Three New Compounds from the Aerial Parts of *Caragana sinica*

by **Shuguang Wang**, **Dayou Ma**, and **Changqi Hu***

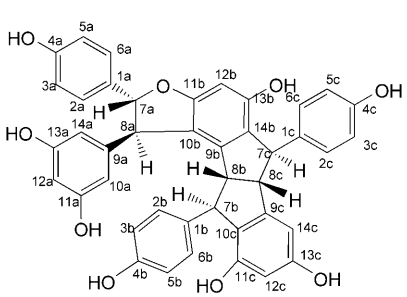
Department of Chemistry of Natural Drugs, School of Pharmacy, Fudan University, Shanghai 200032, People's Republic of China
(phone: + 86-021-54237564; e-mail: shuguang3@shmu.edu.cn)

Two new stilbenoids, carasiphenol C (**1**) and Carasiphenol D (**2**), and one new dihydroaurone, carasinaurone (**3**), were isolated from the aerial parts of *Caragana sinica*, along with three known substances. Their structures were elucidated on the basis of spectroscopic methods and X-ray-diffraction analysis. Compound **2** showed significant biological activity against *Pyricularia oryzae*.

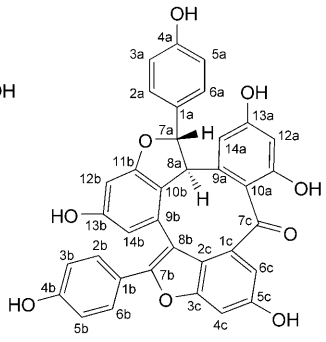
Introduction. – *Caragana sinica* (BUCHOLZ.) REHD. (Leguminosae) is widely distributed in China. Its dried root named 'Jinquegen' [1] has been used as a folk medicine. In the previous papers, we reported that the AcOEt extract of the root of *Caragana sinica* showed the effects of stimulating the proliferation, differentiation, and maturation of cultured osteoblasts *in vitro* [2]. In our lab, many oligostilbenes, some of which exhibited estrogenic activity, were isolated from this plant [3]. The aerial parts of the plant have scarcely been used and were always considered a waste product. However, our recent studies on the aerial parts of the plant showed that it can be a rich source of oligostilbenes. In this paper, we report the isolation and structure elucidation of two new oligostilbenes, carasiphenol C (**1**) and carasiphenol D (**2**), and a new dihydroaurone, carasinaurone (**3**), together with three known compounds, (−)-ampelosin F, caraphenol A [4], and carasinols A [5], from the aerial parts of *Caragana sinica*. Carasiphenol D (**2**) showed significant activity against the mycelial growth of *Pyricularia oryzae*. The minimum inhibitory concentration (*MIC*) of **2** was 0.017 μM.

Results and Discussion. – Carasiphenol C (**1**) was isolated as a yellowish, amorphous, optically active powder. Its molecular formula, $C_{42}H_{32}O_9$, was deduced from the HR-ESI-MS (*m/z* 681.2130 ($[M - H]^+$, $C_{42}H_{33}O_9^+$)). The structure of **2** was established by the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) and their comparison with those of ampelopsin H [6], a tetramer of resveratrol, and confirmed by HMBC and NOE data (*Fig. 1*).

The ^{1}H-NMR spectrum of **1** exhibited signals for three 4-hydroxy-substituted phenyl moieties at δ 7.22 (*d*, J = 8.5 Hz, 2 H), 6.86 (*d*, J = 8.5 Hz, 2 H), 6.98 (*d*, J = 8.3 Hz, 2 H), 6.70 (*d*, J = 8.3 Hz, 2 H), 6.44 (*d*, J = 8.6 Hz, 2 H) and 6.35 (*d*, J = 8.3 Hz, 2 H); for two 3,5-dihydroxy-substituted phenyl moieties at δ 6.25 (*d*, J = 2.3 Hz, 2 H), 6.31 (*t*, J = 2.1 Hz, 1 H), 6.61 (*d*, J = 1.3 Hz, 1 H), and 6.17 (*d*, J = 1.3 Hz, 1 H), and for two aliphatic protons of a dihydrobenzofuran moiety at δ 5.25 (J = 7.5 Hz, 1 H) and 4.82 (J = 7.5 Hz, 1 H). The ^{13}C-NMR spectrum exhibited the signals of six aliphatic and 36 aromatic C-atoms. All these data suggested that **1** could be a resveratrol trimer.[1]) ^{1}H- and ^{13}C-NMR features of **1** were similar to those of a segment of ampelopsin H [6], except that ampelopsin H was a tetramer. The planar structure of **1** was determined by a HMBC



1[1])



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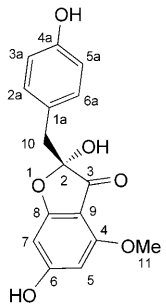

3[1])

Table 1. *^{1}H-NMR Data* ((D_6)Acetone, 500 MHz) *for* **1**–**3**[1]). δ in ppm, J in Hz.

	1	**2**		**3**
H–C(2), H–C(6a)	7.22 (*d*, *J* = 8.5)	7.11 (*d*, *J* = 8.2)	H–C(5)	5.91 (*d*, *J* = 1.3)
H–C(3a), H–C(5a)	6.86 (*d*, *J* = 8.5)	6.72 (*d*, *J* = 8.6)	H–C(7)	5.97 (*d*, *J* = 1.3)
H–C(7a)	5.25 (*d*, *J* = 7.5)	5.98 (*s*)	H–C(10)	3.01 (*s*)
H–C(8a)	4.82 (*d*, *J* = 7.5)	4.81 (*s*)	H–C(11)	3.74 (*s*)
H–C(10a)	6.25 (*d*, *J* = 2.3)		H–C(2b)	7.02 (*d*, *J* = 8.3)
H–C(12a)	6.31 (*d*, *J* = 2.1)	6.79 (*d*, *J* = 1.9)	H–C(6b)	7.02 (*d*, *J* = 8.3)
H–C(14a)	6.25 (*d*, *J* = 2.3)	6.85 (*d*, *J* = 1.9)	H–C(3b)	6.62 (*d*, *J* = 8.5)
H–C(2b), H–C(6b)	6.44 (*d*, *J* = 8.6)	7.57 (*d*, *J* = 8.7)	H–C(5b)	6.62 (*d*, *J* = 8.5)
H–C(3b), H–C(5b)	6.35 (*d*, *J* = 8.4)	6.83 (*d*, *J* = 8.7)		
H–C(7b)	4.51 (br. *s*)			
H–C(8b)	3.66 (*d*, *J* = 5.9)			
H–C(12b)	6.25 (*s*)	6.50 (*d*, *J* = 2.1)		
H–C(14b)		6.25 (*d*, *J* = 2.1)		
H–C(2c)	6.98 (*d*, *J* = 8.3)			
H–C(6c)	6.98 (*d*, *J* = 8.3)	6.66 (*d*, *J* = 2.1)		
H–C(4c)		6.44 (*d*, *J* = 2.1)		
H–C(3c)	6.70 (*d*, *J* = 8.3)			
H–C(5c)	6.70 (*d*, *J* = 8.3)			
H–C(7c)	4.61 (br. *s*)			
H–C(8c)	3.61 (*d*, *J* = 5.9)			
H–C(12c)	6.17 (*d*, *J* = 1.3)			
H–C(14c)	6.61 (*d*, *J* = 1.3)			

experiment (*Fig. 1,a*). Significant correlations *H*–C(8b)/C(7c) and C(9c), *H*–C(8c)/C(9b) and C(7b), *H*–C(7c)/C(9b) and C(8b) indicated the presence of a five-membered ring (denoted by C_1). Significant correlations *H*–C(7b)/C(9c), *H*–C(7c)/C(8c), *H*–C(8b)/C(9c), *H*–C(8b)/C(10c), and *H*–C(8c)/C(7b) indicated the presence of another five-membered ring (noted by C_2), and the two rings C_1 and C_2 were connected at C(8b) and C(8c). This kind of connection is the same as that observed in pallidol [7]. Significant correlations *H*–C(8a)/C(9b) and C(11b) indicated that C(8a) was connected to C(10b). All above data confirmed the planar structure of **1** as shown in *Fig. 1,a*. The NOEs H–C(8a)/H–C(2a,6a) and H–C(7a)/H–C(10a,14a) indicated a *trans* orientation of H–C(7a) and H–C(8a) (*Fig. 1,b*). Similarly, the NOEs

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

Table 2. *^{13}C-NMR Data* ((D$_6$)Acetone, 125 MHz) *for* **1**–**3**[1]). δ in ppm.

	1	**2**		**1**	**2**		**3**
C(1a)	133.5	133.4	C(10b)	116.6	124.3		
C(2a), C(6a)	129.6	129.0	C(11b)	163.1	156.9	C(2)	108.1
C(3a), C(5a)	116.5	116.4	C(12b)	97.1	103.5	C(3)	195.3
C(4a)	158.7	158.9	C(13b)	155.6	160.5	C(4)	162.6
C(7a)	95.1	89.3	C(14b)	126.1	109.5	C(5)	95.1
C(8a)	57.4	48.2	C(1c)	138.0	139.1	C(6)	168.7
C(9a)	145.8	139.6	C(2c)	129.4	125.3	C(7)	92.3
C(10a)	107.8	120.3	C(6c)	129.4	107.6	C(8)	173.4
C(11a)	160.3	156.1	C(3c)	116.2	159.9	C(9)	105.6
C(12a)	102.6	97.3	C(5c)	116.2	159.7	C(10)	47.2
C(13a)	160.3	157.3	C(4c)	156.2	102.3	C(11)	58.8
C(14a)	107.8	109.5	C(7c)	53.7	199.7	C(1a)	126.5
C(1b)	137.0	123.4	C(8c)	60.4		C(2a)	132.7
C(2b), (6b)	129.4	129.3	C(9b)	150.7		C(6a)	132.7
C(3b), (5b)	115.6	116.3	C(10c)	123.3		C(3a)	116.4
C(4b)	156.3	158.9	C(11c)	159.6		C(5a)	116.4
C(7b)	50.4	150.8	C(12c)	102.8		C(4a)	157.8
C(8b)	60.3	114.9	C(13c)	155.6			
C(9b)	145.4	135.3	C(14c)	103.6			

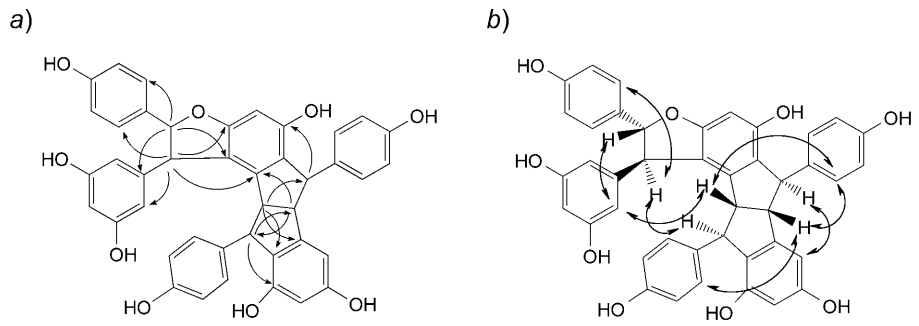

Fig. 1. *Significant* a) *HMBC and* b) *NOE correlations for carasiphenol C* (**1**)

H–C(8c)/H–C(2c,6c) and H–C(7c)/H–C(14c) were in accord with a *trans* orientation of H–C(7c) and H–C(8c). Also the NOE interactions H–C(8a)/H–C(7b), H–(8b)/H–C(10a,14a) and H–C(8b)/H–C(2b,6b) suggested that H–C(8c) and H–C(7b), as well as H–C(7b) and H–C(8b) would have a *trans* orientation. The NOE interactions H–C(8b)/H–C(2c,6c), H–C(8c)/H–C(2c,6c) and H–C(8c)/H–C(2b,6b) determined the configuration of H–C(8c) and H–C(8b) to be *cis*; H–C(7b) and H–C(8a) were determined to be *cis*.

Carasiphenol D (**2**) was isolated as a yellowish amorphous powder. Its molecular formula, $C_{35}H_{22}O_9$, was established by HR-ESI-MS (*m/z* 587.1318 ($[M + H]^+$, $C_{35}H_{23}O_9^+$)). The UV spectrum (λ_{max} 326 nm) revealed the presence of a strongly conjugated system in the structure. The structure of **2** was deduced from its spectral data (*Tables 1* and *2*), their comparison with those of caraphenol A [4] (*Table 3* and *Fig. 2*), a resveratrol trimer which has also been isolated from the roots of this plant, and confirmed by HMBC and NOESY data (*Fig. 3*).

Table 3. *NMR Data* ((D$_6$)Acetone) *of Caraphenol A and Carasiphenol D.* δ in ppm.

	Caraphenol A[1])		Carasiphenol D[1]) (**2**)	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1a)		133.5		133.4
H–C(2a), H–C(6a)	7.22 (*d*, *J* = 8.5)	129.0	7.11 (*d*, *J* = 8.2)	129.0
H–C(3a), H–C(5a)	6.86 (*d*, *J* = 8.5)	116.5	6.72 (*d*, *J* = 8.6)	116.4
C(4a)		158.7		158.9
H–C(7a)	5.25 (*d*, *J* = 7.5)	95.1	5.98 (*s*)	89.3
H–C(8a)	4.82 (*d*, *J* = 7.5)	57.4	4.81 (*s*)	48.2
C(9a)		145.8		139.6
C(10a)		107.8		120.3
C(11a)		160.3		156.1
H–C(12a)	6.31 (*d*, *J* = 2.1)	102.6	6.79 (*d*, *J* = 1.9)	97.0
C(13a)		160.3		157.3
H–C(14a)	6.25 (*d*, *J* = 2.3)	107.8	6.85 (*d*, *J* = 1.9)	109.5
C(1b)		122.7		123.4
H–C(2b), H–C(6b)	7.26 (*d*, *J* = 8.7)	129.4	7.57 (*d*, *J* = 8.7)	129.3
H–C(3b), H–C(5b)	6.70 (*d*, *J* = 8.7)	116.2	6.83 (*d*, *J* = 8.7)	116.3
C(4b)		156.3		158.9
C(7b)		149.4		150.8
C(8b)		114.5		114.9
C(9b)		135.3		135.3
C(10b)		118.9		124.3
C(11b)		163.5		156.9
H–C(12b)	6.52 (*d*, *J* = 2.1)	98.3	6.50 (*d*, *J* = 2.1)	103.5
C(13b)		160.7		160.5
H–C(14b)	6.32 (*d*, *J* = 2.1)	109.6	6.25 (*d*, *J* = 2.1)	109.5
C(1c)		139.7		139.1
C(2c)		120.5		125.3
H–C(6c)	6.94 (*d*, *J* = 8.3)	126.1	6.66 (*d*, *J* = 2.1)	107.6
C(3c)		155.2		159.9
C(5c)		157.2		159.7
H–C(4c)	6.81 (*d*, *J* = 1.8)	96.4	6.44 (*d*, *J* = 2.1)	102.3
H–C(7c) or C(7c)	4.87 (br. *s*)	45.7		199.7
H–C(8c)	5.92 (br. *s*)	87.9		
C(9c)		132.5		
H–C(10c)	7.05 (*d*, *J* = 8.6)	128.3		
H–C(11c)	6.71 (*d*, *J* = 8.6)	115.9		
C(12c)		158.2		
H–C(13c)	6.71 (*d*, *J* = 8.6)	115.9		
H–C(14c)	7.05 (*d*, *J* = 8.6)	128.3		

Carasiphenol D (**2**) was soluble in acetone, AcOEt, MeOH, and dimethylsulfoxide but was insoluble in distilled H_2O. Carasiphenol (**2**) inhibited the mycelial growth of *P. oryzae* by curling and swelling effect (*MIC* = 0.017 μM), indicating that **2** may act *via* a mechanism similar to the antifungal mechanisms of rhizoxin, which is a commercial fungicidal agent [8]. Therefore, **2** isolated from *C. sinica* may prove to be a valuable antifungal agent against rice-blast disease.

The ^{1}H-NMR spectrum of **2** exhibited signals for two 4-hydroxy-substituted phenyl groups at δ 7.57 (*d*, *J* = 8.7 Hz, 2 H), 6.83 (*d*, *J* = 8.7 Hz, 2 H), and 7.11 (*d*, *J* = 8.2 Hz, 2 H), and 6.72 (*d*, *J* = 8.6 Hz, 2 H), for three 3,5-dioxy-1,2-phenylene moieties at δ 6.50 (*d*, *J* = 2.1 Hz, 1 H), 6.25 (*d*, *J* = 2.1 Hz, 1 H), 6.44 (*d*, *J* = 2.1 Hz, 1 H),

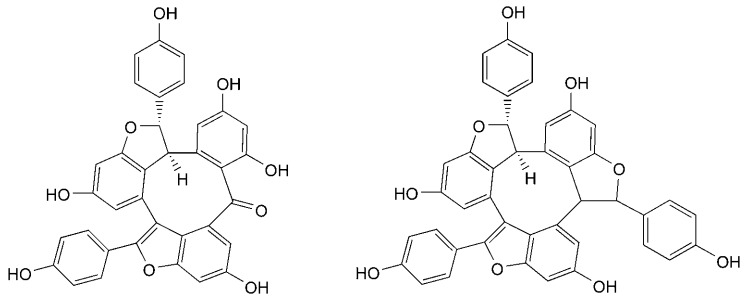

Fig. 2. *Comparison of carasiphenol D* (**2**) *and caraphenol A*

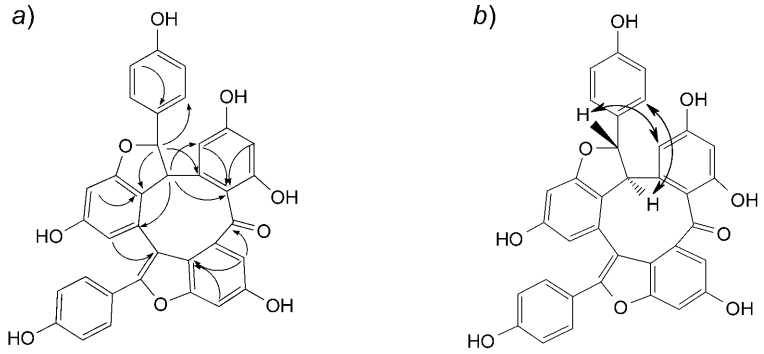

Fig. 3. *Significant* a) *HMBC and* b) *NOE correlations for carasiphenol D* (**2**)

6.66 (*d*, *J* = 2.1 Hz, 1 H), 6.79 (*d*, *J* = 1.9 Hz, 1 H), and 6.85 (*d*, *J* = 1.9 Hz, 1 H), and for two aliphatic protons of a dihydrobenzofuran moiety at δ 5.98 (br. *s*, 1 H) and 4.81 (br. *s*, 1 H). The ^{13} C-NMR spectrum exhibited the signals of two aliphatic and 32 aromatic C-atoms and an isolated carbonyl group (δ 199.7). All these data suggested that **2** could be a stilbene compound. ^{1}H- and ^{13} C-NMR features of **2** were similar to those of a segment of caraphenol A [4] (*Table 3*), a resveratrol trimer (*Fig.2*).

In the HMBC spectrum of **2**, significant correlations H–C(8a)/C(10a) and C(9b), H–C(14b)/C(8b), H–C(6c)/C(2c) and C(7c), and H–C(14a)/C(10a) indicated the presence of a nine-membered ring (*Fig. 3*,*a*). The relative configuration of **2** was established on the basis of the ^{1}H-NMR and NOESY data. In the ^{1}H-NMR spectrum, the coupling constant *J*(7a,8a) of *ca.* 0 Hz suggested that the bond angle between H–C(7a) and H–C(8a) is nearly 90°. According to a molecular model of **2**, *trans* orientation of H–C(7a) and H–C(8a) was deduced. Together with the NOEs interactions H–C(8a)/H–C(2a,6a) and H–C(7a)/H–C(14a) (*Fig. 3*,*b*), which also indicated a *trans* orientation for H–C(7a) and H–C(8a) [4], the relative configuration of **2** was established.

Carasinaurone (**3**) was isolated as pale yellow cubic crystals. Its molecular formula $C_{16}H_{14}O_6$ was established by HR-ESI-MS (*m/z* 341.0428 ($[M + K]^+$, $C_{16}H_{14}KO_6^+$)). The structure of **3** was established by spectroscopic means (*Tables 1* and *2*, and *Fig. 4*) and comparison with the data of dihydroaurone [9], which has a planar structure, and its configuration was deduced from the X-ray crystallographic analysis (*Fig. 5*).

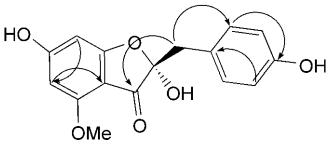

Fig. 4. *Significant HMBC correlations of carasinaurone* (**3**)

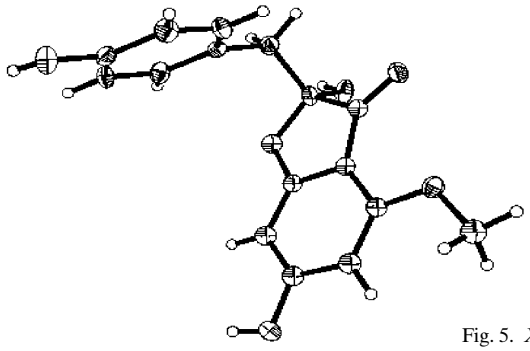

Fig. 5. *X-Ray structure of carasinaurone* (**3**)

The ^{1}H-NMR spectrum exhibited signals for a 4-hydroxy-substituted phenyl group at δ 7.02 (*d*, $J = 8.3$ Hz, 2 H) and 6.62 (*d*, $J = 8.5$ Hz, 2 H), for a 5-hydroxy-3-methoxy-1,2-phenylene group at δ 5.91 (*d*, $J = 1.3$ Hz, 1 H) and 5.97 ($J = 1.3$ Hz, 1 H), for two CH_2 protons at 3.01 (*s*), and for three Me protons at δ 3.74 (*s*). In the HMBC spectrum (*Fig. 4*), significant correlations *H*−C(10)/C(2b,6b), C(3), and C (2), H−C(5)/C(7) and C(9), and *H*−C(7)/C(5) and C(9) were observed, all above data indicating that **3** could be a dihydroaurone derivative [9] with a planar structure.

X-Ray crystallographic analysis of carasinaurone (**3**)[1])[2]): The compound crystallized in the monoclinic space group P_{21}/C with molecules of composition $C_{16}H_{14}O_6$ ($Z = 4$); accurate cell constants are: $a = 10.2922$ (14), $b = 9.2173$ (14), $c = 14.173$ (2) Å; $\beta = 90.497(3)°$; $V = 1344.5$ (3) Å^3. All reflections were collected on the *CCD* area detector diffractometer, MoK_a radiation (λ 0.71073 Å); maximum 2θ value 25.10°; independent reflections 2387, observed reflections 1797 ($|F|^2 \geq 2\,\delta\,|F|^2$) $F(000) = 632$. The structure was resolved by direct methods (SHELXS-97), expanded by using SHELXS-97/2, and refined by full-matrix least-squares calculations. H-Atoms were fixed at calculated positions. The final indices were $R_f = 0.0436$, $R_w = 0.1099$.

This work was supported by the *National Natural Science Foundation of China* (Grant No. 30270155).

Experimental Part

General. M.p.: *Kofler* micro melting-point apparatus; uncorrected. Optical rotations: *Jasco P-1020* polarimeter; MeOH solns. UV Spectra: *Shimadzu UV-240* spectrophotometer λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 783* IR spectrophotometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-400* spectrometer; δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. HR-ESI-MS: *AB-QSTAR-Pulsar* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *C. sinica* (BUCHOLZ.) REHD. were collected in September, 2001, from Zhongxiang County, Hubei Province, China, and identified by Professor *Zhi-Jian Feng*, Department of Biology, Shanghai East China Normal University. A Voucher specimen (CS-HZ-1106) is deposited in the Herbarium of Materia Medica, Department of Chemistry of Natural Drugs, School of Pharmacy, Fudan University, People's Republic of China.

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[2]) CCDC 245803 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif from the *Cambridge Crystallographic Data Centre*.

Extraction and Isolation. The air-dried and powdered aerial parts of *Caragana sinica* (BUCHOLZ.) REHD. (70 kg) were macerated with 95% EtOH at r.t. The solvent was evaporated to yield a residue (5 kg), which was successively partitioned between H_2O and petroleum ether and AcOEt. The AcOEt extract (230 g) was subjected to CC Silica gel (1.5 kg, 200–300 mesh), $CHCl_3$/MeOH (10:1, 9:1, 4:1, 7:3 and 3:2), MeOH): *Fractions A–F. Fr. C* was subjected to CC (silica gel (300–400 mesh), petroleum ether/Et_2O 3:2): caraphenol A (47 mg), (−)-ampelosin F (6.0 g), and carasinaurone (**3**; 93 mg). The latter was purified by crystallization from MeOH/H_2O. From *Fr. D*, carasiphenol D (**2**; 18 mg) and carasinol A (37 mg) were obtained by CC (silica gel, petroleum ether/Et_2O 2:3; and then *Sephadex LH-20* $CHCl_3$/MeOH 1:3). Carasiphenol C (**1**; 10 mg) was obtained from *Fr. E* by CC (silica gel, petroleum ether/Et_2O 2:3; then reversed-phase silica gel (*RP-18*), MeOH/H_2O 2:3).

Carasiphenol C (= 1-(3,5-Dihydroxyphenyl)-1,2,6,6a,11,11a-hexahydro-2,6,11-tri(4-hydroxyphenyl)indeno[1′,2′:2,3]indeno[S,A-b*]furan-5,8,10-triol*; **1**): Brown amorphous powder. M.p. 216–218°, $[\alpha]_D^{20} = +36.8$ ($c = 0.281$, MeOH). UV (MeOH): 278 (3.2). IR (KBr): 3356, 1605, 1532, 1460, 1320, 1205, 1098, 1016, 883. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 681.2130 ($[M + H]^+$, $C_{42}H_{33}O_9^+$; calc. 681.2125).

*Carasiphenol D (= 5,5a-Dihydro-2,7,9,12,-tetrahydroxy-5,15-bis(4-hydroxyphenyl)-10*H*-benzo[8,9]cyclonona[1,2,3*-c,d:*4,5,6*-c′,d′*]bisbenzofuran-10-one;* **2**): Pale yellow amorphous powder. M.p. > 240°. $[\alpha]_D^{25} = +47.9$ ($c = 0.4$ MeOH). UV (MeOH): 326 (3.5). IR (KBr): 3330, 1710, 1640, 1548, 1466, 1280, 1203, 1129, 1057, 825. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2.* HR-ESI-MS: 587.1339 ($[M + H]^+$, $C_{35}H_{23}O_9^+$; calc. 587.1342).

Carasinaurone (*= 2-(4-Hydroxybenzyl)-2,6-dihydroxy-4-methoxybenzofuran-3(2*H*)-one;* **3**): Pale yellow cubic crystals. M.p. > 240°, $[\alpha]_D^{25} = +38.9$ ($c = 0.356$, MeOH), UV (MeOH): 272 (1.7). IR (KBr): 3373, 1680, 1598, 1516, 1430, 1357, 1206, 1120, 834, 790. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2.* HR-ESI-MS: 341.0427 ($[M + K]^+$, $C_{16}H_{14}KO_6^+$; calc. 341.0427).

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Received August 4, 2004