Norlignans from Rhizomes of *Curculigo sinensis*

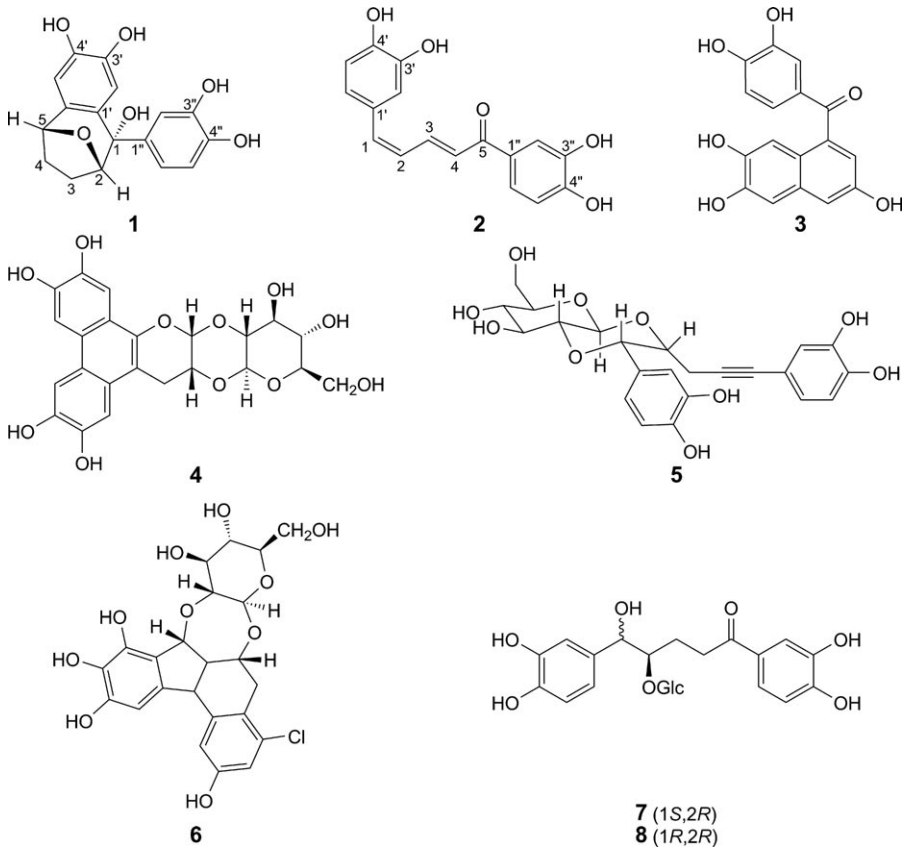
by **Ning Li***[a]), **Tian-Ming Wang**[a]), **Kai-Jin Wang***[a]), and **You-Xing Zhao**[b])

[a]) School of Life Sciences, Anhui University, Hefei 230039, P. R. China
(e-mail: ln0110@sina.com; wkjahla@sina.com)
[b]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

Two novel norlignans, sinensigenins A and B (**1** and **2**, resp.), were isolated from the rhizomes of *Curculigo sinensis*, together with six known norlignans, crassifogenin B (**3**), cucapitoside (**4**), crassifoside B (**5**), crassifoside H (**6**), curculigine (**7**), and isocurculigine (**8**). Their structures were established on the basis of spectral evidence and comparisons with literature data. All of these compounds were isolated from this plant for the first time.

Introduction. – The plants of genus *Curculigo* (Hypoxidaceae), comprising *ca.* 20 species, are distributed in the tropical and subtropical zone. Of them, seven species occur in China [1]. We have studied several species of the genus *Curculigo* concerning the chemical constituents of the rhizomes [2–7]. From the species so far examined, we isolated some norlignans from the rhizomes of *C. capitulata* [4] and *C. crassifolia* [3][5–7] of Yunnan Province, and *C. breviscapa* of Guangxi Province [8]. Norlignans are biosynthetically generated by the coupling of two phenyl-containing C_3-units (cinnamic acid and cinnamyl alcohol) with the loss of the terminal C-atom of the side chain [9]. So far, phytochemical studies are lacking in *C. sinensis.* As a part of our ongoing work on the genus *Curculigo* plants from China, we undertook the phytochemical work on *C. sinensis.* In this study, two novel norlignans, sinensigenins A and B (**1** and **2**, resp.), were isolated from the rhizomes of *C. sinensis*, together with six known norlignans, crassifogenin B (**3**) [3], cucapitoside (**4**) [10], crassifoside B (**5**) [3], crassifoside H (**6**) [8], curculigine (**7**), and isocurculigine (**8**) [4]. Their structures were established by spectroscopic and mass-spectrometric analyses, especially 2D-NMR techniques (^{1}H,^{1}H-COSY, HSQC, HMBC, NOESY), and comparison with literature data. All of these compounds were isolated from this plant for the first time.

Results and Discussion. – Sinensigenin A (**1**) was obtained as a white powder. The molecular formula $C_{17}H_{16}O_6$ was deduced from HR-FAB-MS (negative-ion mode; *m*/*z* 315.0872; calc. 315.0868) and ^{13}C-NMR (DEPT) data, indicating ten degrees of unsaturation. The IR spectrum indicated the presence of OH groups (3405 cm^{-1}). The ^{1}H-NMR spectrum exhibited signals for four CH_2 H-atoms (δ(H) 1.52–1.55 (*m*, H_a–C(3)), 2.17–2.22 (*m*, H_b–C(3)), 2.11–2.14 (*m*, CH_2(4))) and for two O-bearing CH groups (δ(H) 4.06 (br. *s*, H–C(5)), 4.56–4.58 (*m*, H–C(2)); *Table*). The ^{1}H-NMR spectrum of **1** also exhibited signals of five low-field aromatic H-atoms. Three of them were assigned to H–C(2″) (δ(H) 6.93 (*d*, J = 2.0)), H–C(5″) (δ(H) 6.75 (*d*, J = 8.0)),



and H–C(6″) (δ(H) 6.85 (*d*, J = 8.0, 2.4)), which suggested the existence of 1,3,4-trisubstituted aromatic ring. The remaining two aromatic H-atoms were assigned to H–C(2′) (δ(H) 6.04, *s*), and H–C(5′) (δ(H) 6.74, *s*) in a 1,3,4,6-tetrasubstituted aromatic ring. Analysis of the ^{1}H- and ^{13}C-NMR data (*Table*), and HSQC spectra revealed that **1** contains two aromatic rings, including four O-bearing olefinic C-atoms (δ(C) 145.3 (C(3′)), 145.7 (C(4′)), 145.8 (C(3″)), and 145.7 (C(4″))) and eight non-O-bearing olefinic C-atoms (δ(C) 112.6 (C(2′)), 115.4 (C(5″)), 116.1 (C(2″)), 117.5 (C(5′)), 120.0 (C(6″)), 125.5 (C(1′)), 135.3 (C(1″)), and 136.6 (C(6′))), as well as five aliphatic C-atoms including one O-bearing quaternary C-atom (δ(C) 86.2 (C(1))), two O-bearing CH groups (δ(C) 71.6 (C(5)) and 81.0 (C(2))), and two CH_2 groups (δ(C) 26.0 (C(3)) and 38.3 (C(4))).

The ^{1}H,^{1}H-COSY correlations of H–C(2)/H–C(3), H–C(3)/H–C(4), and H–C(4)/H–C(5) showed the connectivity C(2)–C(3)–C(4)–C(5), which was further confirmed by HMBCs (*Fig.*) of H–C(2)/C(4), H–C(4)/C(3), and H–C(5)/C(3). The HMBC experiments showed the long-range couplings of H–C(2″)/C(1) and H–C(6″)/C(1), suggesting that the 1,3,4-trisubstituted aromatic ring was connected with C(1). The HMBCs of H–C(2′)/C(1) and H–C(2)/C(1′) indicated the linkage of

Table. *^1H- and ^{13}C-NMR Data of* **1** *and* **2** *in CD_3OD.* δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
1	86.2 (*s*)		143.7 (*d*)	6.94 (*d*, J = 11.2)
2	81.0 (*d*)	4.56–4.58 (*m*)	125.4 (*d*)	6.91–6.94 (overlap)
3	26.0 (*t*)	1.52–1.55 (*m*), 2.17–2.22 (*m*)	146.2 (*d*)	7.48–7.52 (overlap)
4	38.3 (*t*)	2.11–2.14 (*m*)	124.6 (*d*)	7.14 (*d*, J = 14.8)
5	71.6 (*d*)	4.06 (br. *s*)	191.2 (*s*)	
1′	125.5 (*s*)		130.0 (*s*)	
2′	112.6 (*d*)	6.04 (*s*)	114.6 (*d*)	7.03 (*d*, J = 2.0)
3′	145.3 (*s*)		146.7 (*s*)	
4′	145.7 (*s*)		148.4 (*s*)	
5′	117.5 (*d*)	6.74 (*s*)	116.5 (*d*)	6.76 (*d*, J = 8.0)
6′	136.6 (*s*)		121.7 (*d*)	6.91 (*dd*, J = 8.0, 2.0)
1″	135.3 (*s*)		131.5 (*s*)	
2″	116.1 (*d*)	6.93 (*d*, J = 2.0)	116.2 (*d*)	7.46 (*d*, J = 2.0)
3″	145.8 (*s*)		146.7 (*s*)	
4″	145.7 (*s*)		152.7 (*s*)	
5″	115.4 (*d*)	6.75 (*d*, J = 8.0)	115.9 (*d*)	6.85 (*d*, J = 8.2)
6″	120.0 (*d*)	6.85 (*dd*, J = 8.0, 2.4)	123.4 (*d*)	7.48 (*dd*, J = 8.0, 2.0)

C(1) to C(1′). The correlations H–C(5)/C(6′), H–C(5)/C(1′), H–C(5′)/C(5), and H–C(4)/C(6′) indicated the linkage of C(5) to C(6′). The linkage of C(2) and C(5) to an O-atom was established by the HMBCs of H–C(2)/C(5) and H–C(5)/C(2), and the low-field chemical shift of C(2) and C(5), at δ(C) 81.0 and 71.6, respectively (*Table*). The clear NOESY correlation between H–C(2) and H–C(5), but not between H–C(2) and aromatic H-atoms, indicated the *cis*-relationship of H–C(2), H–C(5), and HO–C(1), which was further confirmed by the NOESY correlations H_a–C(3)/H–C(5), H_b–C(3)/H–C(2″), and H_b–C(3)/H–C(6″). Thus, the structure of sinensigenin A was deduced as shown in the *Figure*.

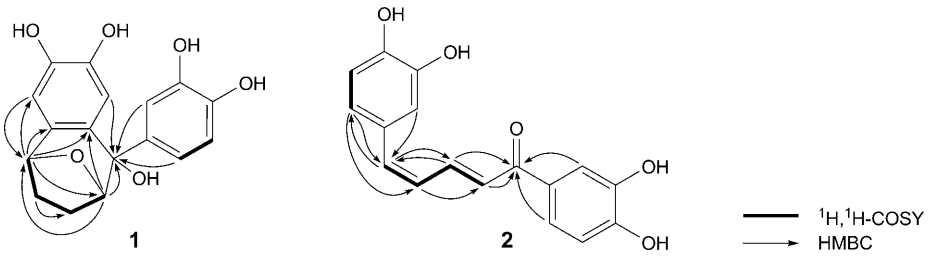


Figure. *Selected 2D-NMR correlations of* **1** *and* **2**

Sinensigenin B (**2**) was obtained as yellow powder. Its molecular formula was determined as $C_{17}H_{14}O_5$, indicating eleven degrees of unsaturation, on the basis of ^{13}C-NMR (DEPT) data and the *pseudo*-molecular-ion peak $[M-H]^-$ at m/z 297.0771 in HR-FAB-MS (negative-ion mode; calc. 297.0762). The IR spectrum showed the presence of OH groups (3424 cm^{-1}) and a conjugated CO group (1599 cm^{-1}). The ^{1}H-NMR spectrum (*Table*) exhibited signals for four unsaturated CH groups (δ(H)

6.94 (*d*, *J* = 11.2), 6.91–6.94 (overlap), 7.48–7.52 (overlap), and 7.14 (*d*, *J* = 14.8)), which were assigned to the H-atoms of two conjugated C=C bonds. The ^{1}H-NMR spectrum of **2** also exhibited signals of six aromatic H-atoms. Three of them were assigned to H–C(2′) (δ(H) 7.03 (*d*, *J* = 2.0)), H–C(5′) (δ(H) 6.76 (*d*, *J* = 8.0)), and H–C(6′) (δ(H) 6.91 (*d*, *J* = 8.0, 2.0)), suggesting the existence of a 1,3,4-trisubstituted benzene ring. The remaining three aromatic H-atoms were assigned to H–C(2″) (δ(H) 7.46 (*d*, *J* = 2.0)), H–C(5″) (δ(H) 6.85 (*d*, *J* = 8.2)), and H–C(6″) (δ(H) 7.48 (*d*, *J* = 8.0, 2.0)) in another 1,3,4-trisubstituted benzene ring, in which H–C(2″) and H–C(6″) were shifted downfield due to an *ortho* CO group (IR $\tilde{\nu}$(C=O) 1599 cm^{-1}, δ(C) 191.2). Analysis of the ^{1}H- and ^{13}C-NMR (*Table*) and HSQC spectra revealed that **2** contains two aromatic rings, including four O-bearing olefinic C-atoms (δ(C) 146.7 (C(3′)), 148.4 (C(4′)), 146.7 (C(3″)), and 152.7 (C(4″))) and eight non-O-bearing olefinic C-atoms (δ(C) 114.6 (C(2′)), 115.9 (C(5″)), 116.2 (C(2″)), 116.5 (C(5′)), 121.7 (C(6′)), 123.4 (C(6″)), 130.0 (C(1′)), and 131.5 (C(1″))), as well as two C=C bonds (δ(C) 143.7 (C(1)), 125.4 (C(2)), 146.2 (C(3)), and 124.6 (C(4))) and one C=C atom (δ(C) 191.2 (C(5))).

The connectivity C(1)=C(2)–C(3)=C(4) was deduced from the ^{1}H,^{1}H-COSY correlations H–C(2)/H–C(3), and H–C(3)/H–C(4), and the HMBCs of H–C(1) with C(3), and of H–C(3) with C(1). The coupling constants (*J*(1,2) = 11.2, *J*(3,4) = 14.8 Hz) indicated the *cis*-relationship of H–C(1) and H–C(2), and the *trans*-relationship of H–C(3) and H–C(4). The norlignan sequence Ph–CH=CH–CH=CH–CO–Ph was established by the HMBCs of H–C(2′) and H–C(6′) with C(1), and of H–C(2″), H–C(6″), and H–C(3) with C(5), along with two H-atom spin systems (H–C(2)/H–C(3), and H–C(3)/H–C(4)) deduced from the ^{1}H,^{1}H-COSY correlations (*Fig.*).

The structures of six known norlignans also isolated from *C. sinensis*, *i.e.*, crassifogenin B (**3**) [3], cucapitoside (**4**) [10], crassifoside B (**5**) [3], crassifoside H (**6**) [8], curculigine (**7**), and isocurculigine (**8**) [4], were corroborated by comparison of their spectroscopic data with those reported in the literature. All of these compounds were isolated for the first time from this plant.

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* gel (25–100 μm, *Pharmacia Fine Chemical Co. Ltd.*); *MCI* gel *CHP-20P* (75–150 μm, *Mitsubishi Chemical Co.*); *Chromatorex ODS* (100–200 mesh, *Fuji Silysia Chemical Co. Ltd.*); silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical Co. Ltd.*, P. R. China). TLC: silica gel; visualization by spraying with 5% H_2SO_4 in EtOH, followed by heating. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *UV-2401PC*; λ_{max} (log ε) in nm, in MeOH. IR Spectra: *Nexus 870-FT-IR*, $\tilde{\nu}_{max}$ in cm^{-1}, KBr pellets. NMR Spectra: *Bruker AM 400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); chemical shifts δ in ppm rel. to Me_4Si as internal standard, coupling constants *J* in Hz. FAB-MS and HR-FAB-MS: *VG Autospec-3000* mass spectrometer using glycerol as matrix in negative-ion mode; in *m*/*z*.

Plant Material. The plant material was collected in Jinping, Yunnan Province, China, in May 2008, and identified by Prof. *Kai-Jin Wang*, the School of Life Sciences, Anhui University, where a voucher specimen (No. 20080501) was deposited.

Extraction and Isolation. The fresh rhizomes of *C. sinensis* (30 kg) was cut into small pieces and extracted with 95% EtOH under reflux for 3 h to afford a dark-brown residue (2.0 kg, 850 ml) upon removal of the solvent under reduced pressure. The residue was suspended in H_2O and then passed though a *D101* resin column eluted sequentially with H_2O, followed by 20, 40, 60, 80, and 95% aq. MeOH to afford *Frs. 1–6*. *Fr. 3* (620 g) was resubjected on a *D101* resin to afford *Fr. 3-1* and *Fr. 3-2*, and the *Fr. 3-2* (340 g) was subjected on CC (SiO_2; $CHCl_3$/MeOH 14:1 to 7:3) to afford *Frs. 3-2-1–3-2-6*. *Fr. 3-2-2* (35 g) was separated by CC (*Sephadex LH-20*; H_2O/MeOH 1:0 to 0:1; and then *MCI*; H_2O/MeOH 1:0 to 0:1) to yield **6** (43 mg). *Fr. 3-2-5* (40 g) was subjected to CC (*ODS*; H_2O/MeOH 1:0–0:1) to give a mixture of **7** and **8** (22 mg), and **8** (65 mg). *Fr. 3-2-6* (48 g) was separated by CC (*Sephadex LH-20*; EtOH; and then *MCI*; H_2O/MeOH 1:0 to 0:1) to give **1** (48 mg), **4** (67 mg), and **5** (41 mg) consecutively. *Fr. 4* (480 g) was subjected to CC (*Sephadex LH-20*; H_2O/MeOH 1:0 to 0:1) to afford *Frs. 4-1–4-7*, and then *Fr. 4-6* (43 g) was separated by CC (*Sephadex LH-20*; EtOH/H_2O 1:0 to 0:1; and then *MCI*; H_2O/MeOH 1:0 to 0:1) to give **2** (6 mg). *Fr. 4-7* (12.5 g) was also separated by CC (*Sephadex LH-20*; EtOH; and then *MCI*; H_2O/MeOH 1:0 to 0:1) to afford **3** (54 mg).

Sinensigenin A (=*(5*R**,8*S**,9*S**)-9-(3,4-Dihydroxyphenyl)-6,7,8,9-tetrahydro-5*H*-5,8-epoxybenzo[7]annulene-2,3,9-triol*; **1**). White-grey powder. $[\alpha]_D^{25} = -75.7$ ($c = 0.12$, MeOH). UV (MeOH): 205 (4.22), 285 (3.43), 376 (2.84). IR (KBr): 3405 (OH), 2950, 1609, 1518, 1442, 1358, 1278, 1200, 1159, 1116, 1074, 1033, 881, 786. ^{1}H- and ^{13}C-NMR: *Table.* HR-FAB-MS (neg.): 315.0872 ($[M-H]^-$, $C_{17}H_{15}O_6^-$; calc. 315.0868).

Sinensigenin B (=*(2*E*,4*Z*)-1,5-Bis(3,4-dihydroxyphenyl)penta-2,4-dien-1-one*; **2**). Yellow powder. UV: 205 (4.42), 280 (3.95), 383 (4.02). IR (KBr): 3424 (OH), 1599 (C=O), 1514, 1442, 1365, 1281, 1114, 1041, 814. ^{1}H- and ^{13}C-NMR: *Table.* HR-FAB-MS (neg.): 297.0771 ($[M-H]^-$, $C_{17}H_{13}O_5^-$; calc. 297.0762).

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