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Antibacterial Activity of a Novel Sesquiterpene Coumarin from *Ferula sinaica*

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Ferulsinaic acid (**1**), a novel sesquiterpene coumarin has been isolated from the roots of *Ferula sinaica*. The structure elucidation was determined by HRMS, ^{1}H and ^{13}C 1D and 2D NMR spectral data.

Key Words: *Ferula sinaica*, Apiaceae, Roots, Sesquiterpene coumarins.

INTRODUCTION

Ferula genus belongs to the family Apiaceae with some 130 species distributed throughout the Mediterranean area and Central Asia[1]. Several species of *Ferula* have been used in folk medicine; *e.g.*, *Ferula communis* L., its subspecies and varieties have been used as agents against hysteria and to treat dysentery[2], *Ferula jaeschkeana Vatke* has been applied to wounds and bruises[3] and *Ferula tingitana* L. has proved to be a good source of ammoniac, an oleo-gum resin used in medicine[4]. The widespread sesquiterpene compounds in this genus are characteristic dauicanes, humulanes, himachalanes, germacranes, eudesmanes, and guainanes[5].

In continuation of our interest and the chemical constituents of the Egyptian medicinal plants[6,7], the roots of *F. sinaica* L. are reinvestigated to afford a novel sesquiterpene coumarin named ferulsinaic acid (**1**).

EXPERIMENTAL

^{1}H NMR 500 MHz, ^{13}C NMR 125 MHz with $CDCl_3$ as internal standard; MS: Jeol JMSD-500 instrument; CC: silica gel, Sephadex LH-20 (Pharmacia), Tokyo Pearl HW-40 (Tosho); silica gel (Si60, Hibar TR250-25, Merck), ODS (RP-18, Hibar Rt250-25, Merck).

Plant material: The fresh roots of *Ferula sinaica* (1.5 kg) were collected from Sinai, Egypt in March 2001. The fresh roots were cutted into slices, air-dried and powdered.

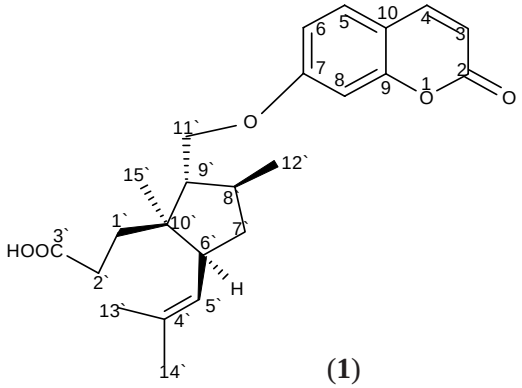
Extraction and isolation: About 1.5 kg of the air-dried powdered roots of *F. sinaica* were extracted with methylene chloride-methanol (1:1) system. 50 g of the crude extract was fractionated over silica gel column (1300 g) using gradient elution with methelene chloride-hexane-methanol mixture concentrated and monitored by TLC. Fraction A (CH_2Cl_2: hexane, 1:1) was further applied to a Sephadex LH-20 column, eluted with methylene chloride-hexane-methanol mixture (8:5:1) to give ferulsinaic acid (**1**) (10 mg) .

Bioassay: The antibacterial activity of ferulsinaic acid was determined against Gram-negative strains (*Serratia* sp., *Pseudomonas* sp., *Escherichia coli*) and Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), obtained from culture collection of Bacteriological Laboratory, Department of Microbiology, Faculty of Pharmacy, El-Minia University, Egypt, using Whatman filter paper No. 1, 1 cm. Diameter, disc diffusion assay methods. Five replicates were performed for the compound with two concentrations (200 and 400 μg/mL) of compound was done. Discs were soaked in the test compound for 30 s, evaporated, then overload on the surface of the nutrient agar media cultured with the tested bacterium. All plates were incubated at 30°C for 48 h . Ampicillin (purchased from ADWIC Comp., Egypt) and amoxillin (purchased from ADCO Comp., Egypt) were used as a reference compounds.

Ferulsinaic acid (**1**) obtained as white amorphous powder $[\alpha]_D$ - 4.5(c 0.67, $CHCl_3$). HRFABMS, $[M]^+$ at m/z 399.21699 (calcd. 399.21716). The IR spectrum showed absorption bands at 2968, 1726 ν(C=O, coumarin), 1711 ν(COOH), 1614 and 1556.

RESULTS AND DISCUSSION

The air-dried roots of *Ferula sinaica* was extracted with methylene chloride-methanol (1:1) system at room temperature. The crude extract was partitioned by successive chromotographic separation to afford a novel sesquiterpene coumarin named ferulsinaic acid (**1**).



(**1**)

Ferulsinaic acid (**1**), had white amorphous powder $[\alpha]_D$ -4.5 (c 0.67, $CHCl_3$). Its molecular formula $C_{24}H_{30}O_5$ was established by HRFABMS, $[M+H]^+$ at m/z 399.21699 (calcd. 399.21716). The IR spectrum showed absorption showed absorption bands at 2963, 1726 ν(C=O, coumrin), 1711 ν(COOH), 1614 and 1560 cm^{-1}. The structure of (**I**) was established by ^1H NMR and ^{13}C NMR spectra (Table-1). The multiplicities of the carbons were determined by the DEPT and HMQC as following: Seven quaternary carbons, nine tertiary carbons, four secondary carbons and four primary carbons.

TABLE-1
^1H (500 MHz, $CDCl_3$) AND ^{13}C (125 MHz, $CDCl_3$) NMR
SPECTRAL DATA FOR COMPOUND **1**

	δ_H (*J* in Hz)	δ_C (multiplicity)[a]
2		161.2 s
3	6.24 (d, *J* = 9.4 Hz)	113.2 d (CH)
4	7.63 (d, *J* = 9.4 Hz)	143.4 d (CH)
5	7.36 (d, *J* = 8.5 Hz)	128.7 d (CH)
6	6.83 (dd, *J* = 8.5, 2.2 Hz)	112.6 d (CH)
7		162.1 s
8	6.82 (d, *J* = 2.2 Hz)	101.2 d (CH)
9		156.0 s
10		112.5 s
1′	1.70 (t, *J* = 8.2 Hz)	33.0 t (CH_2)
2′	2.73 (m)	29.2 t (CH_2)
3′		179.9 s
4′		132.1 s
5′	5.12 (dqq, *J* = 10, 1.3, 1.3Hz)	125.4 d (CH)
6′	2.51 (ddd, *J* = 10, 1.3, 1.3Hz)	49.3 d (CH)
7′	1.19 (ddd, *J* = 12.5, 9.5, 6.5Hz) 1.93 (ddd, *J* = 12.5, 6.5, 6.5Hz)	40.0 t (CH_2)
8′	1.83 (m)	36.5 d (CH)
9′	1.75 (ddd, *J* = 9, 7.5, 5Hz)	53.2 d (CH)
10′		47.0 s
11′	3.97 (dd, *J* = 9.5, 5Hz) 4.00 (dd, *J* = 9.5, 7.5Hz)	69.5 t (CH_2)
12′	1.14 (s)	20.8 q (CH_3)
13′	1.63 (br, s)	18.1 q (CH_3)
14′	1.72 (br, s)	26.2 q (CH_3)
15′	0.92 (d, *J* = 6.6 Hz)	21.0 q(CH_3)

*Multiplicity was determined by DEPT experiments.

^{1}H NMR and ^{13}C NMR suggested the presence of umbelliferone moiety in the skeleton from the signals at δ_H 7.63 (δ_C 143.4, d, J = 9.4 Hz, H-4), δ_H 7.36 (δ_C 128.7, d, J = 8.5 Hz, H-5), δ_H 6.83 (δ_C 112.6, dd, J = 8.5, 2.2 Hz, H-6), δ_H 6.82 (δ_C 101.2, d, J = 2.2 Hz, H-8) and δ_H 5.24 (δ_C 113.2, d, J = 9.4 Hz, H-3).

The ^{1}H NMR data of the sesquiterpene part exhibited a different pattern compare to the previously reported sequiterpene coumarins in the genus *Ferula*. In ^{1}H-^{1}H COSY spectrum the two olefinic methyl at δ 1.63 (H-13´) and 1.72 (H-14´) showed an allylic coupling with a downfield proton at δ 5.12 (H-5´), which itself correlate with a proton at δ 2.51 (H-6´). HMQC and HMBC supported the presence of the moiety: $(CH_3)_2$ = CH–CH that attached to a five member ring, H-12 and H-13 correlated with C-4 and C-5, H-14 correlated with C-1, C-6, C-9 and C-10, H-15 correlated with C-7 and C-8, H-6 with C-1 and H-11 with C-9 and C-10. Additionally, the carboxylic carbon at δ_C 179.9 (C-3´) showed a correlation with H-1´ (δ_H 1.70) in HMBC which supported the presence of $HCOOCH_2CH_2$ group in the molecule. This accumulated data suggested the rearranged 3,4-seco-drimane structure of compound (**1**). The methyl doublet at δ_H 0.92 (H-13) showed correlation with a multiplet at δ_H 1.83 (H-8´) in the ^{1}H-^{1}H COSY. Moreover, this methyl correlate with a ddd at δ_H 2.51 (H-6´) and two dd at δ_H 3.97 and 4.00 (H-11a´ and H-11b´, respectively) in HMBC.

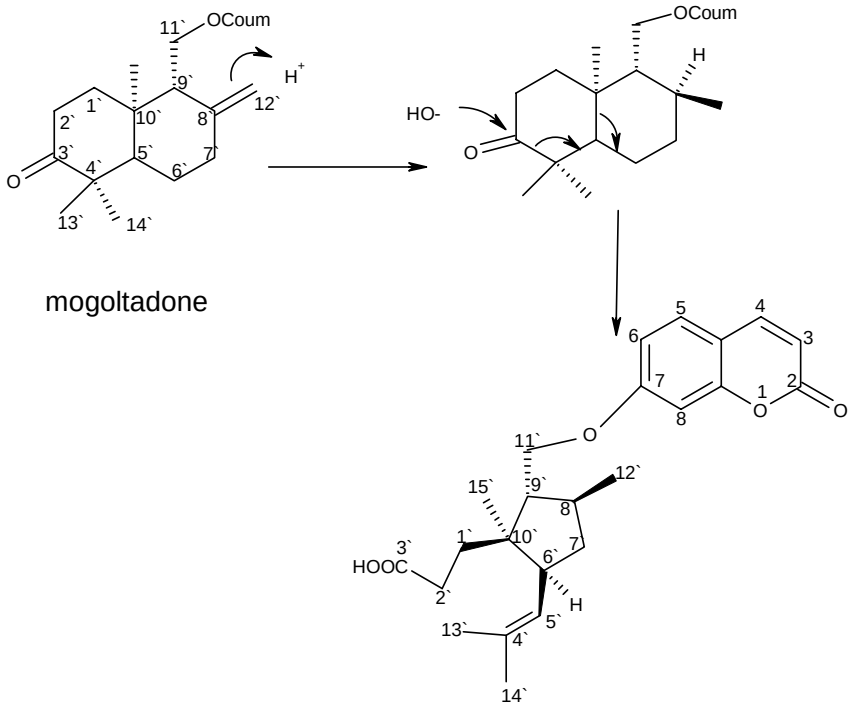


Fig. 1. Possible biogenetic formation of compound **1**

The stereochemistry of **1** was deduced from the NOSEY experiments; H-15´ showed correlation with H-6´ and H-11´, correlated with H-8´ showed correlation with H-15´ and H-11´ and H-9´ correlated with H-12´ and H-7_{β}. The biogenesis of **1** may proceed through mogoltadone like galbanic acid[8], (Fig. 1). The difference between both biogenesis pathway that ferulsinaic acid did not subject to the transformation of H-15´ from C-10´ to C-9´. This phenomenon has also been observed in a previously reported sesquiterpene coumarin from the same genus which appears to be characteristic of the species. Additionally, compound **1** is of a particular interest since it is first member of new class of sesquiterpene coumarin from the genus *Ferula*.

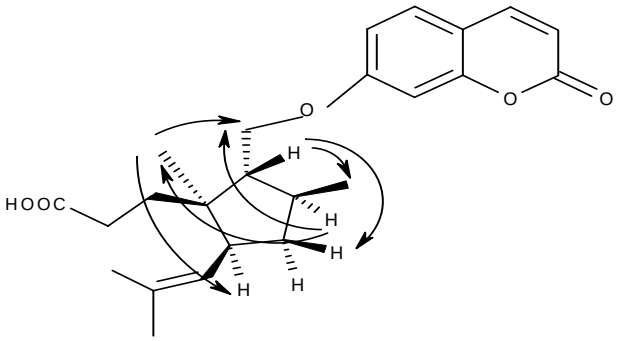


Fig. 2. Important NOE correlation of **1**

TABLE-2
ANTIMICROBIAL ACTIVITIES OF COMPOUND **1**
(DRY DMSO AS SOLVENT)

Test organism	Ferulsinaic acid[c]	*Ampicillin*[d]	*Amoxillin*[d]
Gram-Positive Strain			
Bacillus cereus	10[a] 18[b]	10[a]	N[a]
Staphylococcus aureus	N[a] 5[b]	8[a]	N[a]
Gram-Negative Strain			
Serratia sp.	10[a] 18[b]	11[a]	13[a]
Pseudomonas sp.	11[a] 18[b]	11[a]	13[a]
Escherichia coli	10[a] 17[b]	11[a]	13[a]

[a]Values show the zone of inhibition in mm; conc. of the samples was 200 µg/mL, [b]Values show the zone of inhibition in mm; conc. of the samples was 400µg/mL, [c]Data are the mean of five measurements with neglected standard errors, [d]Reference antibiotics were carried out at 200 µg/mL only, N = No effect.

Antibacterial screening: *In vitro*, screening experiments for antibacterial activities of ferulsinaic acid was subjected to biological testing. To substantiate the antibacterial results, we screened compound (**1**) against an assortment of two Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Serratia* sp., *Pseudomonos* sp., *Escherichia coli*) using ampicillin and amoxillin as a reference standard . The minimum inhibitory concentrations (MICs, µg/mL) were determined using standard agar dilution method[9]. The MIC value is summarized in Table-2.

From the obtained data, it is clear that ferulsinaic acid posses high activity against Gram-positive strain, particulerly *Bacillus cereus*. Also, posses high activity against Gram-negative strains, as shown in Table-2. Our results are in agreement with those reported earliear by Joklik *et al.*[10], they reported that some antibiotics such as ampicillin and amoxillin have been developed as inhibitors of cell wall synthesis of bacterial cell. So, ferulsinaic acid has the common structural feature of penicillins exhibit antibacterial activities.

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