Oligomeric Stilbenes from *Caragana chamlagu* LAMARK Root

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Caraganaphenol A (1), a new tetrameric stilbene, was isolated from the root of *Caragana chamlagu* LAMARK (Leguminosae) along with (+)-α-viniferin (2) and kobophenol A (3). The stereostructure of 1 was suggested using NMR techniques (^{1}H-detected heteronuclear multiple quantum coherence (HMQC), ^{1}H-detected heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser effect (NOE) difference spectra).

Key words *Caragana chamlagu*; Leguminosae; caraganaphenol A; kobophenol A; tetrameric stilbene; 2D NMR

The dried roots of *Caragana chamlagu* LAMARCK (Leguminosae) have been used in Korea and China as a folk medicine effective against neuralgia, rheumatism, and arthritis.

We previously reported[1] the isolation of a trimeric stilbene, (+)-α-viniferin (**2**), as anti-inflammatory principle from the roots of this plant. In a continuing survey of oligostilbenes as part of a phytochemical study on this plant, we wish to report the isolation and structure elucidation of a new tetrameric stilbene, caraganaphenol A (**1**), along with two known stilbene oligomers, (+)-α-viniferin (**2**) and kobophenol A (**3**).[2] Two isolated tetrameric stilbenes (**1** and **3**) were not observed on to have an anti-inflammatory effect by the carrageenin-induced paw edema method.

Acetone extract of the dried roots of *C. chamlagu* was chromatographed on a silica gel, a Sephadex LH-20, and then a reversed phase column to give a small amount of **1** together with (+)-α-viniferin (**2**) and **3**.

Kobophenol A (**3**) was isolated as colorless needles, mp 148.5—151 °C, $[\alpha]_D$ +199.4°. The field desorption mass spectrum (FDMS) of **3** gave a molecular ion peak at *m/z* 924. By extensive NMR experiment, **3** was identified as kobophenol A,[2] which was isolated earlier from *Carex pumila* THUMB.

Caraganaphenol A (**1**) was obtained as a reddish powder and showed $[\alpha]_D$ +188.5°. The UV and IR spectra showed a maximum absorption at 285 nm and strong bands at 3400 (OH) and 1601 cm^{-1} (aromatic C=C), respectively, which bands were virtually the same as those of polyphenols. The field desorption MS (FDMS) of **1** gave a molecular ion peak at *m/z* 922. The determination of its molecular formula by high-resolution MS (HR-EIMS) was unsuccessful because its molecular ion could not be observed due to the non-volatility of **1**. However, fifty-six carbons and forty-two protons in total were observed in the ^{13}C- and ^{1}H-NMR spectra, respectively. The presence of ten hydroxy groups was indicated by the ^{1}H-NMR and the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC)[3,4] spectra shown in Table 1.

Methylation of **1** with Me_2SO_4/K_2CO_3 in dry acetone afforded a decamethyl ether (**1a**), FDMS *m/z* 1062 (M^+), revealing the existence of ten methoxy groups in the ^{1}H-NMR spectrum.

Taking into consideration its molecular weight given by the FDMS, the molecular formula of **1** was deduced to be $C_{56}H_{42}O_{13}$. The degree of unsaturation was estimated to be thirty-six by its molecular formula. Thirty-three unsaturations were assigned to eight benzene rings and to a carbonyl group, leaving the final three unsaturations to be accommodated by three rings. Analysis of the ^{13}C- and ^{1}H-NMR spectra, the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC)[4] and the HMQC experiments revealed the existence of four 4-hydroxyphenyl groups (A1, B1, C1 and D1), two 3,5-dihydroxyphenyl groups (A2 and D2), a 3-hydroxy-5,6-disubstituted phenyl group (B2) and a 3-hydroxy-2,5,6-trisubstituted phenyl group (C2). By comparisons of the ^{1}H-NMR and ^{13}C-NMR data in **1** and **3**, the partial structure of rings A1, A2, A3, B1, B2 and B3 in **1** was estimated to be the same as that of **3** and was ascertained by analysis of HMQC and HMBC spectra. Observation of a long-range coupling from the proton signal at δ_H 7.25 on C1 ring to a carbonyl carbon at δ_C 196.0 showed that a 4-hydroxybenzene (C1) attached to

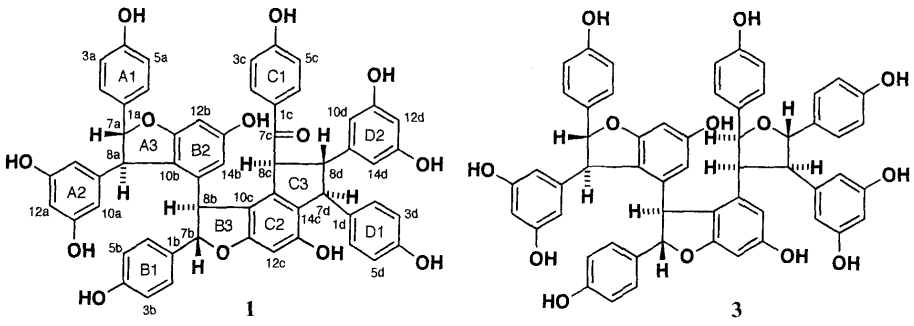

Chart 1

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Table 1. NMR Data of Caraganaphenol A (**1**)[a]

	^{13}C				^{1}H			
	Units a	b	c	d	a	b	c	d
1	129.9	130.5	126.8	135.0	—	—	—	—
2, 6	127.9	126.8	130.6	128.3	6.80 d (8.6)	6.42 d (8.9)	7.25 d (8.9)	6.76 d (8.5)
3, 5	115.0	114.6	114.5	114.6	6.65 d (8.6)	6.47 d (8.9)	6.52 d (8.9)	6.54 d (8.5)
4	157.2	156.7	161.4	155.2	9.41 s[b]	9.22 s[b]	10.18 s[b]	9.04 s[b]
7	93.6	91.3	196.0	56.4	4.94 d (7.9)	5.03 d (2.4)	—	4.07 d (5.5)
8	54.1	48.2	55.8	61.1	3.94 d (7.9)	3.89 d (2.4)	4.44 d (6.1)	3.04 dd (6.1, 5.5)
9	145.5	139.5	139.2	146.3	—	—	—	—
10	104.8	117.4	117.2	104.8	5.78 br s	—	—	5.83 d (2.1)
11	158.4	160.0	160.5	158.4	8.92 s[b]	—	—	9.10 s[b]
12	101.2	94.9	97.0	101.0	5.83 br s	5.77 d (2.1)	6.30 s	6.04 t (2.1)
13	158.4	158.4	154.5	158.4	8.92 s[b]	9.07 s[b]	9.83 s[b]	9.10 s[b]
14	104.8	107.2	123.5	104.8	5.78 br s	5.93 d (2.1)	—	5.83 d (2.1)

a) Measured in DMSO-d_6 with TMS as internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; br, broad. *b*) Indicate hydroxyl proton signals.

a carbonyl group. A three spin system from the signals at δ_H 4.07 (H-7d) to the doublet at δ_H 4.44 (H-8c) through the line at δ_H 3.04 (H-8d), which showed an obvious linear vicinal coupling pattern, was assigned by spin-decoupling experiments. The three spin systems were assembled together with a pentasubstituted benzene and three substituents (C1, D1 and D2 rings). A 1-oxo-4-hydroxybenzene (C1 + a carbonyl group) was attached to C-8c by tracing a long-range spin network from H-8c (δ_H 4.44) to C-7c (δ_C 195.0). The signal of H-8c (δ_H 4.44) showed a cross peak with the line of C-9c (δ_C 139.2), which in turn coupled to H-7d (δ_H 4.07). The signal of H-7d showed long-range correlations with C-9d and C-14c (δ_C 123.5). These results together with a three spin system in the ^{1}H-NMR spectrum (*vide supra*) led to the formation of a five membered ring (C3). D1 (a 4-hydroxybenzene) and D2 (3,5-dihydroxybenzene) were located at C-7d and C-8d, respectively, by the observation of long-range correlations from H-7d (δ_H 4.07) to C-1d (δ_C 135.0) and C-2d and 6d (δ_C 128.3), and from H-8d (δ_H 3.04) to C-9d and C-10d and 14d (δ_C 146.3).

Further, the structure was supported by two fragments with *m/z* 391 and 671 in EIMS of 1a (Fig. 1). Thus, the planar structure of caraganaphenol A was determined as shown in Chart 1.

The relative stereochemistry of **1** was assigned from results of NOE difference experiments (Fig. 2). Irradiation at the signal of H-8a enhanced the signal intensity of H-2a, 6a, H-8c, and H-10d, 14d, so that A1 ring, C1 ring, D2 ring, and H-8c are present in the same orientation (α-configuration). On irradiation at the signal of H-8c, NOE enhancements were observed at the signals of H-8b, H-7d, and H-10d, 14d, indicating that those had the same direction as H-8c. Further, NOE enhancement was observed between H-2d, 6d and H-2c, 6c. Thus, it was found that C1 and D1 rings have the same relative configuration. The stereochemistry of H-7 and H-8 positions in units A, B, and D are all *trans* because NOEs appeared between H-8a and H-2a, 6a, H-7b and H-14b, and H-7d and H-10d, 14d. On the basis of these findings, the configuration of **1** was shown as relative-(7a*R*, 8a*R*, 7b*S*, 8b*S*, 7c*S*, 8c*S*, 7d*S*, 8d*R*).

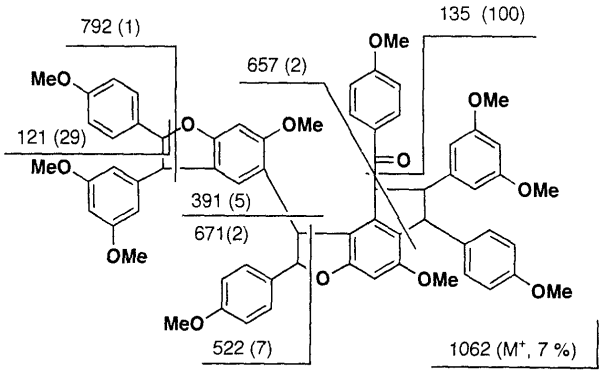

Fig. 1. Assignments for EIMS Fragmentations of Deca-*O*-methyl Caraganaphenol A (**1a**)

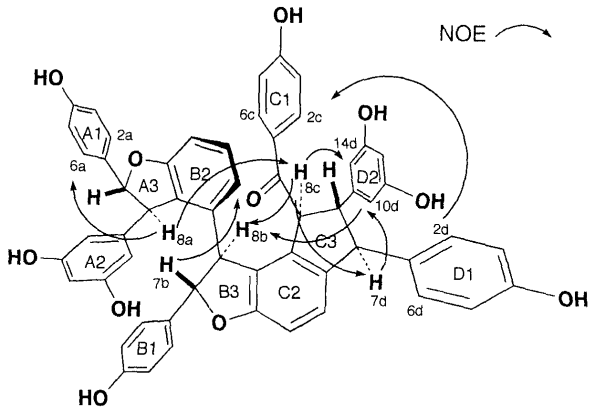

Fig. 2. Stereostructure of Caraganaphenol A (**1**)

Experimental

All the melting points were taken on a Yanagimoto micromelting-point apparatus and are uncorrected. UV spectrum was obtained in MeOH with a Hitachi 200-10 spectrophotometer, and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM GX-400 spectrometer using tetramethyl silane (TMS) as an internal standard. MS were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out on Wako gel C-200 (Wako Pure Chemical Co., Ltd.), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.), and Cosmosil 75 C_{18}-OPN (Nacalai Tesque Co., Ltd.).

Extraction and Isolation The dried roots (3.4 kg) of *Caragana chamlagu* obtained from a market in Korea were extracted with Me_2CO

(14 l × 4) at room temperature. The acetone extract was concentrated *in vacuo* to give a brown mass (44.0 g). The extract was chromatographed on silica gel eluted with a C_6H_6–Me_2CO mixture to give fractions 1—26. Fraction 20 (1.086 g) was applied on a Sephadex LH-20 column and developed with MeOH to give (+)-α-viniferin (**2**) (529 mg). Fraction 23 (7.614 g) was subjected to a silica gel column eluted with a stepwise gradient of $CHCl_3$–MeOH from 96 : 4 to 94 : 6 to give **3** (4.126 g) and **1** (31.2 mg).

Caraganaphenol A (1) Reddish amorphous solid, $[\alpha]_D^{23}$ +188.5° (c=0.785, MeOH). FDMS m/z: 922 (M^+). UV λ_{max}^{MeOH} nm (log ε): 208 (5.10), 225 sh (4.93), 285 (4.36), 294 sh (4.21). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650 sh, 1601, 1511, 1448, 831. CD ($c=1.39\times10^{-5}$, MeOH) $\Delta\varepsilon^{23}$: 209 (80.0), 254 sh (12.1), 286 (−5.8), 300 (5.0), 329 (−3.6). ^{1}H- and ^{13}C-NMR: see Table 1.

Caraganaphenol A Decamethyl Ether (1a) Compound **1** (5.0 mg) in dry Me_2CO (2.5 ml) was treated with Me_2SO_4 (0.12 ml) and K_2CO_3 (100 mg) and kept at 80 °C for 3 h. The reaction mixture was added to NH_4OH (0.1 ml) and H_2O (20 ml) and extracted with Et_2O (20 ml × 3). The extract was evaporated *in vacuo* and chromatographed by preparative thin layer chromatography with *n*-hexane–AcOEt (3 : 2) to give **1a** (3.7 mg), yellowish solid, FDMS m/z: 1061 (M^+-H). EIMS fragmentation is shown in Fig. 1. ^{1}H-NMR ($CDCl_3$) δ: 3.42 (1H, dd, J=6.0, 5.2 Hz), 3.57 (3H, s, OMe), 3.70 (9H, s, OMe × 3), 3.71 (3H, s, OMe), 3.73 (3H, s, OMe), 3.74 (1H, d, J=2.4 Hz), 3.78 (3H, s, OMe), 4.22 (1H, d, J=8.0 Hz), 4.28 (1H, d, J=4.4 Hz), 4.39 (1H, d, J=5.2 Hz), 4.54 (1H, d, J=4.4 Hz), 5.22 (1H, d, J=4.4 Hz), 5.24 (1H, d, J=8.0 Hz), 5.92 (1H, d, J=2.4 Hz), 6.17 (1H, d, J=2.0 Hz), 6.23 (2H, d, J=2.0 Hz), 6.34 (1H, t, J=2.0 Hz), 6.44 (1H, s), 6.59 (2H, d, J=2.0 Hz), 6.60 (2H, d, J=8.8 Hz), 6.61 (2H, d, J=8.8 Hz), 6.70 (2H, d, J=8.8 Hz), 6.72 (2H, d, J=8.4 Hz), 6.74 (2H, d, J=8.8 Hz), 7.00 (2H, d, J=8.4 Hz), 7.02 (2H, br s, J=8.8 Hz), 7.42 (2H, d, J=8.8 Hz).

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