(+)-α-Viniferin, an Anti-inflammatory Compound from *Caragana chamlagu* Root

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The roots of *Caragana chamlagu* LAMARCK (Leguminosae) are used as an anti-neuralgic, anti-rheumatic, anti-arthritic, *etc.* in the folk medicine of Korea. An ether extract was fractionated with monitoring of the anti-inflammatory activity, and the active principle was elucidated as (+)-α-viniferin on the basis of spectroscopic data, including two-dimensional nuclear magnetic resonance and circular dichroism spectra.

Keywords *Caragana chamlagu*; Leguminosae; anti-inflammatory principle; (+)-α-viniferin; 2D-NMR

The dried roots of *Caragana chamlagu* LAMARCK (Leguminosae) have been used in the folk medicine of Korea as an effective anti-neuralgic, anti-rheumatic and anti-arthritic. Kim *et al.*[1,2] have reported the anti-inflammatory action of the crude drug as an ether extract using the carrageenin-induced paw edema method, and have isolated a mixture of four sterols, cholesterol, brassicasterol, campesterol and β-sitosterol, from the non-saponified fraction of a methanolic extract, although the active principle was not identified.

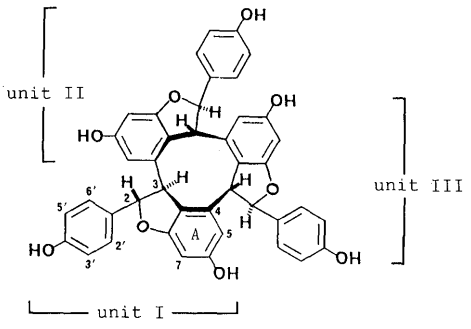

Chart 1

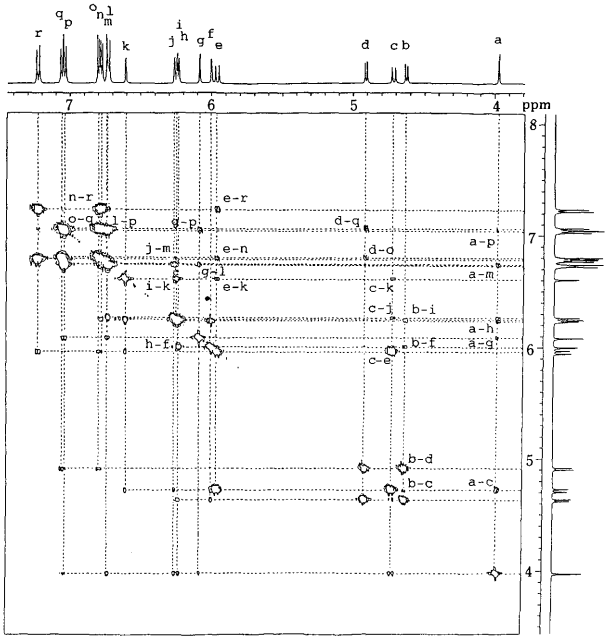

Fig. 1. Contour Map of the ^1H–^1H COSY Spectrum of (+)-α-Viniferin (2)

In this paper, we report the isolation and structural elucidation of the anti-inflammatory principle.

The dried roots of *Caragana chamlagu* were extracted successively with *n*-hexane, ether and methanol, and the ether extract found to have activity by using the carrageenin-induced hind paw edema method in mice,[3] was fractionated with monitoring of anti-inflammatory activity by oral administration, using the procedure shown in Chart 2. As a result, compound **2** was isolated as the active principle.

Compound **2**, obtained as colorless prisms, mp 231—233 °C, $C_{42}H_{30}O_9 \cdot 5/2\,H_2O$, $[\alpha]_D^{24}$ +50.7° (EtOH), gave a positive coloration in the $FeCl_3$ reaction. The field desorption mass spectrum (FD-MS) of **2** showed *m/z* 701 ($M^+ + Na$) and 678 (M^+). The ultraviolet (UV) and infrared (IR) spectra were virtually the same as those of polyphenols, showing a maximum at 285 nm and strong absorptions at 3400 and 1613 cm^{-1} (aromatic C=C), respectively. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2** showed six doublets (δ 46.4—95.6) due to six aliphatic carbon atoms, twelve doublets (δ 96.9—128.6) due to aromatic carbon atoms, and a total of eighteen quaternary aromatic carbon atoms (δ 118—161.7) including nine singlets (δ 158.2—161.7) assigned to quaternary aromatic carbons bearing oxygen. The proton nuclear magnetic resonance (^1H-NMR) spectrum of **2** showed the presence of six methines (δ 3.97—6.07), three 1,2,3,5-tetrasubstituted benzene rings and three 1,4-disubstituted benzene rings. The ^1H-NMR spectrum indicated that the methine protons at δ 3.97 (H_a) and 6.07 (H_g) have a torsion angle of about 90°, and the remaining two pairs of vicinally coupled methine protons showed doublet signals at δ 4.61 (H_b) and 4.90 (H_d) (each d, J=6.4 Hz), 4.71 (H_c) and 5.95 (H_e) (each d, J=9.7 Hz). The

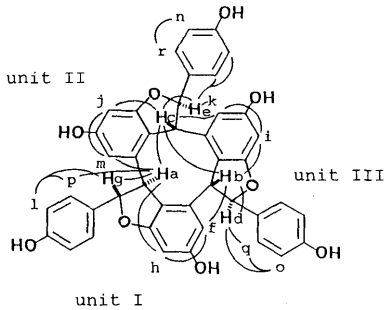

Fig. 2. Proton Assignments Based on the ^1H–^1H COSY Spectrum
The curved lines indicate ^1H–^1H coupling.

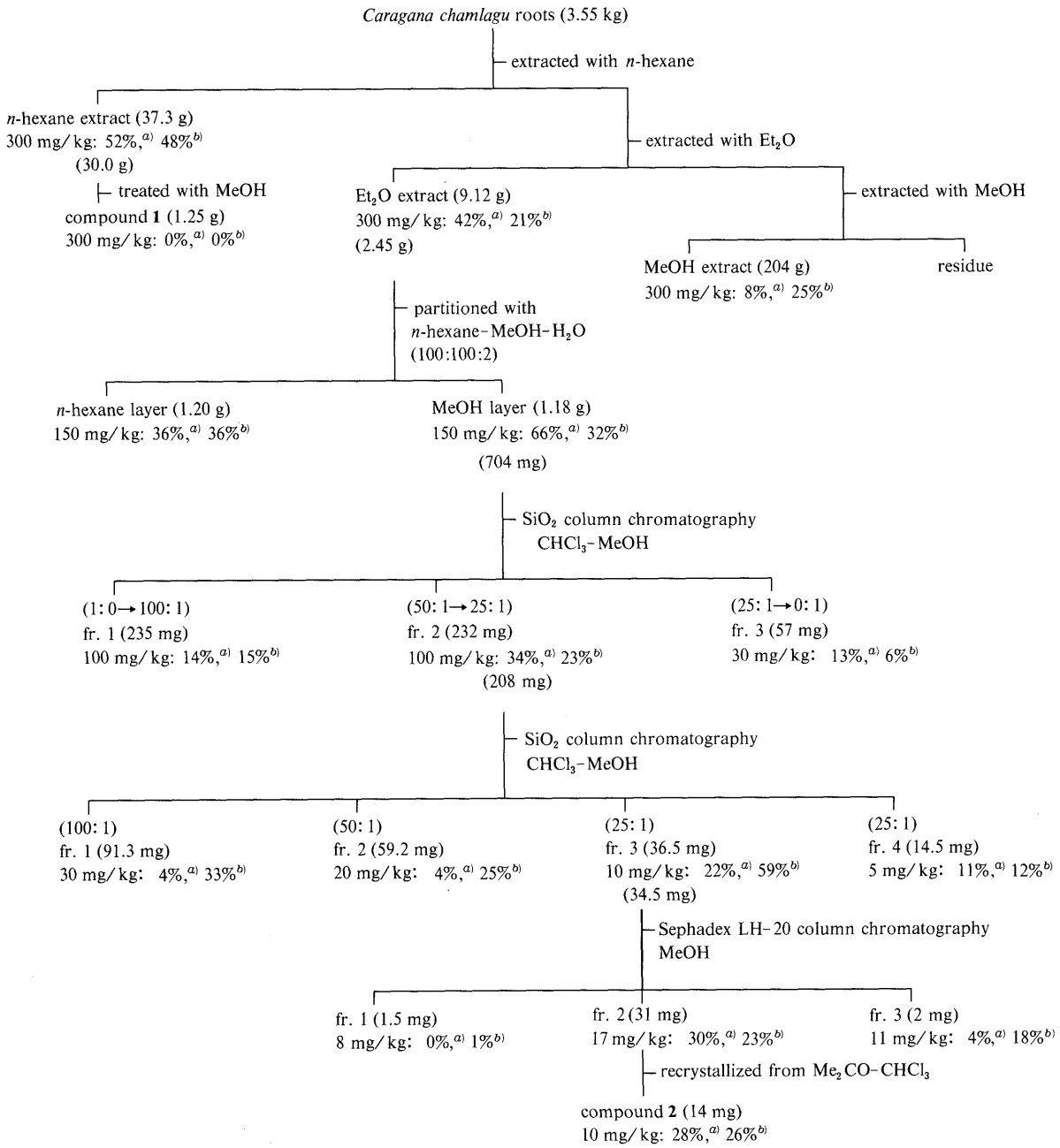

Chart 2. Procedure for Isolation of Anti-inflammatory Principle from *Caragara chamlagu* Roots

a, *b*) Dose and inhibition ratio; inhibition ratio of edema was determined at the times *a*) 1 h; *b*) 5 h after carrageenin administration.

TABLE I. ^{1}H- and ^{13}C-NMR Data for **2** in Me_2CO-d_6

Carbon No.	H ppm (*J* in Hz) and C ppm					
	Unit I		Unit II		Unit III	
2	6.07 (br s)	86.4	5.95 (d, *J*=9.7 Hz)	90.0	4.90 (d, *J*=6.4 Hz)	95.6
3	3.97 (br s)	46.4	4.71 (d, *J*=9.7 Hz)	52.8	4.61 (d, *J*=6.4 Hz)	55.6
3a	—	118.8	—	120.9	—	119.7
4	—	141.2	—	139.7	—	138.7
5	5.99 (d, *J*=1.8 Hz)	108.5	6.724 (d, *J*=1.8 Hz)	106.2	6.59 (d, *J*=1.8 Hz)	105.8
6	—	159.30	—	159.34	—	160.8
7	6.22 (d, *J*=1.8 Hz)	98.0	6.25 (d, *J*=1.8 Hz)	96.6	6.22 (d, *J*=1.8 Hz)	96.9
7a	—	161.6	—	160.6	—	161.7
1′	—	132.0	—	132.3	—	132.5
2′,6′	7.03 (d, *J*=8.5 Hz)	128.08	7.22 (d, *J*=8.5 Hz)	128.15	7.08 (d, *J*=8.5 Hz)	128.6
3′,5′	6.722 (d, *J*=8.5 Hz)	115.7	6.77 (d, *J*=8.5 Hz)	116.1	6.79 (d, *J*=8.5 Hz)	116.1
4′	—	157.8	—	158.2	—	158.3

TABLE II. Long-Range Correlated ^{13}C and ^{1}H Signals in the COLOC Spectrum (Me_2CO-d_6) of **2**

Carbon No.	^{13}C Signals (δ ppm) and correlated ^{1}H signals (δ ppm) (position in units I, II and III)					
	Unit I		Unit II		Unit III	
2	86.4	I-2′,6′ (7.03)	90.0	II-3 (4.71) II-2′,6′ (7.22)	95.6	III-3 (4.61) III-2′,6′ (7.08)
3	46.4	II-5 (6.724)	52.8	II-2 (5.95) III-5 (6.59)	55.6	I-5 (5.99)
3a	118.8	I-3 (3.97) III-3 (4.61) I-5 (5.99) I-2 (6.07) I-7 (6.22)	120.9	II-3 (4.71) II-7 (6.25) II-5 (6.724)	119.7	III-3 (4.61) II-3 (4.71) III-5 (6.59)
4	141.2	III-3 (4.61) III-2 (4.90)	139.7	I-3 (3.97) II-3 (4.71) I-2 (6.07)	138.7	II-3 (4.71) II-2 (5.95)
5	108.5	III-3 (4.61) I-7 (6.22)	106.2	II-7 (6.25)	105.8	II-3 (4.71) III-7 (6.23)
6	159.30	I-5 (5.99)	159.34	II-5 (6.724)	160.8	III-7 (6.23) III-5 (6.59)
7	98.0	I-5 (5.99)	96.6	II-5 (6.724)	96.9	III-5 (6.59)
7a	161.6	I-3 (3.97) I-2 (6.07)	160.6	II-3 (4.71) II-7 (6.25)	161.7	III-3 (4.61) III-7 (6.23)
1′	132.0	I-3 (3.97) I-2 (6.07) I-3′,5′ (6.722)	132.3	II-3 (4.71) II-3′,5′ (6.77)	132.5	III-3 (4.61) III-3′,5′ (6.79)
2′,6′	128.08	I-2 (6.07)	128.15	II-2 (5.95)	128.6	III-2 (4.90)
3′,5′	115.7		116.1		116.1	
4′	157.8	I-3′,5′ (6.772) I-2′,6′ (7.03)	158.2	II-2′,6′ (7.22)	158.3	III-3′,5′ (6.79) III-2′,6′ (7.08)

two-dimensional nuclear magnetic resonance (2D-NMR) spectra were measured in order to confirm the structure and the assignments of proton and carbon signals. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum (Fig. 1) of **2** clarified the relationships between the three methine signals [δ 3.97 (H_a), 4.71 (H_c), 4.61 (H_b)] and the six meta-coupled signals [δ 5.99 (H_f), 6.22 (H_h); 6.724 (H_m), 6.25 (H_j); 6.59 (H_k), 6.23 (H_i)], the three signals of methine with attached oxygen [δ 6.07 (H_g), 5.95 (H_e), 4.90 (H_d)] and the six 4-hydroxyphenyl proton signals [δ 7.03 (H_p), 6.722 (H_l); 7.22 (H_r), 6.77 (H_n); 7.08 (H_q), 6.79 (H_o)] with cross-peaks due to usual spin-coupling and long-range coupling ($^{4}J_{H-H}$ and $^{5}J_{H-H}$) (Fig. 2). Thus, the plane structure of **2** was found to be a ring structure having three 2-phenyl-2,3-dihydrobenzofuran units, as shown in Fig. 2, and all the proton signals were able to be assigned to the three units (units I, II, and III) according to the coupling beginning from the resonance of H_a (H-3 in unit I) (Table I). The assignments of all methine and quaternary carbon signals for **2** were achieved as shown in Table II by making use of the ^{13}C–^{1}H heteronuclear shift correlation 2D spectrum (CH-COSY) and the ^{1}H–^{13}C long-range coupling correlation 2D spectrum (COLOC) (Table II). The stereochemical configuration of **2** was determined from the 2D nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum (Fig. 3). The appearance of cross peaks due to H_a (δ 3.97) and H_p (δ 7.03), and H_c (δ 4.71) and H_r (δ 7.22) indicated that the configurations at H-2 and H-3 in units I and II are *trans* (Fig. 4). Two cross peaks between H_a (δ 3.97) and H_d (δ 4.90), and H_c (δ 4.71) and H_b (δ 4.61) showed them to exist on the same side in the plane structure. Consequently, the configuration at H_b and H_d is *trans* as shown in the drawing. The signals of H_g (δ 6.07)

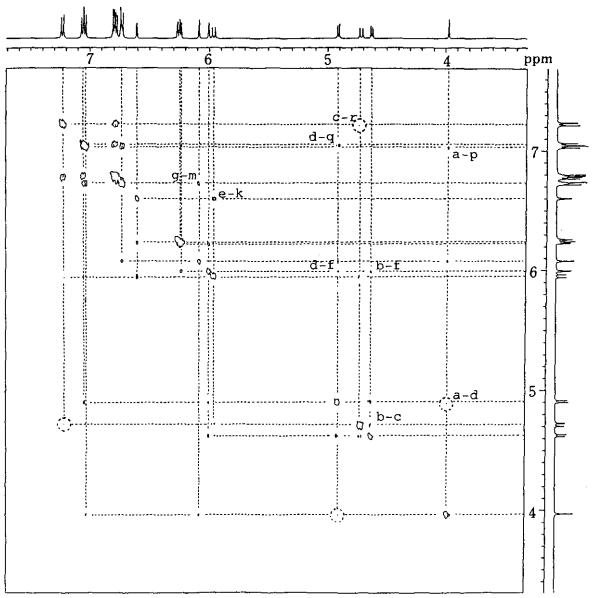

Fig. 3. Contour Map of the 2D-NOE Correlation Spectrum of (+)-α-Viniferin (**2**)

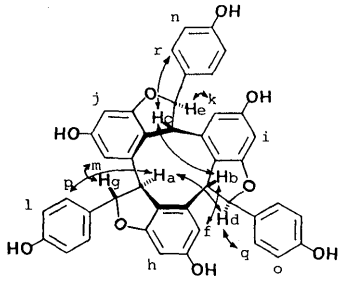

Fig. 4. H–H Connectivities as Revealed by the 2D-NOESY Spectrum

and H_e (δ 5.95) appeared at low field, so that they were considered to be located at horizontal positions with respect to the aromatic ring A in units I and III, respectively. The proton signal of H_a (δ 3.97) appears at higher field than H_c (δ 4.71) and H_b (δ 4.612), because this atom lies above the plane of the aromatic ring A (Chart 1) in unit III. From the above results the stereostructure of **2** was concluded to be as shown in Chart 1, and this structure is the same as that of α-viniferin, which was isolated as a phytoalexin from grapevine (*Vitis vinifera*).[4] Compound **2** has similar spectral data to α-viniferin, except that the Cotton effects of the circular dichroism (CD) spectrum showed opposite curves. Therefore, compound **2** is an enantiomer of α-vaniferin, and should be named (+)-α-viniferin.

Compound **1**, obtained as colorless needles, mp 89—91 °C, was identified by IR, ^{13}C-NMR, and high-resolution MS as a mixture of glycerol-α-lignocerate ($C_{27}H_{54}O_4$), glycerol-α-cerotate ($C_{29}H_{58}O_4$), and glycerol-α-montanate ($C_{31}H_{62}O_4$), the ratio of which was calculated by gas liquid chromatography (GLC) to be 1.6 : 9.9 : 1.

This is the first report of a stilbene oligomer with anti-inflammatory properties.

Experimental

All melting points were taken on a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV and CD spectra were obtained on a Hitachi 200-10 and JASCO J-600 spectrophotometers, and IR spectra were recorded on a JASCO IRA-2 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were taken on JEOL GX-400 and JEOL FX-100 spectrometers, respectively, using tetramethylsilane as an internal standard. The MS were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out with silica gel (Wako gel C-200, Wako Pure Chemical Industry Ltd.) or Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.).

Assay Procedure for Anti-inflammatory Activity The anti-inflammatory activity of test samples was evaluated based on the mouse hind paw edema method as follows. A group of 5 male mice of ddY-strain (6 weeks old) (Shizuoka Laboratory Animal Center, Shizuoka, Japan) was used at each dose level. The sample in 5% gum arabic suspension (10 ml/kg) was administered *p.o.* to mice, then after 30 min, 2% carrageenin in physiological saline solution (25 μl) was injected into the subplantar tissue of the right hind paw, and saline solution (25 μl) into that of the left hind paw. The difference in foot-pad thickness between the right and left feet was measured with a dial caliper gauge every hour.

Extraction The powdered roots (3.55 kg) of *Caragana chamlagu* obtained from a market in Korea were extracted with *n*-hexane (15 l × 3), Et_2O (15 l × 3), and MeOH (15 l × 3) under reflux, successively. The extracts were separated as shown in Chart 1.

Glyceride (1) Recrystallization (MeOH) gave colorless needles, mp 89—91 °C. IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 2860, 1730, 1330, 1320, 1310, 1295, 1280, 1270, 1255, 1240, 1230, 1215, 1200, 1190, 1180. ^{1}H-NMR (pyridine-d_5) δ: 0.88 (3H, t, J = 5.4 Hz, Me), 1.32 (44H, s, CH_2 × 22), 1.65 (2H, m, CH_2), 2.37 (2H, t, J = 7.33 Hz, CH_2COO), 4.14 (2H, d, J = 5.4 Hz, H-3), 4.49 (1H, q like, J = 4.9 Hz, H-2), 4.71 (2H, dd, J = 4.9, 2.0 Hz, H-1). ^{13}C-NMR (pyridine-d_5) δ: 14.2 (q), 22.9 (t), 25.3 (t), 29.4 (t), 29.6 (t), 29.7 (t), 32.1 (t), 34.4 (t), 64.2 (t), 66.6 (t), 70.9 (d), 173.4 (s). High-resolution MS *m/z*: Calcd for $C_{27}H_{54}O_4$: 442.4018. Found: 442.4018; Calcd for $C_{29}H_{58}O_4$: 470.4332. Found: 470.4344; Calcd for $C_{31}H_{62}O_4$: 498.4644. Found: 498.4592. GLC: 1% OV-1 on Chromosorb WAW DMCS 80—100 mesh (3 mm × 0.5 m) column temperature, 100—300 °C (increased at the rate of 4 °C/min); carrier gas, N_2 (flow rate, 50 ml/min). The components was identified as glycerol-α-lignocerate (retention time (t_R), 32.3 min), glycerol-α-cerotate (t_R 34.8 min), glycerol-α-montanate (t_R 37.5 min), the ratio of which was calculated from the GLC as 1.6 : 9.9 : 1.

(+)-α-Viniferin (2) Recrystallization (*n*-hexane–acetone) gave colorless plates, mp 231—233 °C, $[\alpha]_D^{24}$ +50.7° (c = 1.02, EtOH). *Anal.* Calcd for $C_{42}H_{30}O_9 \cdot 5/2\,H_2O$: C, 69.70; H, 4.18. Found: C, 69.88; H, 4.29. UV λ_{max}^{EtOH} nm (log ε): 210 (4.92), 228 sh (4.79), 278 sh (3.96), 285 (4.11), 294 sh (3.96). CD (c = 0.00124, EtOH) $\Delta\varepsilon^{20}$: 228 (+19.1), 240 (0), 249 (−15.7), 275 sh (−0.8), 292 sh (−4.9), 299 (−9.7). IR ν_{max}^{KBr} cm^{-1}: 3400, 1613, 1597, 1514, 829, 805 sh, 774, 763. FD-MS *m/z*: 701 (M^+ + Na), 678 (M^+). The ^{1}H- and ^{13}C-NMR data are listed in Tables I and II.

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