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Studies on the Korean Indigenous Plants.* Isolation of 1-eicosanoyl cafferate from *Echinosophora koreensis*

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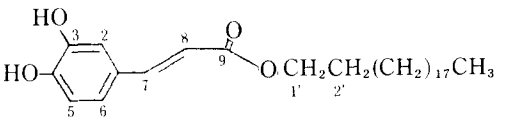
Abstract 1-Eicosanoyl cafferate, mp 109-110°, was isolated from the underground parts of *Echinosophora koreensis* together with hexacosanol, mp 75-6°. 1-Eicosanoyl cafferate was isolated for the first time from plant source.

Keywords *Echinosophora koreensis*, Leguminosae, 1-Eicosanoyl cafferate, Hexacosanol.

Echinosophora koreensis Nakai(Leguminosae) is indigenous to Korea and the only species of this genus of the family Leguminosae. Earlier investigations on this plant showed the presence of sterols, coumarin and triterpenoids[1] and flavonoids[2]. Further studies on the underground parts of this plants has resulted in the isolation of a new compound, 1-eicosanoyl cafferate together with hexacosanol.

Repeated chromatographic separations of ether soluble portion of the MeOH extract over silica gel and LiChroprep^R RP-8 yielded compound **1** as needles, mp 109-110°. The IR spectrum of **1** showed the presence of hydroxyl at 3490 and 3320 cm^{-1}, α,β-unsaturated ester carbonyl at 1688 cm^{-1}, aromatic at 1609 and 1536 cm^{-1} and polymethylene groups at 715 cm^{-1} which suggested to be that aromatic ester of higher alcohol. This was further corroborated by its UV spectrum which was virtually identical to that of ethyl caffeate[3]. The NMR spectrum of **1** showed signals for three aromatic protons at δ 6.94(1H, d, J=7.2 Hz, H-5), 7.03(1H, brd, J=7.2 Hz, H-6) and 7.08(1H, brs, H-2) and shows a pair of one proton doublets appearing at δ 6.25 and 7.57(1H each, J=15.9 Hz, H 8 and 7) typical of a trans olefin. In the region of upfield, the n-alkyl ester functional group was observed which appeared methyl signal at δ 0.87(3H, brt, J=6 Hz), $(CH_2)_n$ at 1.26(34 H, s), $-COOCH_2\underline{CH_2}$ at 1.67(2 H, m), and $-COO\underline{CH_2}$ at 4.18(2 H, t, J=6 Hz)[4,5]. These data indicated that **1** was a caffeic acid ester of higher alcohol.

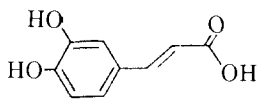


1

$CH_3(CH_2)_n\overset{2}{C}H_2\overset{1}{C}H_2OH$

2 n=23

3 n=17



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The higher alcohol was deduced as eicosanol (**3**) from the mass spectrum of **1**. The ester **1** on alkaline hydrolysis yielded eicosanol (**3**) and caffeic acid (**4**) which were identified by direct comparison with authentic samples. Therefore the compound **1** was identified as eicosanoyl cafferate that hitherto had not been found in plants.

The second compound (**2**) was crystallized from petroleum ether to give colorless flakes, mp 75-6°. The IR spectrum of this compound indicated the presence of hydroxyl at 3350 cm^{-1} and polymethylene at 726 and 716 cm^{-1} typical of higher alcohols. The NMR spectrum of **2** was similar to compound **1**; methyl signal at δ 0.88 (3H, brt, J=6 Hz), long chain methylene protons at δ 1.25(46 H, s), methylene protons adjacent to primary hydroxyl group at δ 1.52(2 H, m) and methylene protons adjacent to an oxygen at δ 3.63(2 H, m). This data suggested that **2** was hexacosanol. This assumption was supported by mass spectrum of **2**. It showed an ion peak at m/z 364

*Part 2 in the series "Korean indigenous plants". For part 1 see ref (1).

in the high mass region which corresponded to the loss of one molecule of H_2O from the molecular ion[6]. Other fragment peaks for $[C_nH_{2n+1}O^+-H_2O]$ and $[M^+-(C_nH_{2n}+H_2O)]$ ions[6] strongly supported that **2** was hexacosanol. Direct comparison with an authentic sample established its identity.

EXPERIMENTAL METHODS

Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. NMR spectra were recorded on a Varian FT-80A spectrometer and are given in ppm(δ) downfield from an internal TMS standard. Mass spectra were determined on a Hewlett-Packard 5985B GC/MS system at 70 eV using direct inlet system. UV spectra were runned with Gilford System 2600 spectrophotometer.

Plant collection and extraction

This was carried out as described previously[1].

Fractionation and isolation

The MeOH extract was partioned between ether and water. The ether fraction was chromatographed over silica gel and eluted with $CHCl_3$ and then MeOH-$CHCl_3$ (1 : 49) to give subfractions 1 to 3 and 4 and 5, respectively. Subfraction 5 was concentrated and allowed to stand at room temperature. The precipitate was filtered and the filtrate was subjected to column chromatography on a prepacked LiChroprep^R RP-8 reversed phase column with MeOH-H_2O (8 : 2) to afford compound **1** and **2**. Compound **1** was crystallized from MeOH as needles. Compound **2** was crystallized from petroleum ether as colorless flakes.

***Compound* 1**

mp 109-110°. IR $\nu_{max}^{KBr}cm^{-1}$ 3490, 3320(OH), 2922, 2850, 1475(CH), 1688, 1285, 1170 (α,β-unsaturated ester carbonyl), 1609, 1536(aromatic C=C), 975(trans double bond), 715[$(CH_2)_n$]. UV λ_{max}^{MeOH} nm(log ε) 221(3.84), 236(sh, 3.69), 246(3.71), 303(3.85), 330(3.96). NMR($CDCl_3$, TMS) δ 0.87(3H, brt, J=6Hz, CH_3), 1.26[34 H, s, $(CH_2)_n$], 1.67(2H, m, H-2′), 4.18(2 H, t, J=6 Hz, H-1′), 6.25(1 H, d, J=15.9 Hz, H-8), 6.94(1 H, d, J=7.2 Hz, H-5), 7.03(1H, brd, J=7.2 Hz, H-6), 7.08(1 H, brs, H-2), 7.57(1 H, d, J=15.9 Hz, H-7). MS, *m/z*(rel. int.) 460(M^+, 1.0), 404($M^+-C_4H_8$, 22.0), 260(1.1), 245(0.8), 236(2.6), 219(3.0), 182(29.7), 181(17.7), 180 (caffeic acid, 100), 163(56.0), 145(10.7), 136(180-CO_2, 19.0), 135(163-CO, 12.3), 134(12.2), 123(11.3), 97(5.9), 83(8.4), 69(13.4), 55(15.9).

***Hydrolysis of* 1**

1 (30 mg) was hydrolized with 5% KOH in EtOH for 3 hr at 60-70°. The reaction mixture was partitioned with ether and water. The ether layer was concentrated and the residue was crystallized from petroleum ether to afford colorless flakes. mp 63-5°, which was identified as eicosanol (**3**) by direct comparison with an anthentic sample. The aqueous layer was acidified with d-HCl and extracted with ethylacetate. After evaporation the residue was crystallized from MeOH-H_2O to yield caffeic acid (**4**) as pale yellow powder, mp 220-4°, and direct comparison with an authentic sample established its identity.

***Compound* 2**

mp 75-76° [Lit.[7] mp 78°]. IR $\nu_{max}^{KBr}cm^{-1}$ 3350, 1060(OH), 2922, 2855, 1475, 1465(CH), 726, 716[$(CH_2)_n$]. NMR($CDCl_3$, TMS) δ 0.88(3H, brt, J=6Hz, CH_3), 1.25[46 H, s, $(CH_2)_n$], 1.52(3 H, m, H-2 and OH), 3.63(2 H, m, H-1). MS, *m/z* (rel. int.) 364(M^+-H_2O, 1.5), 336(3.8), 308(0.9), 250(0.7), 237(0.9), 223(1.7), 209(1.9), 195 (2.2), 181(2.9), 167(4.0), 153(5.9), 139(10.3), 125(24.5), 111(48.2), 97(95.0), 83(100), 69 (78.4), 57(65.0), 55(58.3), 43(33.7), 41(20.1).

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