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Minor compounds from the stem bark of Chinese mangrove associate *Catunaregam spinosa*

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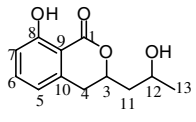
A new dihydroisocoumarin 3-(2-hydroxypropyl)-8-hydroxyl-3,4-dihydroisocoumarin (**1**) and a new iridoid 3-deoxyartselaenin C (**5**), together with six known compounds scopoletin (**2**), scoparone (**3**), morindolide (**4**), pinoresinol (**6**), medioresinol (**7**), and secoisolariciresinol (**8**) were isolated from the stem bark of Chinese mangrove associate *Catunaregam spinosa* Tirveng. The structures of **1** and **5** were determined by extensive spectroscopic analysis, including 1D and 2D NMR data. All compounds except 2 were obtained from *C. spinosa* for the first time. And it was also the first time lignans were obtained from *Catunaregam* sp.

1. Introduction

Mangrove associates are mostly distributed in tidelands of tropical and semitropical areas. The Mangrove associate *Catunaregam spinosa* (Thunb.) Tirveng that was named as *Randia spinosa* (Thunb.) Bl., *Gardenia spinosa* (Thunb.), *Randia dumetorum* (Retz.) Lam in the past, respectively, belongs to the Rubiaceae family. It is well known in folk medicine for its antispasmodic, antidysenteric, anti-inflammatory and antifertility properties (Varshney et al. 1978; Hamerski et al. 2003) in Indian and Brazil. Previous chemical research on the species resulted in the isolation of triterpene and saponins (Sati et al. 1987), iridoid glucosides (Hamerski et al. 2003) and coumarin glucosides (Sati et al. 1989). During the course of further investigation on the chemical constituents of the Chinese mangrove associate *C. spinosa*, two new compounds, named 3-(2-hydroxypropyl)-8-hydroxyl-3,4-dihydroisocoumarin (**1**), 3-deoxyartselaenin C (**5**) along with six known compounds scopoletin (**2**) (Yu et al. 2003), scoparone (**3**) (Brow et al. 1975), morindolide (**4**) (Yoshikawa et al. 1995), pinoresinol (**6**) (El-Hassan et al. 2003), medioresinol (**7**) (Abe and Yamauchi 1988) and secoisolariciresinol (**8**) (Agrawal and Rastogi 1982) were obtained. All compounds except **2** were obtained from *C. spinosa* for the first time. And three lignans were obtained from the genus for the first time. This paper deals with the isolation and structure elucidation of these new compounds.



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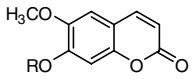

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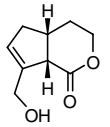
3 R = CH_3



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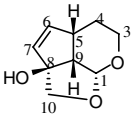

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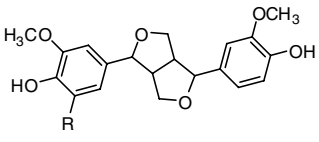
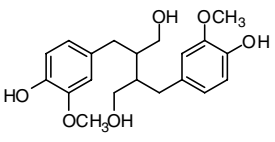


6 R = H
7 R = OCH_3



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2. Investigations, results and discussion

Compound **1** exhibited a molecular ion peak at *m/z* 223.0968 $[M + H]^+$ in its positive mode HR-ESIMS, corresponding to the molecular formula $C_{12}H_{14}O_4$ (calcd 223.0970). The ^1H NMR and ^{13}C NMR data (Table 1) were assigned using 1D and 2D techniques. The data were very similar to those published for mellein (Bi et al. 2006), except for the presence of a prolonged side chain in **1** consisting of two more carbon atoms, one of which was a hydroxylated methine and the other a methyl group. This suggested the structure given and was consistent with the 2D results. The compound is therefore 3-(2-hydroxypropyl)-8-hydroxyl-3,4-dihydroisocoumarin with undetermined stereochemistry at the two chiral centers.

Compound **5** had the molecular formula $C_9H_{12}O_3$ as established by HR-ESIMS ($[M + H]^+$ at *m/z* 169.0864). The ^1H and ^{13}C NMR (DEPT) data (Table 2) were again assigned using 1D and 2D techniques. The data for **5** were similar to those published for artselaenin C (Su et al.

Table 1: ^1H, ^{13}C and selected HMBC NMR data for compound 1[a, b]

Position	δ_H	δ_C	HMBC
1	–	169.6 (C)	–
3	4.81 m	78.3 (CH)	C-11, C-12, C-10
4α	3.00 dd (11.9, 15.9)	33.0 (CH_2)	C-11, C-3, C-9, C-5, C-10
4β	3.01 dd (2.5, 15.9)		C-11, C-3, C-9, C-5, C-10
5	6.71 d (7.5)	118.0 (CH)	C-4, C-9, C-7, C-6, C-10
6	7.42 dd (7.5, 8.4)	136.3 (CH)	C-10, C-8
7	6.90 d (8.4)	116.3 (CH)	C-9, C-5, C-8, C-1
8	–	162.2 (C)	–
9	–	108.3 (C)	–
10	–	139.3 (C)	–
11β	1.86 ddd (5.4, 7.6, 14.8)	43.5 (CH_2)	C-13, C-4, C-12, C-3
11α	2.12 ddd (7.5, 10.6, 14.8)		C-13, C-4, C-12, C-3
12	4.16 m	65.2 (CH)	C-3
13	1.29 d (6.2)	23.9 (CH_3)	C-11, C-12, C-3
8-OH	10.95 s	–	C-9, C-7, C-8

[a] ^1H NMR and ^{13}C NMR data were determined at 500 MHz and 125 MHz separately in $CDCl_3$;
[b] Chemical shifts values δ are in ppm, and coupling constant values J in Hz

Table 2: ^1H, ^{13}C , ^1H-^1H COSY and HMBC NMR data for compound 5[a, b]

Position	δ_H	δ_C	COSY	HMBC
1	5.46 (d, 7.2)	100.3 (CH)	H-9, H-7	C-9, C-5, C-3
3α	3.68 (m)	56.2 (CH_2)	H-4β, H-3β	C-5, C-1
3β	3.55 (m)		H-3α	C-5, C-1, C-4
4α	1.62 (m)	25.5 (CH_2)	H-4β,	C-9
4β	1.94 (m)		H-4α, H-5, H-3α	C-5, C-3, C-6
5	3.29 (1H, m)	39.1 (CH)	H-4β, H-9	C-9
6	5.79 (dd, 5.4, 1.7)	140.4 (CH)	–	C-4, C-5, C-9, C-8, C-7
7	5.76 (dd, 5.4, 2.3)	134.5 (CH)	–	C-5, C-9, C-8, C-6
8	–	93.5 (C)	–	–
9	2.37 (m)	45.2 (CH)	H-5, H-1	C-5, C-8, C-7, C-6
10α	3.91 (d, 9.4)	70.9 (CH_2)	H-10β	C-9, C-8, C-1, C-7
10β	3.58 (d, 9.4)		H-10α	C-9, C-8, C-7

[a] ^1H NMR and ^{13}C NMR data were determined at 500 MHz and 125 MHz separately in $CDCl_3$;
[b] Chemical shifts values δ are in ppm, and coupling constant values J in Hz

1998) except for the presence of an additional hydroxyl group at C-3 in the latter. Assuming the usual stereochemistry for iridoids with both H-5 and H-9 in the β-position, H-1 (δ_H 5.46) could also be determined to in the β-position since it exhibited a strong NOE interaction with H-9 (δ_H 2.37). Due to the rigidity of the molecule, the lack of an NOE interaction between H-9 (δ_H 2.37) and H-10 (δ_H 3.91, δ_H 3.58), indicated the β-configuration of the 8-OH group. Thus the structure of **5** was elucidated as 3-deoxyartselaenin C.

3. Experimental

3.1. General procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter with MeOH as solvent. UV spectrum were measured with a Beckman coulter™ DU® 640 nucleic acid and protein analyzer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as an internal reference. ESIMS were collected on MDS SCIEX API 2000 LC/MS/MS instrument and VG Auto-Spec-3000 spectrometer for HR-ESIMS. Si gel (100–200 mesh and 200–300 mesh) for column chromatography was obtained from the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Precoated Si gel plates were used for analytical TLC.

3.2. Plant material

The stem bark of *Catunaregam spinosa* Tirveng was collected in February 2006 from Sanya, Hainan Province, P.R. China. The specimen was identified by Professor Si Zhang, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen has been deposited in the South China Sea Institute of Oceanology, Chinese Academy of Sciences (accession number: GKLMMM020).

3.3. Extraction and isolation

The air-dried and powered plant material (dry weight 9 kg) was extracted with 95% EtOH at room temperature for three times, then with 50% heated EtOH for three times. After evaporation of the EtOH, the two residues (860 g) were suspended together in H_2O, and extracted successively with petroleum ether, EtOAc, and BuOH for three times, respectively. The EtOAc fraction was chromatographed on Si gel using $CHCl_3$-MeOH (stepwise, 0–50% MeOH), to yield 7 fractions. Fraction 3 eluted with petroleum ether-acetone (stepwise, 10%–80% acetone) was further chromatographed on Si gel to yield A–J fractions. Fraction B eluted with chloroform-acetone (8% acetone) yielded **1** (7 mg) and **3** (3 mg). Fraction C was purified by Sephadex LH-20 ($CHCl_3$/MeOH: 50/50) to yield **5** (12 mg). Fraction D eluted with chloroform-methanol (3% cholorform) yielded **8** (8 mg). Fraction F eluted with petroleum ether-ethyl acetate (65%–35% petroleum ether) yielded **4** (8 mg). Fraction G was purified by Sephadex LH-20 ($CHCl_3$/MeOH: 50/50) to yield **2** (15 mg). Fraction I and J, separately, eluted with petroleum ether and ethyl acetate (65% petroleum ether) yielded **6** (34 mg) and **7** (12 mg).

3.4. 3-(2-Hydroxypropyl)-8-hydroxyl-3,4-dihydroisocoumarin (1)

Amorphous powder; $[\alpha]_D^{20}$ – 61.5° (c = 1.0×10^{-3}, MeOH); UV (MeOH) λ_{max} 218, 247, 313 nm; IR (KBr) υ_{max} 3250, 1673, 1600, 1465 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 223 $[M + H]^+$, 240 $[M + NH_4]^+$, 245 $[M + Na]^+$, 445 $[2M + H]^+$; HR-ESIMS *m/z* 223.0968 $[M + H]^+$ (calcd for $C_{12}H_{15}O_4$, 223.0970).

3.5. 3-Deoxyartselaenin C (5)

Amorphous powder; $[\alpha]_D^{20}$ + 146.8° (c = 2.2×10^{-3}, MeOH); UV (MeOH) λ_{max} 202 nm; IR (KBr) υ_{max} 1720, 1465, 1383, 1080, 1035 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 2; ESIMS *m/z* 169 $[M + H]^+$, 186

$[M + NH_4]^+$, 191 $[M + Na]^+$, 359 $[2M + H]^+$; HR-ESIMS *m/z* 169.0864 $[M + H]^+$ (calcd for $C_9H_{13}O_3$, 169.0865).

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