibenzylbutane type) as reported in the literature; however, special lignanoid compounds were isolated from *E. acerifolia* for the first time, revealing that *E. acerifolia* displays chemical similarity and uniqueness at the same time, when compared with other species of this genus. The evaluated AchE-inhibitory activities of the natural products from *E. acerifolia* revealed justification for its use in folk medicine and its potential for the treatment of *Alzheimer*'s disease.

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Experimental Part

General. TLC: Silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China); visualization by heating after spraying with 5% H_2SO_4 in EtOH. Column chromatography (CC): *Sephadex LH-20* (*GE Healthcare Bio-Sciences AB*, Sweden), *RP-18* (40–70 μm; *Fuji Silysia Chemical Ltd.*, Japan), and silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China). Semiprep. HPLC: *Agilent 1100* liquid chromatograph (*Zorbax SB-C18*, 9.4 mm × 25 cm, column). UV Spectra: *Shimadzu double-beam 210A* spectrometer. Optical rotations: *Horiba SEPA-300* polarimeter. IR Spectra: *Tensor 27* spectrometer; KBr pellets. NMR Spectra: *Bruker AV-400* or *AVANCE III-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS and HR-ESI-MS: *API QSTAR Pulsar 1* spectrometer; in m/z. HR-EI-MS: *API Qstar Pulsar*; in m/z.

Plant Material. Plant material was collected at Dali City Yunnan Province, P. R. China, in 2010, and identified by Prof. *H. Peng*, and Dr. *Y. Niu* (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (HUANG0006) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The powdered dry stems of *E. aceriflolia* (19 kg) were extracted with EtOH (95%; 3 × 50 l) under reflux for 2 h. The extract was concentrated and suspended in H_2O, followed by successive partition with petroleum ether (PE; 3 × 5 l), AcOEt (3 × 5 l), and BuOH (3 × 5 l), resp. The BuOH extract (1.4 kg) was separated by CC (SiO_2; $CHCl_3$/MeOH 9:1–3:1; × 10 l) to give *Frs. A–C*. *Fr. A* (69 g) was separated by CC (SiO_2; (ϕ 12 × 160 cm); PE/AcOEt 6:1 to 1:1), and subjected to repeated CC (*RP-18*; MeOH/H_2O 3:2, 4:1, 9:1, 1:0) and semiprep. HPLC (MeOH/H_2O 65:35 or 50:50) to yield **1** (4.3 mg), **2** (4.7 mg), **3** (7.4 mg), and **4** (29.8 mg). *Fr. B* (228 g) was separated by CC (SiO_2; (ϕ 16 × 160 cm); PE/AcOEt 3:1 to 1:5) to give *Frs. B1–B3*. *Fr. B1* was subjected to repeated CC (*RP-18*; MeOH/H_2O 1:4, 2:3, 3:2, 4:1, 9:1, 1:0; SiO_2; $CHCl_3$/MeOH 20:1; and *Sephadex LH20*) to give **5** (23.2 mg), **6** (4.3 mg), **7** (23.4 mg), **8** (7.9 mg), and **9** (11.0 mg). *Fr. B2* was subjected to repeated CC (*RP-18*; MeOH/H_2O 1:4, 2:3, 3:2, 4:1, 9:1, 1:0; SiO_2; $CHCl_3$/MeOH, 20:1; and *Sephadex LH20*) to furnish **10** (9.7 mg), **11** (21.0 mg), **12** (13.0 mg), and **13** (24.3 mg); *Fr. B3* was purified by repeated CC (*RP-18*; MeOH/H_2O 1:4, 2:3, 3:2, 4:1, 9:1, 1:0; SiO_2; $CHCl_3$/MeOH, 20:1; and *Sephadex LH20*) to afford **14** (9.6 mg), **15** (5.7 mg), and **16** (6.9 mg). *Fr. C* (187 g) was subjected to repeated CC (*RP-18*; MeOH/H_2O 1:4, 2:3, 3:2, 4:1, 9:1, 1:0; SiO_2; $CHCl_3$/MeOH, 10:1) to give **17** (24.8 mg), **18** (12.4 mg), **19** (7.2 mg), **20** (15.7 mg), **21** (5.5 mg), **22** (6.7 g), and **23** (13.5 mg).

Excocarinol F (*=(12α)-2,9,12-Trihydroxypimara-1,8(14),15-trien-3-one=(4a*R,*4b*R,*6*S,*7*S,*10a*R*)-7-Ethenyl-4a,4b,5,6,7,9,10,10a-octahydro-3,4b,6-trihydroxy-1,1,4a,7-tetramethyl-2(1*H*)-phenanthrenone*; **1**). Colorless amorphous powder. $[\alpha]_D^{31} = +18.64$ (c = 3.00, MeOH). UV (MeOH): 191 (3.35), 211 (3.32), 268 (3.93). IR (KBr): 3418, 2971, 2939, 2876, 1711, 1671, 1649, 1460, 1408, 1374, 1246, 1227, 1066, 1050, 1003, 972, 860. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 355 ($[M+Na]^+$). HR-ESI-MS: 355.1877 ($[M+Na]^+$, $C_{20}H_{28}NaO_4^+$; calc. 355.1885)

Excocarinol G (*=(7β,12α,13α)-7,9,12-Trihydroxypimara-8(14),15-dien-3-one=(4a*S,*4b*S,*6*S,*7*R,*9*S,*10a*R*)-7-Ethenyl-3,4,4a,4b,5,6,7,9,10,10a-decahydro-4b,6,9-trihydroxy-1,1,4a,7-tetramethyl-2(1*H*)-phenanthrenone*; **2**). Colorless amorphous powder. $[\alpha]_D^{32} = -25.51$ (c = 1.04, MeOH). UV (MeOH): 241 (3.61), 207 (3.68), 269 (4.6), 259 (4.6). IR (KBr): 3441, 2959, 2934, 2873, 1724, 1608, 1632, 1456, 1384, 1286, 1121, 1075. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (neg.): 369 ($[M+Cl]^-$). HR-EI-MS: 334.2134 (M^+, $C_{20}H_{30}O_4^+$; calc. 334.2144).

Excoecafolinol A (*=(1*S,*3a*R,*8a*R*)-2,3,3a,4,8,8a-Hexahydro-6-(hydroxymethyl)-3a-methyl-1-(1-methylethenyl)azulen-5(1*H*)-one*; **3**). Colorless amorphous powder. $[\alpha]_D^{28} = -5.76$ (c = 0.23, MeOH). UV (MeOH): 206 (3.58), 252 (3.04). IR (KBr): 3440, 2956, 2875, 1724, 1646, 1452, 1409, 1381, 1287, 1122, 1074, 1000, 889, 827, 749. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 257 ($[M+Na]^+$). HR-ESI-MS: 257.1514 ($[M+Na]^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.1517).

Excoecafolinol B (= *(1*S*,3a*R*,5*R*,8a*R*)-1,2,3,3a,4,5,8,8a-Octahydro-5-hydroxy-3a-methyl-1-(1-methylethenyl)azulene-6-methanol*; **4**). Colorless amorphous powder. $[\alpha]_D^{28} = -5.76$ ($c = 0.23$, MeOH). UV (MeOH): 206 (3.58), 252 (3.04) . IR (KBr): 3440, 2956, 2875, 1724, 1646, 1452, 1409, 1381, 1287, 1122, 1074, 1000, 889, 827, 749. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 259 ($[M+Na]^+$). HR-ESI-MS: 259.1667 ($[M+Na]^+$, $C_{15}H_{24}NaO_2^+$; calc. 259.1673).

Excoecanol A (= *(1*S*,2*R*,3*R*)-1-[(2*S*,3*S*)-3-(3,4-Dimethoxyphenyl)-2-(hydroxymethyl)-8-methoxy-2,3-dihydro-1,4-benzodioxin-6-yl]-3-(4-hydroxy-3-methoxybenzyl)-2-(hydroxymethyl)butane-1,4-diol*; **5**). Colorless amorphous oil. $[\alpha]_D^{17} = +0.74$ ($c = 0.278$, MeOH). UV (MeOH): 280(3.79). IR (KBr): 3440, 2998, 2955, 2938, 2883, 2843, 1699, 1595, 1516, 1463, 1426, 1367, 1330, 1274, 1235, 1154, 1124, 1033, 966, 825. ^{1}H- and ^{13}C-NMR: see *Table 3*. ESI-MS (pos.): 609 ($[M+Na]^+$). HR-ESI-MS: 609.2330 ($[M+Na]^+$, $C_{31}H_{38}NaO_{11}^+$; calc. 609.2335).

Excoecanol B (= *Ethyl (2*R*)-2-Hydroxy-2-[4-(hydroxymethyl)-3-methoxyphenyl]acetate*; **6**). Colorless amorphous powder. $[\alpha]_D^{11} = -12.62$ ($c = 1.50$, MeOH). UV (MeOH): 217(3.96), 232(4.00), 281 (2.66) . IR (KBr): 3429, 2962, 2940, 1726, 1604, 1518, 1465, 1453, 1433, 1372, 1277, 1252, 1211, 1180, 1127, 1036. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 263 ($[M+Na]^+$). HR-ESI-MS: 263.0901 ($[M+Na]^+$, $C_{12}H_{16}NaO_5^+$; calc. 263.0895).

Acetylcholinesterase (AchE)-*Inhibitory Bioassay.* AchE-inhibitory activity of the compounds was evaluated as described in [25] with tacrine as positive control [26]. The mixture contained phosphate buffer (pH 8.0, 1100 µl), test compound soln. (100 µM in DMSO, 10 µl), and AchE soln. (0.04 U/100 µl, 40 µl). After incubation for 20 min (30°), the reaction was initiated by the addition of 20 µl of DTNB (= 5,5′-dithiobis(2-nitrobenzoic acid) (6.25 mM)) and 20 µl of acetylthiocholine. The hydrolysis of acetylthiocholine was monitored at 405 nm after 30 min. All the reactions were performed in triplicate. The inhibition index was calculated according to the equation: % inhibition = $(E-S)/E \times 100$ (E, activity of the enzyme without test compound; S, the activity of enzyme with test compound).

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