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# NEW LONG-CHAINED FERULOYL ESTER FROM THE BARK OF *Cedrelinga catenaeformis**

**H. R. El-Seedi**

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*Tetradecyl ferulate and a new n-alkyl ester of 3-hydroxy-4-methoxy-*trans*-cinnamate (hexacosanylisoferulate) have been isolated from* Cedrelinga catenaeformis *Duke (Leguminoseae). The structures were determined by 1D- and 2D-NMR spectroscopy, mass spectrometry, chemical transformations and finally from unambiguous synthesis. This is the first report of long chained cinnamic acid ester derivative from the genus.*

**Key words**: *Cedrelinga catenaeformis*, Leguminoseae, accelerating gradient chromatography, NMR and MS spectrometry.

One of the largest of the plant families, the Leguminoseae, with 600 genera and 13.000 species, is cosmopolitan. The species show an extraordinary diversity of habits, structures, and biological activities [1]. Naturally occurring ferulic acid ester of higher saturated alcohols have been isolated and characterized from the genera *Bauhina* [2], *Euchresta* [3], and *Erythrina* [4] belonging to Leguminoseae.

*Cedrelinga catenaeformis* Duke (Leguminoseae), with the common name "Majagua or Damagua", is a hardwood species from the American tropical region. Previously, a new dihydrophenanthrapyrans has been isolated from the species [5]. The plant was collected as part of our ongoing project on chemical and pharmacological studies of medicinal plants used in traditional medicine [6, 7]. This paper deals with the isolation and characterization of two long-chained feruloyl ester derivatives from the bark of the tree and the synthesis of the novel compound.

By a combination of chromatographic techniques, mainly MPLC, CC, and prep. TLC on silica gel, compounds **1** and **2** were isolated from the hexane extract of *Cedrelinga catenaeformis*. The spectral properties showed these substances to be esters of ferulic acid. Structure **1** was elucidated from its physical and spectral properties, whereas there was little deviation from the previous published $^1$H NMR data [8] due to solvent effects. The IR spectrum of compound **2** exhibits the presence of the $\alpha$, $\beta$ unsaturated ester carbonyl at 1695 cm$^{-1}$ (C=O), aromatic residue (1610, 1520 cm$^{-1}$), and hydroxyl groups (3340–3350 cm$^{-1}$). The UV spectrum showed bathochromic absorption bands at $\lambda_{max}$ 331, 296, and 238 nm, which are characteristic for ferulic acid derivatives [9]. The $^1$H NMR spectrum indicated the aromatic protons as an ABX system at δ 6.84 d (J = 8.2 Hz), 7.13 dd (J = 8.2 and 2.0 Hz), and 6.86 d (J = 2.0 Hz) with *ortho*, *ortho/meta*, and *meta* coupling, respectively and together with two protons at δ 6.22 d (J = 16 Hz) and 7.59 d (J = 16 Hz) of a *trans*-double bond which confirmed the pattern of 3-hydroxy-4-methoxy-*trans*-cinnamate (isoferuloyl moiety). The signals at δ 4.15 t (J = 6.7 Hz) and 1.68 ppm were assigned to four carbonyl methylene protons and its neighboring methylene, respectively. The $^{13}$C NMR spectrum showed also a furelate derivative with resonance attribute to a C=O group at δ 169.1 ppm and aromatic carbons wich deshielded quaternary carbons attached to oxygen. The presence of a long alkyl chain was detected at δ 22–32 ppm, the deshielded methylene adjacent to the oxocarbonyl function at δ 64.5 ppm, and the terminal methyl group at δ 14.0 ppm. Spectral data are in good agreement with closely related structures published for ferulic acid esters [10, 11].

*Dedicated to Profs. S. El-Kousy, E. E. A. El-Khrisy, and K. B. G. Torssell on the occasion of their 60th, 65th, and 75th birthdays.

Division of Pharmacognosy, Department of Medicinal Chemistry, Biomedical Centre, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden and Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt, fax +46 18 509101, e-mail: hesham.el-seedi@fkog.uu.se. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 214-216, May-June, 2007. Original article submitted March 15, 2006.

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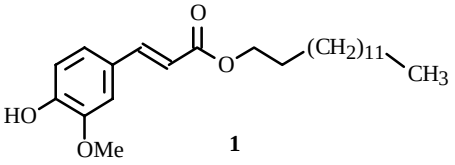
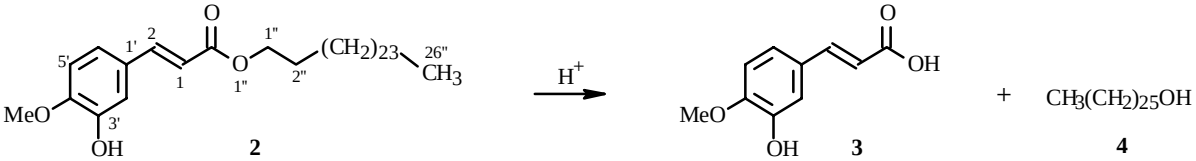
Alkaline hydrolysis yielded isoferulic acid **3** and the free higher alcohol **4** which was proved by co-TLC and mixed melting point test.




**1**




**2** **3** **4**

Final conformation of **2** was carried out via synthesis which proved the compound to be hexacosanyl-3-hydroxy-4-methoxy-*trans*-cinnamate (hexacosanylisoferulate).

Ferulic acid and other related hydroxycinnamic esters are reported to have an important function as protection against herbivores and pathogens [12]. It is possible that the natural products **1**, **2**, and related compounds may play a role in the stress management of halophytic plants. They may be associated with water retention within the plant cells with the hydrophobic long alkyl chain being "anchored" within the cell membrane and the hydrophilic hydroxycinnamate portion remaining within the cell, thus retaining a hydration shell [13]. On the other hand, the biological significance of these types of compounds remain unclear. This will be the goal of our future studies.

## EXPERIMENTAL

1D and 2D NMR spectra were recorded on a 400 MHz Varian VXR-400 NMR instrument. IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrometer. UV spectra were recorded on a Shimadzu UV-160A UV-VIS recording spectrophotometer, and MS spectra were obtained with a Jeol JMS SX102 spectrometer in the positive ion mode. Melting points were determined using a Digital Melting Apparatus (Model 1A 8103, Electrothermal Engineering Ltd., U.K.) and are uncorrected. TLC was performed on precoated aluminum sheets [silica 60 $F_{254}$, 0.25 mm (Merck, Darmstadt, Germany)] and preparative TLC on precoated glass sheets [silica 60 $F_{254}$, 0.5 mm (Merck)], with the detection provided by UV light (254 and 366 nm) and by spraying with vanillin-sulfuric acid reagent followed by heating (120°C).

Medium pressure liquid chromatography (MPLC). This was performed using a SEPARO AB MPLC equipment (Baeckstrom Separo AB, Lidingo, Sweden), also referred to as accelerating gradient chromatography (AGC) with variable-length glass columns with inner diameters of 4 cm, packed with silica gel 60, 40–63 μm (Merck). An FMI Lab pump, model QD (Fluid Metering Inc., Oyster bay, NY) was used at a flow rate of 18–28 mL/min. Fractions of 18 mL were collected with a Gilson Model 201 fraction collector. The columns were eluted with continuous gradients running from hexane, over $CH_2Cl_2$ to EtOAc afforded by a SEPARO constant-volume mixing chamber combined with an open reservoir. Initially, the mixing chamber contained 50 mL non-polar solvent and the reservoir contained the first of 15–20 premixed binary (less polar/more polar solvent) gradient mixtures, of 20–40 mL each, which were successively fed to the reservoir during the separation [14].

**Plant Material**. Dr. F. Ghia at the Reserva Forestal Endesa, Prov. de Pichincha, Ecuador, collected the bark of Cedrelinga catenaeforms. A voucher specimen, F. G. 514, is deposited in the Herbario Economico, Escuela Politecnica Nacional (EPN), Quito, Ecuador.

**Extraction and Isolation**. The plant material was dried at 40°C in the dark in a ventilated hood and ground. The material (8.79 kg) was extracted exhaustively (at room temp.) three times with light petroleum ether and for 5 days each time with occasional stirring. The extracts were evaporated in *vacuum* to give 45.7 g semisolid material.

The petroleum ether fraction (43 g) was chromatographed on silica gel (86 g) using SEPARO MPLC equipment as described above.

The ferulate compounds exhibited a blue fluorescent spot under $UV_{365}$ light on silica gel 60 $F_{254}$, 0.25 mm plate and gave a positive phenol test.

**Tetradecyl-4-hydroxy-3-methoxy-*trans*-cinnamate (tetradecylferulate) 1**. Colorless flakes mp 66–67°C (petroleum ether–benzene), $C_{36}H_{62}O_4$ ($M^+$ 390). UV($\lambda_{max}$, MeOH): 208, 242 and 325 nm. IR ($\nu_{max}$, KBr, $cm^{-1}$) 1616, 1540 $cm^{-1}$

(aromatic residue), 1696 $cm^{-1}$ (C=O), 3425 (OH), 1635 (-C=C-H). $^1H$ NMR (acetone-$d_6$, δ, ppm, J/Hz): 0.89 (3H, t, J = 6.9, terminal methyl), 1.27 (br.s, $(CH_2)_n$), 1.69 (2H, m, $CH_2$-2″), 3.93 (3H, s, OMe-3′), 4.17 (2H, t, J = 6.7, $CH_2$-1″), 4.82 (1H, br.s, OH-3′ exchangeable with $D_2O$), 6.23 (1H, d, J = 16, H-1), 6.86 (1H, d, J = 8.0, H-5′), 6.94 (1H, d, J = 2.0, H-2′), 7.13 (1H, dd, J = 8.0 and 2.0, H-6′) and 7.58 (1H, d, J = 16, H-2). EI MS *m/z* (rel. int.): 390 $[M]^+$ (100), 194 (85), 177 (65), 57 (55).

**Hexacosanyl-3-hydroxy-4-methoxy-*trans*-cinnamate (Hexacosanylisoferulate) 2**. Colorless flakes mp 79–81°C (petroleum ether–benzene), $[\alpha]_D^{22}$ 0°C (*c* 0.71). EI MS *m/z* (rel. int.): 502 $C_{36}H_{62}O_4$ $M^+$ (100), 194 (85), 177 (65), 57 (55). UV ($\lambda_{max}$, MeOH): 331, 296 and 238 nm. IR ($\nu_{max}$, KBr, $cm^{-1}$): 1610, 1520 $cm^{-1}$ (aromatic residue), 1695 $cm^{-1}$ (C=O), 3350 (OH). $^1H$ NMR (acetone-$d_6$, δ, ppm, J/Hz): 0.88 (3H, t, J = 6.9, terminal methyl), 1.23 (br.s, $(CH_2)_n$), 1.68 (2H, m, $CH_2$-2″), 3.89 (3H, s, OMe-4′), 4.15 (2H, t, J = 6.7, $CH_2$-1″), 6.22 (1H, d, J = 16, H-1), 6.84 (1H, d, J = 8.2, H-5′), 6.86 (1H, d, J = 2.0, H-2′), 7.13 (1H, dd, J = 8.2 and 2.0, H-6′), 7.59 (1H, d, J = 16, H-1) and 8.12 (1H, br.s, OH-3′ exchangeable with $D_2O$). $^{13}C$ NMR (acetone-$d_6$, δ ppm): 14.0 (C-26″), 22.4–31.9 (C-2″ to C-25″), 55.9 (4′-OMe), 64.5 (C-1″), 109.7 (C-1), 115.0 (C-5′), 115.7 (C-2′), 123.2 (C-6′), 127.4 (C-1′), 144.8 (C-3′, C-4′, C-2), 169.1 (C=O). EI MS *m/z* (rel. int.): 502 $[M]^+$ (100), 194 (85), 177 (65), 57 (55).

**Acid Hydrolysis.** 21 mg of **2** was refluxed with a solution of 0.2 M KOH in MeOH (12 mL) on a steam bath for 4 hrs under $N_2$. The reaction mixture was diluted with $H_2O$ (12 mL) and extracted with $Et_2O$ three times. The combined extracts were dried over anhydrous $Na_2SO_4$ and evaporated to give 11 mg residue, which was subjected to prep. TLC using petroleum ether–$CH_2Cl_2$ (8:2) as eluent to give hexacosanyl alcohol **4** (7 mg) as colorless plates, mp. 74–76°C. The compound was identified from GC-MS analysis. The aqueous fraction was acidified with dil. $H_2SO_4$ and extracted with EtOAc three times. The combined extracts were dried over anhydrous $Na_2SO_4$ and evaporated to give 9 mg residue, which was subjected to prep. TLC using ($CHCl_3$–MeOH, 9:1, $R_f$ 0.28) as eluent to give isoferulic acid **3** (4 mg), which was identified by $^1H$ NMR spectra and comparison with authentic sample, mixed melting point, and co-TLC.

**Synthesis of Hexacosanyl-3-hydroxy-4-methoxy-*trans*-cinnamate (Hexacosanylisoferulate) 2**. 3-Hydroxy-4-methoxy-*trans*-cinnamic acid (39 mg) and hexacosanyl alcohol (61 mg) were dissolved in dry benzene (50 mL) and one drop of conc. $H_2SO_4$. A Dean Stark apparatus was used to remove the water formed during the reaction. The benzene was evaporated under reduced pressure and the ester formed was purified on TLC using benzene–petroleum ether (9:1) to give **2**, which was elucidated.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