

Ferulic acid ester from *Jatropha podagrica* (Euphorbiaceae)

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Abstract : A new ferulic acid ester, n-heptyl ferulate (**1**) together with 8-hydroxy-6,7-dimethoxycoumarin (**2**), acetylaleuritic acid (**3**) and γ -sitosterol (**4**) were isolated from the stem and roots of *Jatropha podagrica*. Their structures were determined by using 1D and 2D NMR techniques. Compounds **2**, **3** and **4** were cytotoxic towards the HeLa (cervical carcinoma) cell line with IC₅₀ values of 39.9, 35.7 and 15.9 μ g/ml respectively.

Abstrak : Satu ferulik asid ester baru, n-heptil ferulate (**1**) bersama-sama dengan 8-hidroksi-6,7-dimetoksikoumarin (**2**), asid asitilauritolik (**3**) dan γ -sitosterol (**4**) telah diasingkan daripada batang dan akar *Jatropha podagrica*. Struktur-struktur tersebut telah ditentukan dengan menggunakan teknik RMN 1D dan 2D. Sebatian-sebatian **2**, **3** dan **4** didapati sitotoksik terhadap sel HeLa dengan nilai-nilai IC₅₀ masing-masing iaitu 39.9, 35.7 dan 15.9 μ g/ml.

Keywords: *Jatropha podagrica*, Euphorbiaceae, n-heptyl ferulate.

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Introduction

Jatropha species (Euphorbiaceae) are well known for their medicinal values, and as a source of lignans [1], diterpenes [2] and peptides [3]. These compounds are biologically active and possess antimicrobial [4], antitumor and cytotoxic activities [5]. Many species from this genus have been reported to be widely used in traditional medicine especially in the treatment of malaria [6], arthritis, gout, jaundice [7], ulcers [8] and cancer [9]. *Jatropha podagrica* known locally as "Jarak Tapak Gajah" is widely grown in Malaysia as an ornamental plant. This paper reports the isolation and characterisation of n-heptyl ferulate (**1**), the coumarin fraxidin (**2**), acetylaleuritic acid (**3**) and γ -sitosterol (**4**) from *Jatropha podagrica* as well as the biological activity of these compounds.

Experimental

Plant Material

The stem and roots of *Jatropha podagrica* were collected from Sungai Buloh in the state of Selangor, Malaysia. A voucher specimen was deposited at the Biology Department, Universiti Putra Malaysia, Malaysia (voucher specimen no. RG1489)

Extraction and Isolation

The stem and roots of *Jatropha podagrica* (1.0 kg) were ground into powder and extracted in both n-hexane and ethyl acetate. The ethyl acetate extract (4.0 g) was subjected to a silica gel column chromatography and eluted with various solvent

system starting with hexane, followed by hexane-chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate and ethyl acetate-methanol to give 8.0 mg of n-heptyl ferulate (**1**) and 20.0 mg of fraxidin (**2**). The hexane extract (4.5 g) was similarly chromatographed to yield 10.0 mg of acetylaleuritic acid (**3**) and 13.0 mg of γ -sitosterol (**4**).

Characterization

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/ JEOL 400MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. Ultra violet spectra were recorded on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

n-Heptyl ferulate (1): A white gummy solid, mp 70-72°C. UV (CDCl₃) λ_{\max} nm: 244.5, 294.0 319.0. IR ν_{\max} cm⁻¹ (KBr): 3418, 1712, 1632, 1272, 1166. EI-MS m/z (rel. int.): 292 [M⁺, 7], 149 (9), 99 (9), 97 (25), 75 (100), 55 (49). ¹H NMR (500 MHz, CDCl₃), ¹³C NMR, 2D NMR are summarized in Table 1.

Fraxidin (2): Colourless crystals, mp 178-180°C. UV (EtOH) λ_{\max} nm: 215.0, 255.0, 308.5. EI-MS m/z (rel. int.): 222 [M⁺, 100], 207 (39), 189 (5), 179 (12), 161 (14), 151 (14), 133 (11), 123 (36), 108 (8), 95 (19), 79 (17), 63 (10), 51 (34), 41 (7). ¹H NMR (500 MHz, DMSO-d₆): δ 9.82 (1H, brs, 8-OH), 7.88 (1H, d, J =

9.5 Hz, H-4), 6.77 (1H, s, H-5), 6.32 (1H, d, $J = 9.5$ Hz, H-3), 3.82 (3H, s, 7-OMe), 3.78 (3H, s, 6-OMe). ^{13}C NMR (100 MHz, DMSO- d_6): δ 160.9 (C-2), 150.4 (C-6), 145.3 (C-4), 140.7 (C-7), 139.2 (C-8), 139.0 (C-9), 115.1 (C-3), 115.0 (C-10), 100.8 (C-5), 61.2 (7-OMe), 56.6 (6-OMe).

Acetylaleuritic acid (3): Fine white needles, m.p. 278-280°C (Lit. [10]). EI-MS m/z (rel. int.): 498 [M^+ , 1], 483 (2), 438 (4), 423 (6), 377 (3), 344 (4), 329 (6), 301 (2), 287 (4), 269 (8), 248 (28), 234 (78), 203 (23), 189 (84), 135 (24), 119 (32), 107 (23), 95 (23), 81 (22), 69 (39), 43 (100), 41 (27). NMR data are in agreement with lit. values [10].

γ -Sitosterol (4): White needles, mp 132-134°C (Lit. [11]). EI-MS m/z (rel. int.): 414 [M^+ , 55], 396 (24), 381 (17), 354 (5), 329 (34), 303 (23), 273 (10), 255 (23), 231 (15), 213 (27), 199 (12), 187 (10), 173 (15), 159 (28), 145 (34), 133 (29), 119 (29), 107 (48), 95 (48), 81 (56), 57 (59), 43 (100), 41 (56). NMR data are identical to lit. values [11].

Cytotoxicity Assay

The HeLa (cervical carcinoma) cells were cultured in RPMI-1640 medium and maintained as described by Ali *et al.* (1998). Experiments were performed in 96-well microtiter plates (1×10^5 cells mL^{-1}). Cytotoxicity was determined using the microtitration MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Mosmann, 1983) in the presence of the isolated compounds at concentrations of 40.0, 30.0, 20.0, 10.0, 7.5, 5.0 and 2.5 $\mu\text{g}/\text{mL}$. The fraction of surviving cells was measured relative to the untreated cell population by measuring the optical density at 550 nm with the reference wavelength at 630 nm using microplate reader. Cytotoxicity was recorded as the 50% inhibition concentration (IC_{50}) with reference to the untreated control cells.

Results and Discussion

n-Heptyl ferulate (**1**) was isolated as white gummy solid with a mp of 70-72 °C. This compound gave a dark green colour with methanolic ferric chloride, indicating a phenolic compound. The IR spectrum showed absorption bands at ν_{max} 3418 (O-H), 1712 (C=O), 1632 (aromatic ring), 1272 (C-O), 1166 (C-O) cm^{-1} and the EIMS gave a molecular ion peak at m/z 292, corresponding to the molecular formula $\text{C}_{17}\text{H}_{24}\text{O}_4$. The presence of a heptyl group in this compound was revealed by the fragment ion at m/z 99 while the bond cleavage between carbon C-2' and C-3' gave a fragment ion at m/z 149.

The ^1H NMR spectrum of n-heptyl ferulate exhibited a singlet at δ 7.06 for the lone aromatic proton attached to carbon C-2. A pair of doublets at δ 7.10 and δ 6.94 was assigned to the aromatic protons, H-6 and H-5 respectively. The presence of a pair of

trans-coupled olefinic protons in the structure was indicated by the two doublets at δ 7.63 and δ 6.32 which gave similar coupling constant values of 16.0 Hz. These two signals were assigned to the protons H-1' and H-2' respectively. The hydroxy group at C-4 gave a broad singlet at δ 5.89. Meanwhile, a three-proton singlet at δ 3.90 was assigned to the methoxy protons, 3-OCH₃. The heptyl group in the long chain ester moiety also gave a triplet signal at δ 4.21 which was due to proton H-1'', a quintet at δ 1.72 for proton H-2'', overlapped multiplets from δ 1.22 to δ 1.42 for protons, H-3'', H-4'', H-5'' and H-6'', and a triplet at δ 0.90 for the methyl protons, H-7''.

The ^{13}C NMR spectrum indicated a total of 17 carbon signals. The presence of a carbonyl carbon, two oxygenated aromatic carbons, three protonated aromatic carbons, a substituted aromatic carbon, two olefinic carbons, a methoxy carbon, six aliphatic methylene carbons and an aliphatic methyl carbon was supported by DEPT and HMQC spectral data.

The structure of n-heptyl ferulate was determined using the ^1H - ^1H COSY and HMBC spectral data (Table 1). From the HMBC spectrum, it was observed that the lone aromatic proton signal, H-2 (δ 7.06) correlated to the two oxygenated aromatic carbons (δ 147.0 & 148.1), a protonated aromatic carbon (δ 123.3) and an olefinic carbon (δ 144.9) which had to be carbons, C-3, C-4, C-6 and C-1' respectively. The methoxy proton signal (δ 3.90) correlated to the aromatic carbon, C-3 (δ 147.0), while the ^1H - ^1H COSY spectrum showed a coupling between the proton signals at δ 7.10 (H-6) and δ 6.94 (H-5). All these correlations confirmed the presence of a 4-hydroxy-3-methoxyphenyl moiety in the assigned structure.

From the HMBC spectrum, it was observed that the olefinic proton signal, H-1' (δ 7.63) correlated to carbon signals, C-1 (δ 127.3), C-2 (δ 109.5), C-6 (δ 123.3), C-2' (δ 115.9) and C-3' (δ 167.7). Another olefinic proton signal, H-2' (δ 6.32) showed couplings with the carbon signals, C-1 (δ 127.3) and C-3' (δ 167.7). All these spectral data support the presence of a propionic acid moiety attached to the phenyl carbon, C-1.

The heptyl moiety in the side chain gave several correlations in the HMBC spectrum. It was observed that the proton signal, H-1'' correlated to carbon signals, C-3' (δ 167.7), C-2'' (δ 29.0) and C-3'' (δ 26.3) while the proton signal, H-2'' (δ 1.72) correlated to carbon signals, C-1'' (δ 64.9), C-3'' (δ 26.3) and C-4'' (δ 29.6). Correlations were also detected for the methyl proton signal, H-7'' (δ 0.90) with carbon signals, C-5'' (δ 23.0) and C-6'' (δ 32.2). From these correlations, it was concluded that a heptyl group is attached to the carboxylic group of the propionic acid moiety. Hence the structure was assigned as n-heptyl ferulate.

Compounds **1** – **4** were bioassayed against HeLa cells. Compound **1** was inactive while the compounds

2, **3** and **4** gave IC₅₀ values of 39.9 µg/ml, 35.7 µg/ml and 15.9 µg/ml respectively.

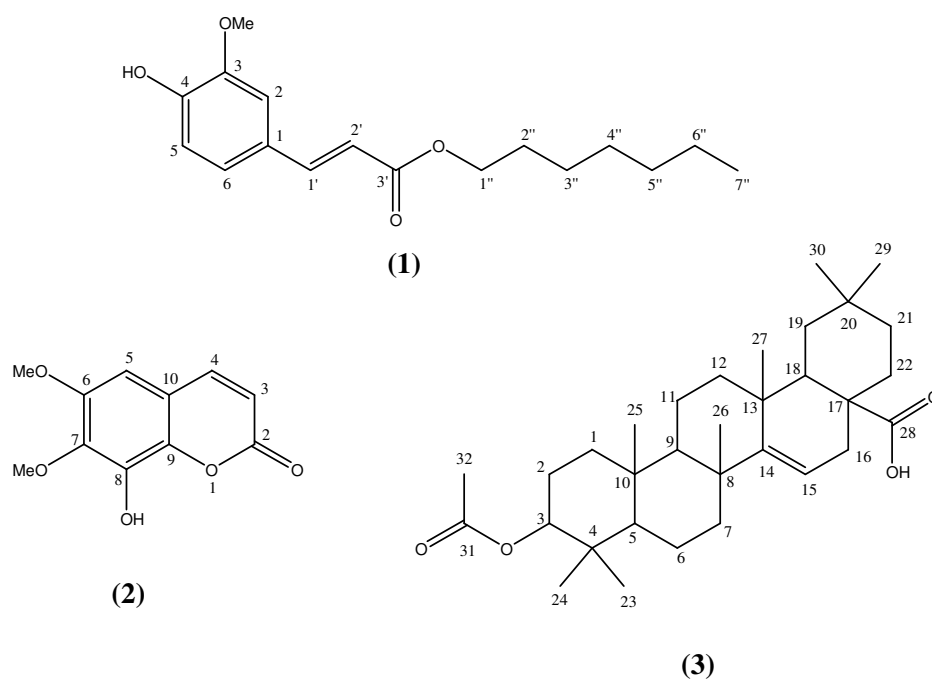


Figure 1 : Structures of compounds 1-3.

Table 1 : ¹H, ¹³C, 2D NMR data for n-heptyl ferulate (**1**)

Position	δ_{H}	δ_{C}	¹ H- ¹ H COSY	HMBC
1	-	127.3	-	-
2	7.06 (1H, s)	109.5	-	C - 3, 4, 6, 1'
3	-	147.0	-	-
4	-	148.1	-	-
5	6.94 (1H, d, $J = 8.0$ Hz)	114.9	H-6	C - 1, 3, 4, 6
6	7.10 (1H, d, $J = 8.0$ Hz)	123.3	H-5	C - 2, 4, 5, 1'
1'	7.63 (1H, d, $J = 16.0$ Hz)	144.9	H-2'	C - 1, 2, 6, 2', 3'
2'	6.32 (1H, d, $J = 16.0$ Hz)	115.9	H-1'	C - 1, 3'
3'	-	167.7	-	-
1''	4.21 (2H, t, $J = 7.0$ Hz)	64.9	H-2''	C - 3', 2'', 3''
2''	1.72 (2H, quin, $J = 7.0$ Hz)	29.0	H-1''	C - 1'', 3'', 4''
3''	1.22-1.42 (2H, m)	26.3	-	-
4''	1.22-1.42 (2H, m)	29.6	-	-
5''	1.22-1.42 (2H, m)	23.0	-	-
6''	1.22-1.42 (2H, m)	32.2	-	-
7''	0.90 (3H, t, $J = 7.0$ Hz)	14.4	-	C - 5'', 6''
3-OMe	3.90 (3H, s)	56.2	-	C - 3
4-OH	5.89 (1H, brs)	-	-	-

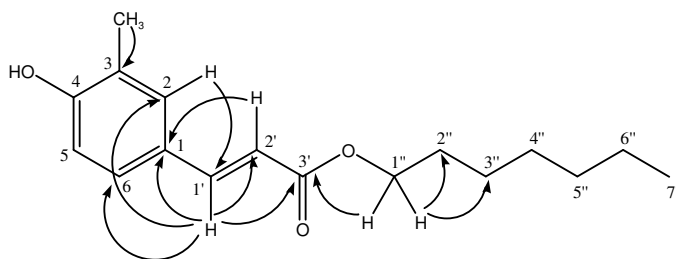


Figure 2 : Selected HMBC correlations

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