Catunaregin and Epicatunaregin, Two Norneolignans Possessing an Unprecedented Skeleton from *Catunaregam spinosa*

by **Guang-Chun Gao**[a][b], **Xiong-Ming Luo**[a], **Xiao-Yi Wei**[c], **Shu-Hua Qi**[a], **Hao Yin**[a], **Zhi-Hui Xiao**[a], and **Si Zhang***[a]

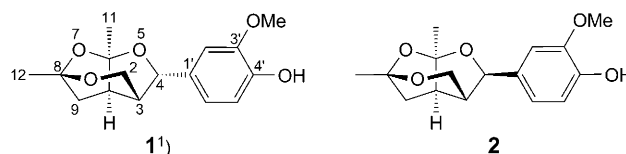
[a]) Guangdong Key Laboratory of Marine Material Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, P. R. China (phone: +86-20-89023103; fax: +86-20-84451672; e-mail: zhsimd@scsio.ac.cn)

[b]) Institute of Plant Protection and Microbiology, Zhejiang Academy of Agriculture Science, Hangzhou, 310021, P. R. China

[c]) Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, 510650, P. R. China

Catunaregin (**1**) and epicatunaregin (**2**), two novel norneolignans with a unique O-bridged furopyran ring, together with three known neolignans, ficusal, balanophonin, and 5″-methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A, were isolated from the stem bark of *Catunaregam spinosa*, a Chinese mangrove associate. Their structures were elucidated by spectroscopic means, including two-dimensional NMR techniques. Compounds **1** and **2** exhibited moderate inhibition against the mammary cancer F10 cell line.

Introduction. – Mangrove plants, growing in tropical and subtropical intertidal estrarine zones, are a potential source of new drugs. *Catunaregam spinosa* T. (Rubiaceae), mainly distributed in southeast Asia and Africa, was used as folk medicine in India and Brazil [1] [2]. The occurrence of coumarin glucosides [3], iridoid glucosides [1], triterpenes, and saponins [4] in this plant were previously reported. In continuation of our studies on the chemical diversity of mangrove plants in Hainan Island, this plant was re-investigated, and two novel norneolignans, catunaregin (**1**) and epicatunaregin (**2**), were isolated from the stem bark, together with three known neolignans, ficusal (**3**), balanophonin (**4**), and 5″-methoxy-4″-*O*-(8-guaiacylglycerol)-buddlenol A. Compounds **1** and **2** possess an unprecedented norneolignan skeleton with an O-bridged furopyran ring. Here, we report the isolation, structure elucidation, and cytotoxicity of these new compounds.



Results and Discussion. – The EtOH extract of the stem bark of *C. spinosa* was subjected to extraction and solvent partitioning as described in the *Exper. Part*. The

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

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resulting AcOEt fraction was subjected to repeated column chromatography (silica gel and *Sephadex LH-20*) to yield two novel norneolignans, compounds **1** and **2**, together with three known compounds, ficusal (**3**) [5], balanophonin (**4**) [6], and 5″-methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A [7].

Catunaregin (**1**) was obtained as a colorless amorphous solid. The molecular formula was established as $C_{16}H_{20}O_5$ from the HR-ESI-MS *quasi*-molecular-ion peak at *m*/*z* 315.1202 ($[M+Na]^+$), inferring seven degrees of unsaturation. Its IR spectrum exhibited absorption bands for a OH group (3411 cm^{-1}) and an aromatic ring (1606 cm^{-1}, 1518 cm^{-1}, and 1454 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (*Table*) in combination with an HSQC spectrum indicated the presence of a 1,3,4-trisubstituted benzene ring (δ(H) 6.82 (*d*, $J=1.7$), 6.89 (*d*, $J=8.1$), and 6.81 (*dd*, $J=8.1$, 1.7); δ(C) 146.6, 145.2, 134.5, 118.9, 114.5, and 108.7), two tertiary Me groups (δ(H) 1.50 (*s*, Me(12)[1])) and 1.61 (*s*, Me(11)); δ(C) 24.8 and 22.6, resp.), a CH_2 group (δ(H) 1.89 (*dd*, $J=3.9$, 12.5, H_α–C(9)) and 2.28 (*d*, $J=12.5$, H_β–C(9)); δ(C) 33.3), two CH groups (δ(H) 2.50 (*ddt*, $J=10.2$, 4.0, 2.1, H–C(3)) and 2.72 (*dd*, $J=10.2$, 3.8, H–C(10)); δ(C) 47.9 and 45.8, resp.), an O-bearing CH group (δ(H) 4.96 (*d*, $J=4.0$, H–C(4)); δ(C) 85.3), an O-bearing CH_2 group (δ(H) 3.77 (*d*, $J=2.0$, CH_2(2); δ(C) 63.0), and an aromatic MeO group (δ(H) 3.90 (*s*, *Me*O–C(3′)); δ(C) 55.9). Furthermore, the ^{13}C- and DEPT-NMR spectra (*Table*) exhibited two quaternary C-atom signals at δ(C) 106.0 (C(8)) and 115.3 (C(6)), assignable to two acetal C-atoms. From the above data, it was deduced that compound **1** contained three rings besides the benzene ring.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *for Compounds* **1** *and* **2** (δ in ppm, *J* in Hz)[1])

	1			**2**	
	δ(H)[a])	δ(H)[b])	δ(C)[a])	δ(H)[a])	δ(C)[a])
H_α–C(2)	3.77 (br. *d*, $J=2.0$)	3.82 (*dd*, $J=11.5$, 3.5)	63.0	3.97 (*d*, $J=2.0$)	63.0
H_β–C(2)	3.77 (br. *d*, $J=2.0$)	3.75 (*d*, $J=11.5$)			
H–C(3)	2.50 (*ddt*, $J=10.2$, 4.0, 2.1)	2.51 (*ddt*, $J=10.0$, 4.0, 3.5)	47.9	2.44 (*ddt*, $J=10.2$, 7.0, 2.0)	45.6
H–C(4)	4.96 (*d*, $J=4.0$)	4.90 (*d*, $J=4.0$)	85.3	5.63 (*d*, $J=7.0$)	86.5
C(6)			115.3		112.3
C(8)			106.0		103.5
H_β–C(9)	2.28 (*d*, $J=12.5$)	2.36 (*d*, $J=13.0$)	33.3	2.04 (*t*, $J=4.7$, 7.5)	26.9
H_α–C(9)	1.89 (*dd*, $J=3.9$, 12.5)	1.96 (*dd*, $J=12.5$, 4.0)			
H–C(10)	2.72 (*dd*, $J=10.2$, 3.8)	2.84 (*dd*, $J=10.0$, 4.0)	45.8	2.78 (*dt*, $J=10.2$, 3.5)	38.0
Me(11)	1.61 (*s*)	1.58 (*s*)	22.6	1.53 (*s*)	20.9
Me(12)	1.50 (*s*)	1.44 (*s*)	24.8	1.50 (*s*)	26.0
C(1′)			134.5		134.5
H–C(2′)	6.82 (*d*, $J=1.7$)	6.79 (*s*)	108.7	6.84 (*d*, $J=1.7$)	108.8
C(3′)			146.6		146.6
C(4′)			145.2		145.2
H–C(5′)	6.89 (*d*, $J=8.1$)	6.89 (*s*)	114.5	6.89 (*d*, $J=8.1$)	114.6
H–C(6′)	6.81 (*dd*, $J=8.1$, 1.7)	6.79 (*s*)	118.9	6.83 (*dd*, $J=8.1$, 1.7)	118.9
*Me*O–C(3′)	3.90 (*s*)	3.87 (*s*)	55.9	3.90 (*s*)	56.0

[a]) Data were recorded in $CDCl_3$. [b]) Data were recorded in CD_3OD.

Analysis of the ^{1}H,^{1}H-COSY spectrum permitted to establish partial structures as shown by the bold lines in *Fig. 1*. In the HMBC spectrum (key correlations depicted in *Fig. 1*), correlations were observed from Me(12) to C(8) and C(9), from CH_2(9) to C(10), C(3), and C(2), and from CH_2(2) to C(8), revealing the connectivities of C(8) to C(12) and C(9), as well as a linkage between C(2) and C(8) *via* an O-bridge to form a pyran ring. Correlations were also observed between Me(11), H–C(3), and CH_2(9) to C(6), and from Me(11) to C(10), indicating the connectivities of C(6) to C(10) and C(11). In addition, long-range correlations were observed from both H–C(4) and H–C(3) to C(1′), from both H–C(2′) and H–C(6′) to C(4), from H–C(2′), H–C(5′), and MeO to C(3′), and from H–C(6′) to C(4′), indicating the presence of a 4-hydroxy-3-methoxyphenyl group and the attachment of this group at C(4). Considering two unsaturation degrees remaining and the ^{13}C-NMR chemical shifts for C(4), C(6), and C(8) ((δ(C) 85.3, 115.3, and 106.0, resp.), the connectivities of C(6) to both C(8) and C(4) *via* O-bridges were exclusively derived even though no HMBC correlation between H–C(4) and C(6) was observed. Accordingly, the constitutional formula of compound **1** was established as shown.

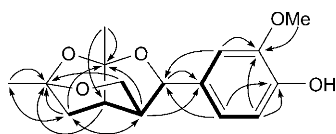


Fig. 1. *^{1}H,^{1}H-COSY* (bold lines) *and selected HMBCs* (arrows) *of* **1** *and* **2**

The relative configuration of compound **1** was assigned by analysis of the ^{1}H-NMR coupling constants and the NOESY spectrum. Since the signals for H_α–C(2) and H_β–C(2) could not be distinguished due to overlapping of the two signals in the ^{1}H-NMR spectrum of **1** in $CDCl_3$, the ^{1}H-NMR spectrum of **1** was remeasured in CD_3OD. In the remeasured ^{1}H-NMR spectrum (*Table*), the signals for H_α–C(2) and H_β–C(2) appeared separately at δ(H) 3.82 (*dd*, $J = 11.5$, 3.5, H_α–C(2)) and 3.75 (*d*, $J = 11.5$, H_β–C(2)). The NOESY spectrum of **2** was thus measured in CD_3OD. In the spectrum (key correlations shown in *Fig. 2*), strong NOE interactions were observed between H_β–C(9) and H_α–C(2), between H–C(4) and H_β–C(2), and between H–C(3) and H–C(10), whereas the interaction between H–C(3) and H–C(4) was weak. This fact, in combination with the vicinal coupling constants, $J = 10.2$ Hz between H–C(3) and H–C(10), and $J = 4.0$ Hz between H–C(3) and H–C(4), showed that the pyran ring was in a boat conformation, and H–C(3), designated to α-orientation, had a *cis* relationship to H–C(10) and a *trans* relationship to H–C(4). Thus, the

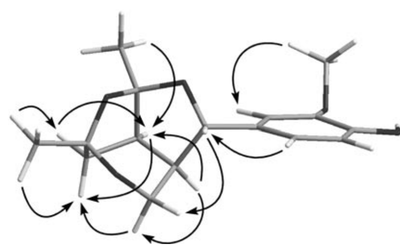


Fig. 2. *Relative configurations and selected NOESY correlations of* **1**

relative configuration of compound **1** was determined as shown in *Fig. 2* (generated by CS Chem & Bio 3D 11.0 using a MM2 force field calculation for energy minimization).

Epicatunaregin (**2**), a colorless amorphous solid, had the same molecular formula $C_{16}H_{20}O_5$ as **1**, as determined from the HR-ESI-MS and ^{13}C-NMR data. Compound **2** resembled **1** to a great extent according to the IR and UV data. The ^{1}H- and ^{13}C-NMR data of compound **2** (*Table*) were closely comparable to those of **1**, except for the chemical shifts of several H-atom and C-atom signals for the heterotricycle residue. For example, H–C(4) and CH_2(2) were shifted downfield by 0.67 and 0.20 ppm, whereas C(10) and C(9) were shifted upfield by 7.8 and 6.4 ppm, respectively, relative to those in **1**. Despite these differences, interpretation of the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra showed that **2** is a diastereoisomer of compound **1**. In the NOESY spectrum of **2** (*Fig. 3*), a strong interaction was observed between H–C(3) and H–C(4) while no NOE between H–C(4) and H–C(10) was observed. Furthermore, the coupling constant ($J = 7.0$ Hz) between H–C(3) and H–C(4) was larger than the one in compound **1** ($J = 4.0$ Hz). These findings showed that compound **2** is the 4-epimer of **1**. This configuration of **2**, in which the 4-Ph group fronted towards the heterotricycle (*Fig. 3*) differing from that of **1**, in which this group oriented away from the heterotricycle residue (*Fig. 2*), was in accordance with the changes in chemical shifts of the H- and C-atom signals relative to those of **1**. Therefore, the structure of **2** was determined as shown.

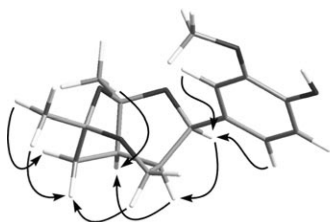
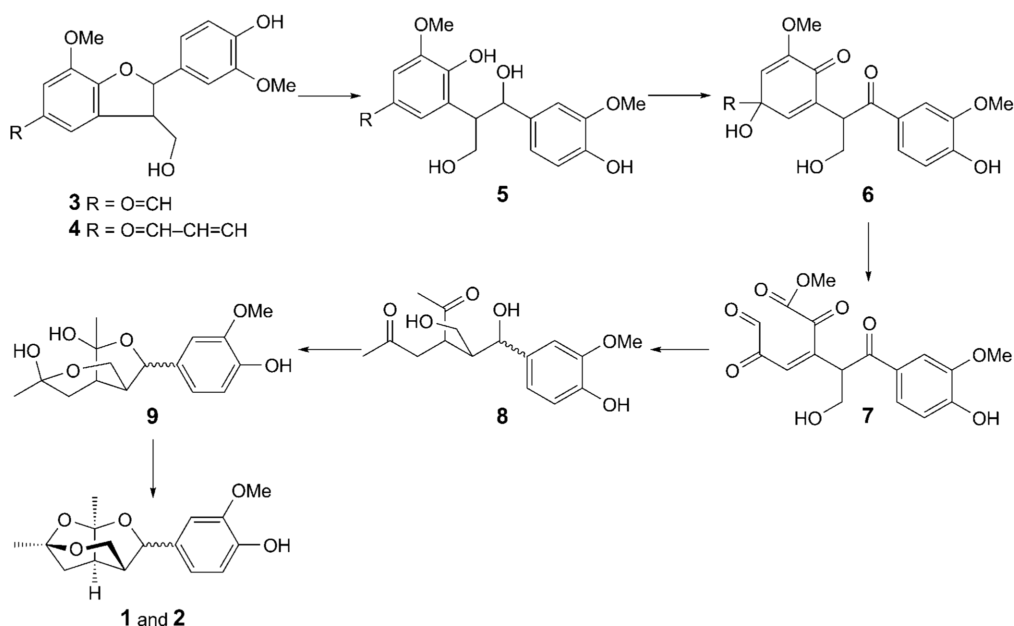


Fig. 3. *Relative configurations and selected NOESY correlations of* **2**

Both **1** and **2** possess a unique O-bridged furopyran ring. They seem to be biogenetically transformed from benzofuran neolignans, such as ficusal (**3**) and balanophonin (**4**), which were also isolated in the present study. The possible transformation mechanism may be rationalized as follows (*Scheme 1*): the furan ring of **3** or **4** was presumably cleaved *via* hydrolysis to provide a 1,2-diphenylpropane-1,3-diol **5** which underwent oxidation, followed by oxidative cleavage to generate, *via* intermediate **6**, intermediate **7**. Partial reduction of **7** afforded a diketone intermediate **8**. The later might be transformed to **1** and **2** *via* hemiacetal **9** through intramolecular nucleophilic addition. The weak optical activity of **1** ($[\alpha]^{20}_{D} = +1.4$ ($c = 0.8$, MeOH)) and the optical inactivity of **2** ($[\alpha]^{20}_{D} = 0$ ($c = 0.1$, MeOH)) suggest that these reactions in the transformation were possibly not enantioselective. Compounds **1** and **2** represent the first two examples of this unprecedented norneolignan skeleton.

Catunaregin (**1**) and epicatunaregin (**2**) were evaluated for cytotoxic activity against two tumor cell lines by the MTT method [8]. As a result of a cytotoxic bioassay, **1** and **2** showed moderate cytotoxicity towards human mammary cancer F10 cell line ($IC_{50} =$

Scheme. *Postulated Intermediates in the Biogenetic Transformation of* **1** *and* **2** *from Ficusal and Balanophonin*



5.33 and 12.76 μg/ml, resp.) compared to the positive control (5-fluorouracil, IC_{50} = 16.81 μg/ml) and no obvious cytotoxicity towards lung cancer HvEvc cell line.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China), *RP-18* silica gel (40–60 mesh; *Merck*); *Sephadex LH-20* (*Pharmacia*). HPLC: *ODS* column (*YMC-Pack ODS-5-A*, 250 × 10 mm i.d., 5 μm; *YMC*); *Waters*-600 HPLC system equipped with a *Waters*-996 photodiode array detector. TLC: precoated silica gel *G* plates (*Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Beckman coulter™ DU® 640* nucleic acid and protein analyzer, λ_{max} in nm. IR Spectra: *Bruker EQUINOX55* spectrometer; in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer; Me_4Si as internal standard; δ in ppm, J in Hz. MS: *MDS SCIEX API 2000 LC/MS/MS* instrument for ESI and *Bruker BioTOF Q* spectrometer for HR-ESI; positive-ion mode; in *m/z*.

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 Prof. *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).

Extraction and Isolation. The air-dried and powered plant material (9 kg) was extracted with 95% EtOH at r.t. for three times (3 × 7 d), after evaporation of the EtOH, the viscous residue (860 g) was suspended in H_2O and extracted successively with petroleum ether (PE), AcOEt, and BuOH for three

times each. The AcOEt fraction (45 g) was fractionated by CC (SiO_2; $CHCl_3$/MeOH 99:1, 98:2, 95:5, 90:10, 80:20, 50:50): *Frs. E1–E7. Fr. E3* (6 g) was subjected to CC (SiO_2; PE/acetone 10:1, 8:1, 6:1, 4:1): *Frs. A–J. Fr. H* was subjected to CC (SiO_2; PE/AcOEt) to give impure **1** and **2**, which were separately purified by CC (*Sephadex LH-20*; MeOH): **1** (9 mg) and **2** (1.7 mg). *Fr. J* was subjected to CC (SiO_2; PE/AcOEt 1:1): ficusal (**3**; 15 mg). *Fr. E6* (8 g) was subjected to CC (SiO_2; $CHCl_3$/acetone 5:1, 3:1, 1:1): *Frs. K–P. Fr. L* was subjected to CC (SiO_2; PE/acetone 1:1): balanophonin (**4**; 10 mg). *Fr. N* was fractioned by CC (*RP-18*; MeOH/H_2O 1:1): *Frs. N1–N3. Fr. N3* (170 mg) was then purified by HPLC (*ODS*; MeOH/H_2O 45:55): 5″-methoxy-4″-*O*-(8-guaiacylglycerol)buddlenol A (4 mg).

Catunaregin (=*4-[(1*R*,3*S*,3a*R*,6*S*,7a*R*)-Hexahydro-1,6-dimethyl-3*H*-1,6-epoxyfuro[3,4-*c*]pyran-3-yl]-2-methoxyphenol*; **1**). Amorphous solid. $[\alpha]_D^{20} = +1.4$ ($c = 0.8$, MeOH). UV (MeOH): 198 (3.16), 218 (3.54), 227 (3.72), 279 (3.19). IR: 3411, 2985, 2937, 1606, 1518, 1454, 1386, 1272, 1238. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 293 ($[M+H]^+$), 315 ($[M+Na]^+$), 331 ($[M+K]^+$), 607 ($[2M+Na]^+$), 623 ($[2M+K]^+$). HR-ESI-MS: 315.1202 ($[M+Na]^+$, $C_{16}H_{20}NaO_5^+$; calc. 315.1203).

Epicatunaregin (=*4-[(1*R*,3*R*,3a*R*,6*S*,7a*R*)-Hexahydro-1,6-dimethyl-3*H*-1,6-epoxyfuro[3,4-*c*]pyran-3-yl]-2-methoxyphenol*; **2**). Amorphous solid. $[\alpha]_D^{20} = 0$ ($c = 0.1$, MeOH), optically inactive. UV (MeOH): 220 (3.90), 223 (3.97), 278 (3.39). IR: 3426, 2984, 2937, 1603, 1516, 1463, 1382, 1274, 1237. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 293 ($[M+H]^+$), 315 ($[M+Na]^+$), 331 ($[M+K]^+$), 623 ($[2M+K]^+$). HR-ESI-MS: 315.1205 ($[M+Na]^+$, $C_{16}H_{20}NaO_5^+$; calc. 315.1203).

Cyctotoxicity Assays. The mammary cancer (F10) and lung cancer (HvEvc) cell suspensions (200 µl) at a density of 1×10^5 cells ml^{-1} were placed in 96-well microtiter plates and incubated for 24 h at 37° in a humidified incubator at 5% CO_2. The tested compound soln. (2 µl in DMSO) at different concentrations was added to each well and further incubated for 72 h under the same conditions. Then, MTT soln. (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 50 µl) was added to each well and incubated for 4 h. The old medium (150 µl) containing MTT was then gently replaced by DMSO. Absorbance was then determined on a *Spectra Max Plus* plate reader at 490 nm.

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