Oligomeric Stilbenes from the Root of *Caragana stenophylla*

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Caragaphenol A (1), a new trimeric stilbene, was isolated from the roots of *Caragana stenophylla* (Leguminosae) along with three known oligomeric stilbenes: (+)-α-viniferin (2), miyabenol C (3) and kobophenol A (4). The stereostructure of 1 was elucidated by one- and two-dimensional (1D- and 2D-)-NMR spectral studies.

Key words *Caragana stenophylla*; Leguminosae; caragaphenol A; trimeric stilbene

Caragana stenophylla belongs to the genus Caragana of family Leguminosae. Some species of the genus Caragana have been used in Chinese traditional medicine for the treatment of asthenia syndrome, vascular hypertension, leucorrhagia, neuralgia, rheumatism, arthritis, bruises and contused wounds.[1,2] It has been reported that oligomeric stilbenes from the genus Caragana have some valuable pharmacological activities: they can be used as naturally occurring protein kinase C inhibitors[3,4] and display a growth inhibitory effect on lung cancer cells.[5,6] They also have the analogous effects of estrogen that can promote the absorption of calcium,[7] which makes it interesting to study more oligomeric stilbenes in the Caragana species. *C. stenophylla* was hitherto uninvestigated for its chemical constituents. In this paper, we report the isolation and structure elucidation of a new trimeric stilbene (**1**), together with three known oligomeric stilbenes (**2**—**4**) from the roots of *C. stenophylla*.

Results and Discussion

Compound **1** was obtained as a reddish amorphous powder, $[\alpha]_D^{20}$ +786.54° (c=0.104, MeOH). Its molecular formula was determined as $C_{42}H_{30}O_9$ by HR-FAB-MS m/z 679.1952 $[M+H]^+$ (Calcd. 679.1968). Its ^{1}H- and ^{13}C-NMR spectral data suggested that compound **1** could be a resveratrol trimor. Absorption bands were observed at 212 and 280 nm in the UV spectrum. The IR spectrum showed characteristic absorption bands from hydroxyl (3400 cm^{-1}) and aromatic rings (1600, 1510, 1440 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) showed the presence of three sets of 4-hydroxy-1-substituted benzyl moieties [δ 7.01 (2H, d, J=8.5 Hz) and 6.71 (2H, d, J=8.5 Hz); 6.91 (2H, d, J=8.5 Hz) and 6.71 (2H, d, J=8.5 Hz); 6.86 (2H, d, J=8.5 Hz) and 6.67 (2H, d, J=8.5 Hz)]; three sets of 3,5-dihydroxy-1,2-disubstituted benzyl moieties [δ 6.52 (1H, d, J=1.5 Hz) and 6.25 (1H, d, J=1.5 Hz); 6.33 (1H, d, J=2.5 Hz) and 5.59 (1H, d, J=2.5 Hz); 6.17 (1H, d, J=2.0 Hz) and 6.01 (1H, d, J=2.0 Hz)]; an olefinic proton [δ 6.38 (1H, s)]; four aliphatic protons of two dihydrobenzofuran rings [δ 5.83 (1H, d, J=3.5 Hz) and 4.44 (1H, d, J=3.5 Hz); 4.54 (1H, d, J=11.5 Hz) and 4.45 (1H, d, J=11.5 Hz)] as well as seven phenolic hydroxyl protons (δ 8.50, 8.39, 8.33, 8.21, 8.13, 8.08, 7.65). The ^{13}C-NMR spectrum exhibited the presence of four aliphatic carbons (δ 90.3, 58.9; 89.6, 51.0), two olefinic carbons (δ 132.5, 135.3) and thirty-six aromatic carbons. All protonated carbons were assigned from the HMQC spectrum. The ^{1}H- and ^{13}C-NMR features of compound **1** were similar to those of (+)-α-viniferin[8] except that compound **1** showed only four alphatic protons for two dihydrobenzofuran moieties in the ^{1}H-NMR spectrum and two olefinic carbons signals more than that of (+)-α-viniferin in the ^{13}C-NMR. Furthermore, compound **1** exhibited seven hydroxy signals and one olefinic proton signal in the ^{1}H-NMR spectrum; these spectroscopic data meant that one furan ring of dihydrobenzofuran was opened in compound **1** compared with (+)-α-viniferin. In the HMBC spectrum of compound **1**, long-range correlations between H-2(6)c and C-7c (δ 132.5), H-7c and C-8c (δ 135.3) indicated that ring C1 was attached to C-7c; long-range correlations between H-2(6)a and C-7a (δ 89.6), H-2(6)b and C-7b (δ 90.3) indicated that ring A1 was attached to C-7a and ring B1 was attached to C-7b. Therefore, the planar structure of compound **1** was concluded to be as shown in Fig. 1.

The relative stereochemistry of **1** was established on the basis of NOE difference spectra. Irradiation at the signal of H-8a enhanced the signal intensity of H-2(6)a, the same as H-7a and H-14a, suggesting that H-7a and H-8a were situated in a *trans* orientation. On irradiation at the signal of H-8b, NOE enhancement was observed at the signal of H-2(6)b, the same as H-7b and H-14b and indicated that H-7b and H-8b were also situated in a *trans* orientation. Furthermore, the NOE enhancement was not observed at the signal of H-8b when irradiating at the signal of H-8a, so the positions of H-8a and H-8b were *trans*. The configuration of

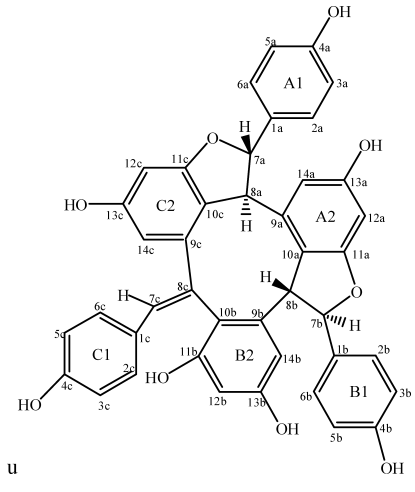

Fig. 1. The Structure of Caragaphenol A

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Table 1. The ^{1}H-, ^{13}C-NMR and HMBC Data of Compound **1** (500, 125 MHz, in CD_3COCD_3)

No.	δ_C	δ_H	HMBC
1a	131.5		H-3a
2(6)a	127.7	7.01 (2H, d, J=8.5 Hz)	H-7a
3(5)a	115.9	6.71 (2H, d, J=8.5 Hz)	
4a	158.2		H-2a
7a	89.6	5.83 (1H, d, J=3.5 Hz)	H-2a
8a	51.0	4.44 (1H, d, J=3.5 Hz)	H-14a, H-7a
9a	141.7		H-7a, H-8a, H-8b
10a	118.3		H-7b, H-8a, H-8b, H-12a, H-14a
11a	160.5		H-7b, H-8b, H-12a
12a	95.6	6.01 (1H, d, J=2.0 Hz)	H-14a
13a	159.7		
14a	104.3	6.17 (1H, d, J=2.0 Hz)	H-8a, H-12a
1b	134.7		H-3b, H-7b
2(6)b	129.8	6.86 (2H, d, J=8.5 Hz)	H-7b
3(5)b	116.0	6.67 (2H, d, J=8.5 Hz)	
4b	158.1		H-2b
7b	90.3	4.45 (1H, d, J=11.5 Hz)	H-2b, H-8b
8b	58.9	4.54 (1H, d, J=11.5 Hz)	H-7b, H-14b
9b	139.4		H-7b, H-8b
10b	116.8		H-7c, H-8b, H-12b, H-14b
11b	157.9		
12b	102.7	6.33 (1H, d, J=2.5 Hz)	H-14b
13b	157.9		
14b	111.1	5.59 (1H, d, J=2.5 Hz)	H-8b, H-12b
1c	130.5		H-3c
2(6)c	131.4	6.91 (2H, d, J=8.5 Hz)	H-3c, H-7c
3(5)c	115.5	6.71 (2H, d, J=8.5 Hz)	
4c	157.7		H-2c
7c	132.5	6.38 (1H, s)	H-2c, H-6c
8c	135.3		H-7c, H-14c
9c	143.7		H-7c
10c	121.5		H-7a, H-8a H-12c, H-14c
11c	159.3		H-7a, H-8a
12c	96.7	6.25 (1H, d, J=1.5 Hz)	H-14c
13c	158.8		
14c	110.6	6.52 (1H, d, J=1.5 Hz)	H-12c

alkene was *Z*-form because NOE enhancement was observed at the signal of H-14c when irradiating at the signal of olefinic proton H-7c. On the basis of these findings, the relative configuration of **1** is shown in Fig. 1.

The known compounds, (+)-α-viniferin (**2**),[8] miyabenol C (**3**)[8] and kobophenol A (**4**)[8] were also isolated and identified by comparison of their spectral data with corresponding literature values.

Experimental

General Procedures Melting points were determined on a Kofler micromelting-point apparatus and are uncorrected. UV spectra were obtained on a Philips PYE Unican Pu 8800 spectrophotometer. IR spectra were recorded as KBr pellets on a Perkin-Elmer 783G infrared spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. ESI-TOF-MS were recorded on a API QSTAR Pulsar I system Q-TOF MS instrument. 1D and 2D NMR spectra were taken on a INOVA AM-500 instrument with TMS as internal standard. Silica gel for column chromatography and TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China.

Plant Meterial The roots of *C. stenophylla* were collected in Baotou city, Innermongolia municipality, People's Republic of China, in June 2002. The voucher specimen (IMP-02-06-24) is deposited in the Laboratory of Phytochemistry of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and Isolation The dried roots of *C. stenophylla* (10 kg) were pulverized and extracted with 95% ethanol under reflux for 3×2 h at 90 °C. The solvent was evaporated *in vacuo* to yield 1 kg residue that was partitioned between H_2O and petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH, successively. The EtOAc extract (300 g) was subjected to a silica gel column eluted with $CHCl_3$–MeOH (10 : 1 to 1 : 10) to give ten fractions. Fraction 5 was applied to a silica gel (300—400 mesh) low pressure column eluted with $CHCl_3$–EtOAc–MeOH (10 : 1 : 0.5) to afford compound **2** (300 mg), **3** (80 mg). Fraction 6 was similarly chromatographed on silica gel and refined with preparative TLC to yield compound **1** (80 mg) which was further purified by a Sephadex LH-20 column eluted with $CHCl_3$–MeOH (1 : 1). Fraction 8 was applied to a silica gel (200—300 mesh) column eluted with $CHCl_3$–EtOAc–MeOH (8 : 2 : 1) to afford compound **4** (500 mg).

Compound **1**: Reddish amorphous powder, mp 263—265 °C; $[\alpha]_D^{20}$ +786.54° (c=0.104, MeOH); UV λ_{max} (MeOH) nm (log ε): 212 (4.67), 285 (4.22); IR (KBr) ν_{max} cm^{-1}: 3400, 1600, 1510, 1440, 1340, 1240, 1170, 1110, 1000, 830; HR-FAB-MS m/z 679.1952 $[M+H]^+$ (Calcd. 679.1968); ^{1}H- and ^{13}C-NMR data, see Table 1.

Acknowledgements Prof. Wen-Yan Lian, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China., is gratefully acknowledged for the plant identification.

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