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Article

Three Stilbene Tetramers from the Roots of *Caragana sinica*

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Three stilbene tetramers, carasinols A—C (**1**—**3**), along with three known substances, leachianol C, cararosinol A and stenophyllol B, were isolated from the roots of *Caragana sinica*. Their structures were elucidated by spectroscopy. It was found that compounds isolated except for stenophyllol B stimulated the proliferation of cultured osteoblasts.

Keywords *Caragana sinica*, Fabaceae, resveratrol, carasinols A—C

Introduction

Caragana sinica (Buc'hoz) Rehd. (Fabaceae) is wildly distributed in China. Its root is used as a Chinese traditional herb medicine for the treatment of hypertension, leukorrhagia and bruises.[1] The effects of stimulating the proliferation of cultured osteoblasts *in vitro* of EtOAc extract of the roots of *C. sinica* and some oligostilbenes were reported previously.[2] Three new resveratrol tetramers, carasinols A—C (**1**—**3**) (Figure 1) were isolated from the roots of *C. sinica* together with three known resveratrol oligomers, leachianol C,[3] cararosinol A[4] and stenophyllol B.[5] The isolated compounds were tested for the activity of stimulating the growth of cultured osteoblasts.

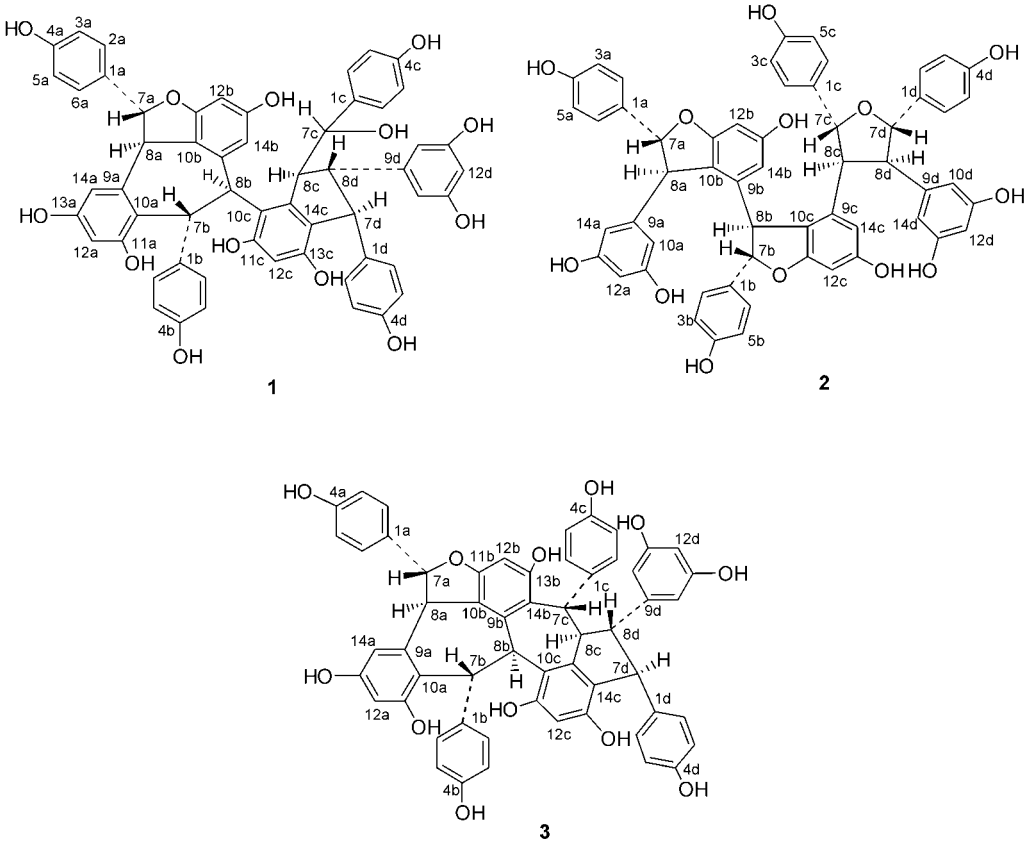

Figure 1 Structures of carasinols A—C (**1**—**3**).

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Results and discussion

Carasinol A (**1**) was obtained as a brown amorphous powder. Its molecular formula as $C_{56}H_{44}O_{13}$ was established by HR-FABMS. The ^{1}H NMR spectrum exhibited signals of four sets of *ortho*-coupled aromatic protons assigned to four 4-hydroxyphenyl groups, one AB_2 system revealing the presence of a 3,5-dihydroxyphenyl group, two sets of *meta*-coupled aromatic protons assigned to two 3,5-dihydroxy-1,2-disubstituted phenyl groups, one singlet of a pentasubstituted benzene ring, two aliphatic protons on one dihydrobenzofuran ring [δ 5.84, 4.29 (d, J=10.6 Hz, each 1H)] and six other aliphatic methine protons. These disclosed that **1** is a resveratrol tetramer with one dihydrofuran unit and one aliphatic hydroxyl moiety at C(7c) according to δ_C 78.8. The planar structure was determined by HMBC spectrum (Figure 2). Significant correlations between H(7d)/C(8c), H(8d)/C(7c), H(8d)/C(9c), H(7d)/C(9c) indicated the presence of a five-membered ring (C_3). Correlations between H(8a)/C(9b), H(8b)/C(10a), H(7b)/C(9a), revealed the presence of a seven-membered ring (B_3). C(8b) was connected with C(14c) regarding the correlations between H(8b)/C(9c), H(8b)/C(13c). Distinct NOEs between H(7d)/H[10(14)d], H(8d)/H[2(6)d], H(8c)/H[10(14)d] showed that H(8d) was *trans* to H(7d), H(8c); NOEs between H(7a)/H(14a), H(8a)/H[2(6)a] revealed H(7a), H(8a) were *trans*. NOEs between H(7b)/H(14b), H(8b)/H[2(6)b] indicated H(7b), H(8b) were *trans*. The seven-membered ring is similar to the seven-membered ring in Hopeaphenol.[6] So the relative stereochemistry of **1** was determined as shown in Figure 3.

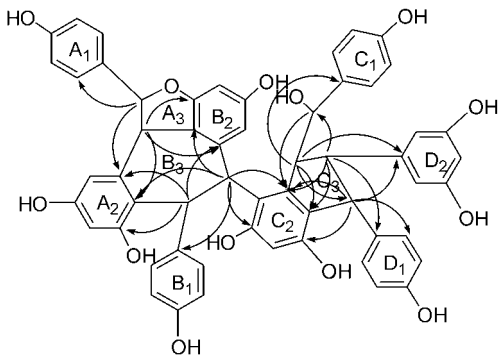

Figure 2 Significant HMBC correlations of **1**.

Carasinol B (**2**), a pale yellow amorphous powder, had the molecular formula as $C_{56}H_{44}O_{13}$ given by HR-FABMS. The ^{1}H NMR spectrum indicated the presence of four 4-hydroxyphenyl groups, two 3,5-dihydroxyphenyl groups and two 3,5-dihydroxy-1,2-disubstituted phenyl groups. In the aliphatic region, four protons on two dihydrobenzofuran rings [δ 5.08, 4.05 (d, J=3.3 Hz, each 1H); δ 4.84, 3.60 (d, J=4.1 Hz, each 1H)] and four successive protons of a tetrasubstituted tetra-hydrofuran were observed. The significant HMBC correlation (Figure 4) revealed the planar structure of **2** was the same as kobophenol A.[7] A significant NOE between H(7a)/H[10(14)a], H(8a)/H[2(6)a] indicated H(7a) and H(8a) were *trans*; NOEs between H(7b)/H(14b), H(8b)/H[2(6)b] indicated the two methine protons on the ring B_3 were *trans*. On ring C_3, distinct NOEs between H(7c)/H(7d), H(7c)/H(14c), H(7c)/H-[10(14)d], H(8c)/H[2(6)c], H(7d)/H[10(14)d], H(8d)/H-[2(6)d] revealed H(7c) and H(7d) were *trans* to H(8c) and H(8d). NOEs between H(8a)/H(8b) and H(8b)/H(8c) gave evidence for the molecular stereochemistry determination as shown in Figure 5. So, the only difference between **2** and kobophenol A is the relative spacial tendency of H(7c).

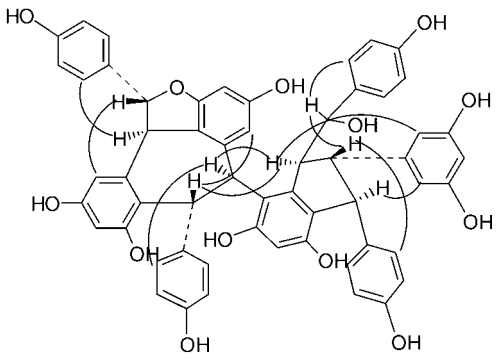

Figure 3 Significant NOEs of **1**.

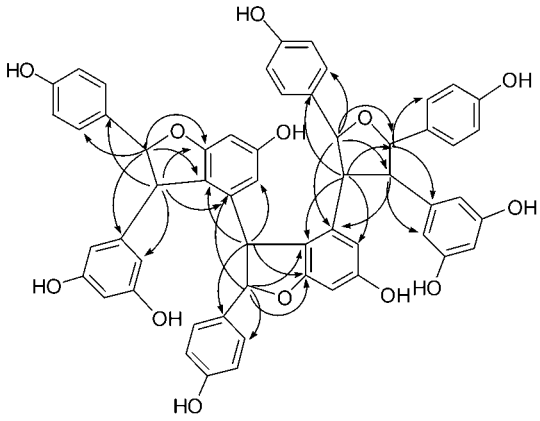

Figure 4 Significant HMBC correlations of **2**.

The molecular formula of Carasinol C (**3**), a yellow amorphous powder, was assigned as $C_{56}H_{42}O_{12}$ by HR-FABMS indicating a resveratrol tetramer. The ^{1}H NMR spectrum showed the presence of four 4-hydroxyphenyl groups, one 3,5-dihydroxyphenyl group, one 3,5-dihydroxy-1,2-disubstituted benzene ring and two pentasubstituted benzene rings. In the aliphatic region, two mutually coupled protons were assigned to one dihydrobenzofuran moiety [δ 5.87, 4.81 (d, J=11.7 Hz, each 1H)] and there were other six aliphatic protons. In the HMBC spectrum (Figure 6), correlations between

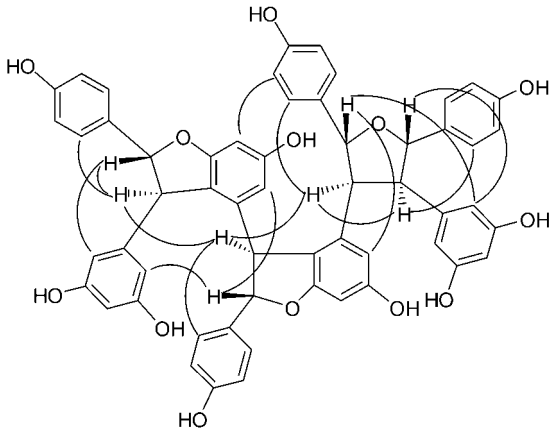

Figure 5 Significant NOEs of **2**.

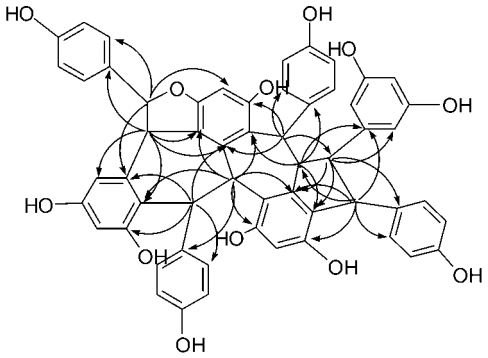

Figure 6 Significant HMBC correlations of **3**.

H(8a)/C(9b), H(7b)/C(9a) indicated the presence of a seven-membered ring (B_3). Another seven-membered ring (C_3) could be inferred from the correlations between H(7c)/C(9b), H(8c)/C(14b), H(8b)/C(9c). Correlations between H(8c)/C(7d), H(7d)/C(9c), H(8d)/C(9c), H(8d)/C(14c) showed the presence of a five-membered ring (D_3). Distinct NOEs between H(7a)/H(14a), H[2(6)a]/H(8a) indicated that two methine protons [H(7a), H(8a)] on the dihydrobenzofuran ring were *trans*. NOEs between H[2(6)b]/H(8a), H(8b)/H[2(6)b], H(8d)/H(7c), H(8d)/H[2(6)d], H(7d)/H[10(14)d], H(8c)/H(8b), H(8c)/H[2(6)d], H(8b)/H[2(6)c] revealed H(8a), H(8b), H(8c) and H(7d) were *cis*. In view of the above observations, the structure of **3** was deduced as shown in Figure 7.

Experimental

General procedure

All UV spectra were measured on a Shimadzu UV-240 spectrophotometer. The IR spectra were taken on a Perkin-Elmer 783 (KBr) spectrophotometer. The optical rotations were determined using a Jasco P-1020 polarimeter in CH_3OH. HR-FABMS data were obtained on a VG AutoSpec 3000 mass spectrometer. The NMR spectra were recorded on a Bruker AM×400 instrument.

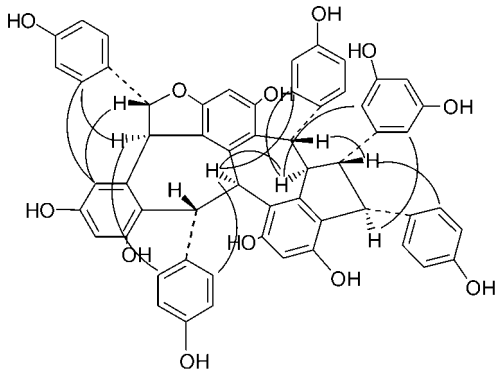

Figure 7 Significant NOEs of **3.**

Plant material

The roots of *C. sinica* (Buc'hoz) Rehd. were collected in August, 1999 from Zhongxiang County, Hubei Province, China and identified by Professor Feng Zhi-Jian, Department of Biology, Shanghai, East China Normal University. A voucher specimen (No. 20917) has been deposited in the herbarium of Institute of Botany, Jiangsu Province and Chinese Academy of Sciences.

Extraction and isolation

The dried and powdered roots (100 kg) were macerated with 90% ethanol at room temperature. The concentrated residue (7.8 kg) was precipitated in water and filtrated. The filtrated solvent was concentrated and applied to macroporous resin washed with water and ethanol successively. The ethanol fraction (360 g) was partitioned between water and EtOAc, *n*-butanol. The *n*-butanol fraction (187 g) was subjected to silica gel with $CHCl_3$-MeOH mixture of increasing polarity. Fr. 4 (31.0 g), after being subjected to a silica gel column eluted with petroleum-EtOAc (1∶4) and another one with cyclohexane-acetone (1∶1), gave stenophyllol B (52 mg). Fr. 6 (8.5 g), after column chromatography on silica gel with cyclohexane-acetone (1∶1.2) and then YWG-C18 eluted with MeOH-H_2O (1∶3), yielded **1** (58 mg), **2** (65 mg) and cararosinol A (63 mg). Fr. 7 (20.2 g) gave **3** (46.5 mg) and leachianol C (27 mg) after passage over an Sephadex LH20 column eluted with acetone and silica gel, eluted with petroleum-EtOAc (1∶5) and $CHCl_3$-EtOAc (1∶4).

Carasinol A (1) Brown amorphous powder, m.p. >240 ℃; $[\alpha]_D^{25}$ −133.4 (*c* 1.509, MeOH); UV (MeOH) λ_{max}: 283 (log ε 4.2) nm; ^{1}H NMR and ^{13}C NMR data are shown in Table 1; IR (KBr) *v*: 3380, 1608, 1512, 1440, 1340, 1222, 1181, 1128, 1005, 832 cm^{-1}; HRFABMS calcd for $C_{56}H_{44}O_{13}$ 923.2704 $[M-H]^-$, found 923.2706.

Carasinol B (2) Pale yellow amorphous powder, m.p. >240 ℃; $[\alpha]_D^{25}$ +181.7 (*c* 2.258, MeOH); UV (MeOH) λ_{max}: 284 (log ε 4.2) nm; ^{1}H and ^{13}C NMR data are shown in Table 1; IR (KBr) *v*: 3380, 610, 1516,

1449, 1240, 1167, 1122, 1000, 830 cm^{-1}; HRFABMS calcd for $C_{56}H_{44}O_{13}$ 923.2704 $[M-H]^-$, found 923.2662.

Carasinol C (3) Yellow amorphous powder, m.p. >240 ℃, $[\alpha]_D^{25}$ –91.3 (*c* 1.013, MeOH); UV (MeOH) λ_{max}: 284 (log ε 4.4) nm; ^1H NMR and ^{13}C NMR data are shown in Table 1; IR (KBr) *v*: 3417, 1613, 1512, 1455, 1344, 1234, 1166, 834 cm^{-1}; HRFABMS calcd. for $C_{56}H_{42}O_{12}$ 905.2598 $[M-H]^-$, found 905.2627.

Stimulating the growth of osteoblasts

The method of MTT[8] was used to observe the activity of stimulating the growth of osteoblasts. It was found that carasinol A—C (**1**—**3**), cararosinol A and leachianol C had the effects of stimulating the proliferation of cultured osteoblasts (the reproduction rate of osteoblasts was raised 22.8%, 10.0%, 26.5%, 17.5% and 19.8% respectively at 1.0 μg/mL than that of the controlled group).

Table 1 ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1**—**3** in acetone-d_6[a]

Site	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a		131.1		134.3		131.1
2(6)a	7.08 (d, *J*=8.6 Hz)	129.8	6.72 (d, *J*=8.5 Hz)	127.8	7.24 (d, *J*=8.6 Hz)	130.1
3(5)a	6.74 (d, *J*=8.6 Hz)	115.9	6.51 (d, *J*=8.5 Hz)	116.0[a]	6.78 (d, *J*=8.6 Hz)	116.1
4a		158.4		158.1		158.6
7a	5.84 (d, *J*=10.6 Hz)	87.9	5.08 (d, *J*=3.3 Hz)	94.8	5.87 (d, *J*=11.7 Hz)	88.1
8a	4.29 (d, *J*=10.6 Hz)	49.5	4.05 (d, *J*=3.3 Hz)	57.0	4.81 (d, *J*=11.7 Hz)	50.9
9a		143.0		148.3		143.4
10a		120.7	5.74 (d, *J*=2.2 Hz)	106.7		118.9
11a		157.6[a]		159.5		158.2
12a	6.37 (d, *J*=2.1 Hz)	102.1	5.90 (t, *J*=2.2 Hz)	102.3	6.54 (d, *J*=1.8 Hz)	102.5
13a		157.7[b]		157.7		158.8
14a	6.34 (brs)	105.6	5.74 (d, *J*=2.0 Hz)	106.7	6.42 (brs)	106.7
1b		135.8		133.5		135.6
2(6)b	7.07 (d, *J*=8.6 Hz)	128.5	6.19 (d, *J*=8.6 Hz)	128.4	7.39 (d, *J*=8.6 Hz)	129.1
3(5)b	6.64 (d, *J*=8.6 Hz)	115.6	6.47 (d, *J*=8.6 Hz)	116.2[b]	6.70 (d, *J*=8.6 Hz)	115.7[a]
4b		156.1		157.6		156.5
7b	5.27 (brs)	42.8	4.84 (d, *J*=4.2 Hz)	94.0	5.73 (d, *J*=3.1 Hz)	40.0
8b	6.50 (brs)	41.7	3.60 (d, *J*=4.2 Hz)	51.7	5.97 (d, *J*=3.1 Hz)	46.2
9b		140.7		144.4		140.8
10b		119.2		120.9		114.9
11b		160.4		163.0		157.9
12b	6.13 (d, *J*=2.2 Hz)	96.4	6.42 (d, *J*=2.1 Hz)	96.4	6.08 (s)	96.4
13b		159.0		161.1		157.7
14b	7.02 (d, *J*=2.2 Hz)	110.9	6.03 (d, *J*=2.1 Hz)	107.4		121.7
1c		137.1		136.3		138.9
2(6)c	6.71 (d, *J*=8.6 Hz)	129.3	6.79 (d, *J*=8.6 Hz)	127.3	7.08 (d, *J*=8.6 Hz)	131.0
3(5)c	6.60 (d, *J*=8.6 Hz)	115.5	6.79 (d, *J*=8.6 Hz)	116.2[c]	6.60 (d, *J*=8.6 Hz)	115.1
4c		157.3		157.3		155.7

Continued

Site	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
7c	4.49 (d, *J*=10.1 Hz)	78.8	5.25 (brs)	86.9	4.33 (d, *J*=10.9 Hz)	50.9
8c	3.75 (d, *J*=10.1 Hz)	60.8	3.35 (dd, *J*=7.6,1.8 Hz)	56.8	4.02 (d, *J*=10.9 Hz)	62.3
9c		149.0		140.0		149.2
10c		122.4		123.7		120.7
11c		153.8		161.7		155.1
12c	6.00 (s)	103.7	6.12 (d, *J*=2.2 Hz)	96.1	6.02 (s)	104.0
13c		157.8		159.9		153.9
14c		121.9	6.73 (d, *J*=2.2 Hz)	108.4		121.8
1d		137.5		131.9		138.6
2(6)d	7.02 (d, *J*=8.6 Hz)	129.5	7.16 (d, *J*=8.6 Hz)	130.1	6.84 (d, *J*=8.6 Hz)	129.0
3(5)d	6.81 (d, *J*=8.6 Hz)	115.5	6.71 (d, *J*=8.6 Hz)	116.5[d]	6.69 (d, *J*=8.6 Hz)	115.6[b]
4d		156.3		158.2		156.2
7d	4.24 (s)	55.1	5.26 (d, *J*=11.1 Hz)	86.1	4.42 (s)	57.3
8d	2.97 (s)	59.5	3.10 (dd, *J*=11.1, 7.6 Hz)	55.8	3.22 (s)	62.0
9d		151.4		139.6		152.5
10(14)d	6.03 (d, *J*=2.2 Hz)	105.8	5.49 (d, *J*=2.1 Hz)	109.3	5.95 (d, *J*=2.2 Hz)	105.1
11(13)d		158.9		158.5		159.2
12d	6.00 (t, *J*=2.2 Hz)	100.9	6.13 (t, *J*=2.1 Hz)	103.3	6.06 (t, *J*=2.2 Hz)	101.0

Acknowledgements

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