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A New Ester Isolated from *Ferula assa-foetida* L.

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A new caffeic acid cinnamyl ester (1) was isolated from the *n*-hexane-soluble fraction of an MeOH extract of the gum resin of *Ferula assa-foetida* L. The structure was determined to be (2*E*)-3,4-dimethoxycinnamyl-3-(3,4 diacetoxyphenyl) acrylate on the basis of spectroscopic data including 1D- and 2D-NMR. Compound 1 showed moderate activity for inhibiting LPS-induced nitric oxide production in murine macrophage RAW264.7 cells, with an IC_{50} value of 54.9 μm.

Key words: *Ferula assa-foetida* L.; Umbelliferae; resin; caffeic acid cinnamyl ester

Plants have been a constant source of drugs and considerable recent emphasis has been placed on finding novel therapeutic agents from medicinal plants. Many people prefer to use medicinal plants rather than chemical drugs. The *Ferula* genus from the family of Umbelliferae is a large genus of about 130 species distributed throughout the Mediterranean area and Central Asia.[1] Several species of this genus have been used in folk medicines,[2] and investigations on the *Ferula* species have indicated antinociceptive, anti-inflammatory and antipyretic effects,[3] contraceptive action,[4,5] and smooth muscle relaxant activity.[6–8] This genus is well documented as a good source of biologically active compounds such as sesquiterpene coumarins[9] and sesquiterpene.[10,11] The *Ferula* genus has been found to be a rich source of gum resin.[1] The gum resins, which are obtained by incising the roots of several species, are used as spices and drugs in many countries. The resins have been reported to cure stomachic, vermifuge and carminative disorders.[12] *Ferula assa-foetida* grows in Kashmir, Iran and Afghanistan. It has an unpleasant smell, is herbaceous and perennial, and grows up to 2 m high.[13] The pharmacological action of *Ferula assa-foetida* has not been studied much. It slightly inhibits the growth of *Staphylococcus aureus* and *Shigella sonnei*, and some of the sulfur compounds show pesticidal activity. A higher dose taken orally causes diarrhoea, meteorism, headaches, dizziness, and enhanced libido.[13] The characteristic features of this plant include the presence of glucuronic acid, galactose, arabinose and rhamnose which have been isolated from the gum,[13] whereas the taste and smells are due to sulfur-containing compounds. Asadisulphide,[14] disulfides, as well as symmetric tri- and tetrasulfides have been isolated,[15] and polysulfide derivatives have recently been reported, as well as umbelliferone, farnesiferol A, B and C, and ferulic acid,[16,17] kamolonol,[18] assafoetidin and ferocolicin,[19] assacoumarin A and B,[14] foetidin,[20] episamarcandin, umbelliprenin and conferol,[21] 5-hydroxyumbelliprenin, 8-hydroxyumbelliprenin and 8-acetoxyumbelliprenin,[22] and episamarcandin acetate.[23] As part of our continuing studies of plants of the *Ferula* genus,[24–28] this present work deals with the isolation of a new caffeic acid cinnamyl ester from the gum resin of *Ferula assa-foetida* and its activity in inhibiting NO production.

Repeated chromatographic separation, involving silica gel and Sephadex LH-20 columns, of the *n*-hexane-soluble fraction of the MeOH extract of the gum resin of *Ferula assa-foetida* L. led to the isolation of new caffeic acid cinnamyl ester **1** (Fig. 1) as a white amorphous powder. The $[M+H]^+$ peak observed at m/z 441.1931 ($[M+H]^+$, calcd. 441.1933) by HR-FAB-MS together with the analysis of the ^{13}C-NMR and DEPT spectra showed its molecular formula to be $C_{24}H_{24}O_8$. The UV spectrum showed absorption maxima at 219, 290 and 331 nm, while the IR spectrum suggested the presence of

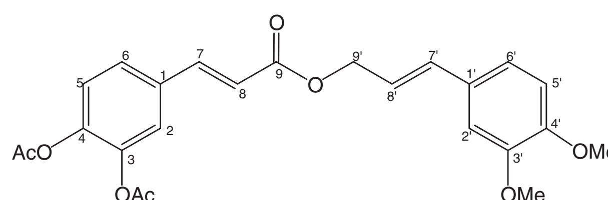

Fig. 1. Structure of Compound **1**.

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1**[a]

C	δ_C	δ_H (J, Hz)	^{1}H–^{1}H COSY	HMBC (H to C)
1	133.3 s			
2	121.3 d	7.14 (d, 2)	H-6	C-6, C-4, C-7
3	141.5 s			
4	139.7 s			
5	123.3 d	7.06 (d, 8.5)	H-6	C-1, C-3
6	111.3 d	7.12 (dd, 8.5, 2)	H-5, H-2	C-2, C-4, C-7
7	144.4 d	7.70 (d, 15.8)	H-8	C-6, C-2, C-9
8	118.1 d	6.44 (d, 15.8)	H-7	C-1
9	166.5 s			
1′	135.3 s			
2′	119.4d	6.99 (d, 1.8)	H-6′	C-6′, C-7′, C-4′
3′	151.4 s			
4′	151.2 s			
5′	122.9 d	6.94 (d, 8.2)	H-6′	C-1′, C-3′
6′	110.3 d	7.01 (dd, 8.2, 1.8)	H-5′, H-2′	C-2′, C7′, C-4′
7′	133.7 d	6.69 (br d, 16, 1.5)	H-8′, H-9′	C-9′, C-6′, C-2′
8′	123.6 d	6.30 (dt, 16, 7)	H-7′, H-9′	C-1′
9′	65.0 t	4.87 (dd, 7, 1.5)	H-8′, H-7′	C-7′, C-9
2 <u>CO</u>CH$_3$	168.7 s			
	169.0 s			
2 CO<u>CH$_3$</u>	20.6 q	2.31 (s)		
		2.32 (s)		
2OCH$_3$	55.9 q	3.85 (s)		C-4′
		3.86 (s)		C-3′

[a]Chemical shift as ppm. Assignment was assigned on the basis of DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectra.

carbonyls at 1772 and 1738 cm^{-1} and a weak band at around 1684 cm^{-1} for the cinnamoyl ester group. The EIMS data exhibited M^{+} at m/z 440 calculated for $C_{24}H_{24}O_8$, a base peak at m/z 247, and other significant peaks at m/z 264 [M + H-3,4-dimethoxycinnamyl moiety]$^{+}$ and 176 [M − H-3-(3,4-diacetoxyphenyl) acrylate moiety]$^{+}$. Assignment of all the protons and carbons of **1** was achieved by studying the ^{1}H–^{1}H COSY, HMQC and HMBC spectra. Inspection of the ^{13}C-NMR spectrum of **1** (Table 1) revealed the presence of twenty four non-equivalence carbons, including two aromatic moieties. An analysis of the DEPT data experiments indicated that **1** possessed two acetoxy metyls at δ_C (20.6), two methoxy methyls (55.9), one oxymethylene (65.0), ten methines, six of which were for the aromatic nucleus (121.3, 123.3, 111.3, 119.4, 122.9, and 110.3) and the other four for olefinic carbons (144.4, 118.1, 133.7, and 123.6), and seven quaternary carbons, one of them being characteristic of a carbonyl ester at δ_C 166.5. The ^{1}H-NMR spectrum (Table 1) suggested the presence of two 1,3,4 trisubsitituteed benzene rings (ABX-type signals at δ_H 7.06 (1H, d, $J = 8.5$ Hz), 7.12 (1H, dd, $J = 8.5, 2.0$ Hz), 7.14 (1H, d, $J = 2.0$ Hz), 6.94 (1H, d, $J = 8.2$ Hz), 6.99 (1H, d, $J = 1.8$ Hz), 7.01 (1H, dd, $J = 8.2, 1.8$ Hz), together with two sets of two *trans*-olefinic proton signals at δ_H 7.70 (1H, d, $J = 15.8$ Hz), 6.44 (1H, d, $J = 15.8$ Hz) and 6.69 (1H, br d, $J = 16.0, 1.5$ Hz), 6.30 (1H, dt, $J = 16, 7$ Hz), in addition to oxymethylene protons at (δ_H 4.87, dd, $J = 7.0, 1.5$ Hz), two methoxyls [δ_H 3.85 (s) and 3.86 (s)] and two acetates [δ_H 2.31 (s) and 2.32 (s)]. Moreover, H-C long-range correlations between H-7 at δ_H 7.70 and carbon C-6 (δ_C 111.3)/C-2 (δ_C 121.3)/C-9 (δ_C 166.5), H-8 at δ_H 6.44 and carbon C-1 (δ_C 133.3), H-7′ at δ_H 6.69 and carbon C-9′ (δ_C 65.0)/C-6′ (δ_C 110.3)/C-2′ (δ_C 119.4), H-8′ at δ_H 6.30 and carbon C-1′ (δ_C 135.3), in addition to H-9′ protons at δ_H 4.87 and carbon C-7′ (δ_C 133.7) and C-9 (δ_C 166.5) were observed in the HMBC spectrum. A comparison of these results with those previously reported[29] suggested that **1** was (2*E*)-3,4-dimethoxycinnamyl-3-(3,4 diacetoxyphenyl) acrylate, a new caffeic acid cinnamyl ester (Fig. 1).

Compound **1** was examined for its dose-response effects on LPS-induced NO production. Excessive production of NO, which is formed by iNOS in macrophages and endothelial cells, is responsible for the inflammatory response and is implicated in the pathogenesis of such inflammatory diseases as septic shock, rheumatoid arthritis, graft rejection, and diabetes.[30] Compound **1** was tested for its effect on NO production in LPS-stimulated RAW264.7 cells with respect to aminoguanidine, an iNOS inhibitor. Compound **1** inhibited LPS-induced NO production in the RAW264.7 cells dose-dependently with an IC_{50} value of 54.9 µm, comparable to that of aminoguanidine (IC_{50} 18.2 µm). The cell viability measured by an MTT assay showed that **1** had no significant cytotoxicity on RAW264.7 cells at its effective concentration for the inhibition of NO production (data not shown).

Experimental

General. NMR spectra were measured with Jeol LA500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] NMR spectrometer in $CDCl_3$. The chemical shift values are

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reported in ppm (δ) units and the coupling constants (J) are in *Hz*. The ^{1}H–^{1}H COSY, DEPT, HMQC, and HMBC NMR spectra were obtained with the usual pulse sequences. Melting point (mp) data were determined with Buchi melting point apparatus and are uncorrected. IR spectra were obtained with a Jasco FT/IR 410 spectrometer, and UV spectra were recorded by a Shimadzu UV-1601 UV–visible spectrophotometer. Low-resolution EI-MS data were collected by a Quattro GC/MS spectrometer having a direct inlet system, and high-resolution FAB-MS data were collected by a Finnigan/Thermo Quest MAT 95XL spectrometer. TLC work was carried out by using plates coated with F254 silica gel 60 (Merck Co.), the TLC plates being visualized was under UV light at 254 and 366 nm and also by spraying a ceric sulphate reagent and heating. Silica gel column chromatography was performed on Merck silica gel 60 (230–400 mesh), and Sephadex LH-20 was used for column chromatography (Pharmacia, 25–100 µm). All solvents were routinely distilled prior to their use, and other chemicals were of commercial grade without purification.

Plant material. F. assa-foetida gum resin, locally known as "Haltet" was purchased from El-Gomhouria Company for Drugs and Equipments, 13 Mahmoud Basuny St., El-Tahrir Sq., Cairo, Egypt and was stored at 4 °C until its extraction.

Extraction and isolation. Dried and coarsely powdered resin (200 g) was extracted three times with MeOH (1 l) at room temp., each time for 24 h, and the extracts were combined and concentrated *in vacuo* at 40 °C. The concentrated extract (55 g) was suspended in H_2O (2 l) and then partitioned five times with 2 l of *n*-hexane to afford an *n*-hexane-soluble syrup after drying. The *n*-hexane-soluble extract (3.3 g) exhibiting a 60.6% inhibitory effect on the production of NO at the concentration of 100 µg/ml was chromatographed over silica gel, using a gradient solvent system of *n*-hexane:EtOAc (10:0-0:1), to give ten fractions (FA1–FA10). Most of the fractions from FA1 to FA9 were mixtures of fatty acids with sulfide components. These fractions (FA1–FA9) were inactive, showing an 8.6% inhibitory effect on the production of NO at the concentration of 100 µg/ml. Fraction FA10 (100 mg), exhibiting a 66.3% inhibitory effect on the production of NO at the concentration of 100 µg/ml, was further chromatographed over silica gel, eluting with *n*-hexane:EtOAc (5:1) to give four subfractions (FA101–FA104) with 11.4%, 13.2%, 68.9% and 14.6% inhibitory effects at the concentration of 100 µg/ml, respectively. Accordingly, the third subfraction, FA103 (40 mg) was purified with Sephadex LH-20 (*n*-hexane:EtOAc = 1:3) to yield **1** (18 mg), with an IC_{50} value of 54.9 µm.

Determination of nitric oxide production. RAW264.7 cells were transferred to 96-well plates at a density of 1×10^5 cells well^{-1}. After 3 h of incubation, the cells were stimulated with LPS (1 µg ml^{-1}) for 24 h in the absence or presence of the compound being tested. As a parameter of NO synthesis, the nitrite concentration was measured in the supernatant of the RAW264.7 cells by the Griess reaction.[31] Briefly, 100 µl of the cell culture supernatant was reacted with 100 µl of the Griess reagent [a 1:1 mixture of 0.1% *N*-(1-naphthyl)ethylenediamine in H_2O and 1% sulfanilamide in 5% phosphoric acid] in a 96-well plate, and the absorbance was measured with a microplate reader (Molecular Devices Co., Menlo Park, CA, USA) at 570 nm. The nitrite concentration in the supernatant was calculated by comparison with a standard sodium nitrite curve.

*(2E)-3,4-Dimethoxycinnamyl-3-(3,4 diacetoxyphenyl) acrylate (**1**).* White amorphous powder; mp 168–169 °C; UV λ_{max} (MeOH) nm (log ε): 219 (4.33), 290 (4.71), 331 (4.57); IR γ_{max} (KBr) cm^{-1}: 1772, 1738, 1684; HR-FAB-MS m/z: 441.1931 ([M + H]$^+$; calcd. 441.1933 for $C_{24}H_{24}O_8$); EIMS m/z (rel. int.): 440 [M]$^+$ (10), 264 (35), 247 (100), 176 (30); ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) data, see Table 1.

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