Iranian Journal of Pharmaceutical Research (2007), 6 (3): 217-221
Received: July 2006
Accepted: December 2006

Original Article

Two New Sesquiterpene Coumarins, Ferusinol and Samarcandin Diastereomer, from *Ferula sinaica*

Ashraf A. El-Bassuony[a], Ahmed A. Gohar[b] and Amal M. Kabbash[c*]

[a] *Basic Sciences Department, Industrial Education College, Beni Suef University, Egypt.*
[b] *Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.*
[c] *Pharmacognosy Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt.*

Abstract

Re-investigation of the methylene chloride extract of the roots of *Ferula sinaica* gave ferusinol, a new sesquiterpene coumarin with a rare carbon skeleton, and samarcandin diastereomer. The structures elucidation were determined by MS, ^{1}H- ^{13}C-1D and 2D NMR spectral data.

Keywords: *Ferula sinaica*; Apiaceae; Sesquiterpene coumarins.

Introduction

The genus *Ferula* 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. Thus, *F. communis* L. and its subspecies and varieties have been used as agents against hysteria and to treat dysentery (2), F. jaeschkeana Vatke has been applied as a herbal contraceptive (3) and *F. 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 daucanes, humulanes, himachalanes, germacranes, eudesmanes, and guaiananes (5). The study of the chemical constituents of this genus has developed rapidly over the last twenty years due to more efficient methods of purification and the availability of sophisticated techniques for structure elucidation and thus several types of sesquiterpene coumarin compounds have been isolated (6-10).

* Corresponding author:
E-mail: amkabbash_99@yahoo.com

Previous works on *F. sinaica* led to isolation of sesquiterpene coumarins, daucanes, bornanes and monoterpenes (6, 11-12). In continuation of our interest in the chemical constituents of the Egyptian medicinal plants, we re-investigated the roots of *F. sinaica* L. and found a new sesquiterpene coumarin with a rare carbon skeleton, named ferusinol, and a diastereomer samarcandin (Figure 1).

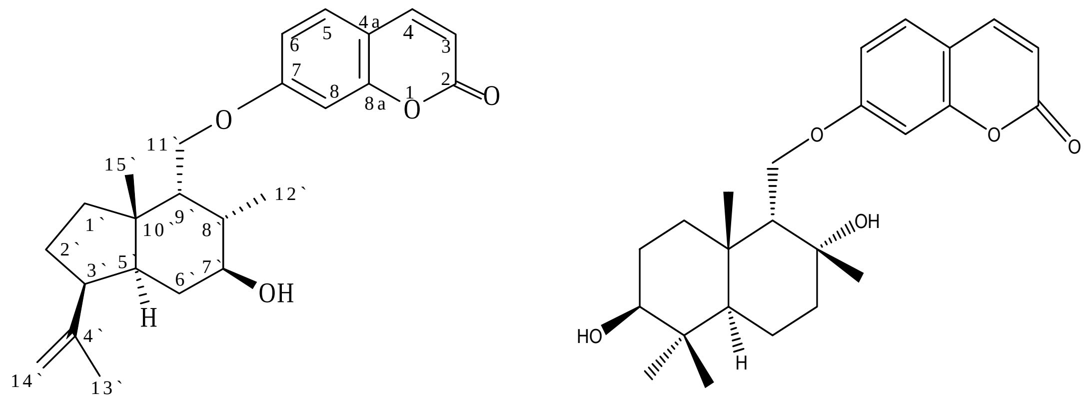
Experimental

General

^{1}H-NMR (500 MHz, $CDCl_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) and 2D spectra were recorded on a JEOL 500 MHz, Lambda spectrometer, TLC: precoated silica gel type 60 (Merck). HPLC was performed in the reversed phase mode on Knauer pump 64 and different refractometer (column: RP-8, 250×25 mm, flow=17 ml/min, elution with MeOH-H_2O, mixtures, refractive index).

Plant material

The fresh roots of *F. sinaica* L. (Apiaceae) (3 kg) were collected from Sinai, Egypt, March 2002. The fresh roots were cutted into slices,



Ferusinol Samarcandin Diastereomer

Figure 1. chemical structures of ferusinol and samarcandin diastereomer.

air-dried and powdered. A voucher specimen (A. A. 111) is deposited at the Department of Botany, Faculty of Science, Beni Suef, Cairo University.

Extraction and separation

The air-dried plant materials (3 kg) were ground and extracted at room temperature with CH_2Cl_2-MeOH (1:1). The extracts were concentrated in vacuum to leave 200 g. residue. This residue was fractionated on flash column, silica gel, using n-hexane, CH_2Cl_2, increased polarity. The fractions eluted with 75% CH_2Cl_2 was refractionated by CC (6 ×120 cm) on silica gel eluting with n-hexane followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100% CH_2Cl_2 then CH_2Cl_2-MeOH up to 15 % MeOH. The 100% CH_2Cl_2 fraction was further purified by CC (2×40 cm), on Sephadex LH-20 eluted with *n*-hexane-CH_2Cl_2-MeOH (6: 4: 1) to give a mixture of **1** (9 mg) and **2** (5 mg). The mixture was purified by HPLC (MeOH-H_2O, 65:35, R_t= 5.6 and 6.0 min).

Bioassay

The antibacterial activity of ferusinol and samarcandin diasteriomer were 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 Botany, Faculty of Science, El-Minia University, Egypt, using Whatman filter paper No. 1, 1 cm diameter, disc diffusion assay methods. Five replicates were performed for the compounds at concentrations 200 µg/ml and 400 µg/ml. Discs were soaked in the test compound for 30 sec, dried and then laid on the surface of nutrient agar medium inoculated with the test bacterium. The plates were incubated at 30 °C for 48 h. Ampicillin (purchased from ADWIC Company, Egypt) and amoxillin (purchased from ADCO Company, Egypt) were used as a reference compounds.

*Ferusinol **1***

^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.26 (1H, d, *J*= 9 Hz, H-3), 7.63 (1H, d, *J*= 9 Hz, H-4), 7.63 (1H, d, *J*= 9 Hz, H-5), 6.83 (1H, dd, *J*= 2.5, 9 Hz, H-6), 6.81 (1H, d, *J*= 2.5 Hz, H-8), 1.10 (1H, m, H-1`$_a$), 0.95 (1H, m, H-1`$_b$), 1.15 (1H, m, H-2`$_a$), 1.95 (1H, m, H-2`$_b$), 2.11 (1H, ddd, *J*= 3.5, 6, 6 Hz, H-3`), 1.41 (1H, ddd, *J*= 3.5, 13, 13 Hz, H-5`), 1.90 (1H, ddd, *J*= 4, 13, 13 Hz, H-6`$_a$), 1.49 (1H, ddd, *J*= 4, 13, 13 Hz, H-6`$_b$), 3.39 (1H, ddd, *J*= 4, 13, 13 Hz, H-7`), 1.96 (1H, dd, *J*= 2.5, 13 Hz, H-8`), 2.23 (1H, ddd, *J*= 2.5, 9, 9 Hz, H-9`), 4.05 (1H, dd, *J*= 7, 9 Hz, H-11`$_a$), 3.97 (1H, dd, *J*= 7, 9 Hz, H-11`$_b$), 1.19 (3H, d, *J*= 7 Hz, H-12`), 1.65 (3H, s, H-13`), 5.05 (1H, br s, H-14`$_a$), 4.91 (1H, br s, H-14`$_b$), 0.93 (3H, s, H-15`); CIMS $(M + H)^+$ *m/z* 383 ($C_{24}H_{30}O_4$) (30 %), 365 $(M + H – H_2O)^+$ (60 %); IR 3345 (OH), 2963, 1726 cm^{-1} (C=O).

*Samarcandin diastereomer **2***

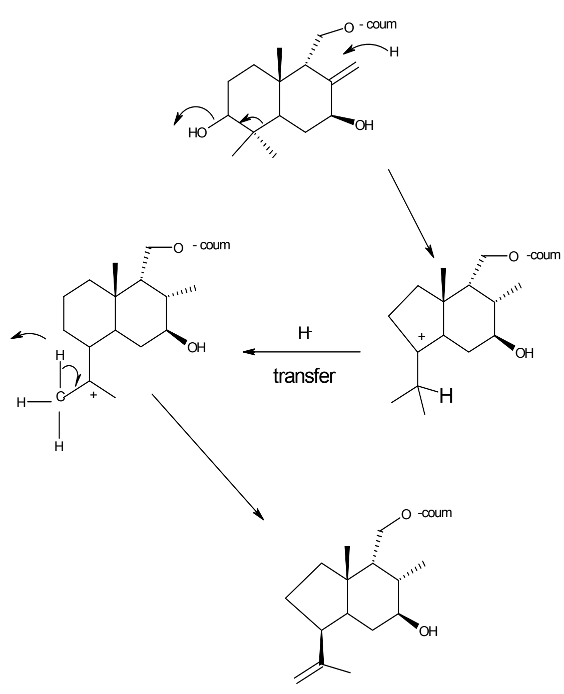
^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.28 (1H, d, *J*

9.55 Hz, H-3), 7.63 (1H, d, *J* 9.55 Hz, H-4), 7.37 (1H, d, *J* 8.6 Hz, H-5), 7.19 (1H, dd, *J* 2.5, 8.6 Hz, H-6), 7.03 (1H, d, *J* 2.5 Hz, H-8), 1.45 (2H, m, H-1`), 2.05 (2H, m, H-2`), 3.42 (1H, dd, *J* 11, 2 Hz, H-3`), 1.57 (1H, dd, *J* 2, 8 Hz, H-5`), 1.15 (2H, m, H-6`), 1.35 (2H, m, H-7`), 2.00 (1H, dd, *J* 4.9, 10.2 Hz, H-9`), 4.66 (1H, d, *J* 6.65 Hz, H-11`$_a$), 4.28 (1H, dd, *J* 6.65, 10.2 Hz, H-11`$_b$), 1.39 (3H, s, H-12`), 1.20 (3H, s, H-13`), 1.04 (3H, s, H-14`), 1.02 (3H, s, H-15`); EIMS $(M)^+$ *m/z* 400 ($C_{24}H_{32}O_3$) (30 %), 382 $(M – H_2O)^+$ (60 %); IR 3450 (OH), 1685 (α - pyrone), 1610, 1595, 1225, 1205 and 1150 cm^{-1}.

Results and Discussion

The CH_2Cl_2-MeOH (1:1) extract of *F. sinaica* was partitioned by successive chromatographic separations to afford two new sesquiterpene coumarins, namely, ferusinol (**1**) and a diastereomer samarcandin (**2**). The structure of **1** was established by the analysis of its ^{1}H-NMR and ^{13}C-NMR spectra, (Table 1), which showed six quaternary carbons, ten tertiary carbons, five secondary carbons and three primary carbons. The ^{1}H-NMR and ^{13}C-NMR spectra suggested the presence of an umbelliferone moiety in the skeleton from the signals at δ_H 7.63 (δ_C 143.4, d, *J*= 9 Hz, H-4), δ_H 7.63 (δ_C 128.7, d, *J*= *9* Hz, H-5), δ_H 6.83 (δ_C 113.0, dd, *J*= 9, 2.5 Hz, H-6), δ_H 6.81 (δ_C 101.1, d, *J*=2.5 Hz, H-8) and δ_H 6.26 (δ_C 113.0, d, *J*= 9 Hz, H-3). The ^{1}H-NMR data (in $CDCl_3$, 500 MHz) of the sesquiterpene part exhibited a different pattern compared to the previously reported sesquiterpene coumarins in the genus *Ferula.* The protons and their sequences could be assigned by ^{1}H-^{1}H COSY as following: the two signals at δ_H 5.05 (br s) and δ_H 4.91 (br s) were characteristic for exomethylene protons (H-14`$_a$, H-14`$_b$). The hydroxylated carbon (C-7`) appeared at $\delta_C$ 70.2 and H-7` at δ_H 3.39 (ddd, *J*= 4, 13, 13 Hz). The signal at δ_H 1.41 was characteristic for H-5` (ddd, *J*= 3.5, 13, 13 Hz), and the two protons H-11`$_a$, H-11`$_b$ appeared as two double doublets at $\delta_H$ 4.05 (*J*= 7, 9 Hz), 3.97 (*J*=7, 9 Hz). The signal at $\delta_H$ 2.23 (ddd, *J*= 2.5, 9, 9 Hz) was assigned for H-9`. The three methyl protons H-12`, H-13` and H-15` appeared as one doublet and two singlets at $\delta_H$ 1.19, 1.65 and 0.93, respectively. The sesquiterpene moiety showed two olefinic carbons at $\delta_C$ 116.9 and 144.1, characteristic for C-14` and C-4`, respectively. The three methyl carbons C-12`, C-13` and C-15` appeared as δ_C 23.0, 24.6 and 17.9, respectively. The other carbon signals are reported in Table 1. The relative stereochemistry of **1** was deduced from NOE experiments; irradiation of H-7` enhanced H-5`, irradiation of H-15` showed an effect on H-11`, irradiation of H-3` enhanced H-5` and irradiation of H-5` enhanced H-7` and H-12`. Ferusinol had a molecular formula $C_{24}H_{30}O_4$ indicated by CIMS, $(M + H)^+$ at *m/z* 383 (30 %), $(M + H – H_2O)^+$ at *m/z* 365 (60 %). The proposed biosynthetic route of ferusinol (**1**) is given in Scheme 1.

^{1}H-NMR of compound **2,** aided with ^{1}H-^{1}H COSY, showed two signals at δ_H 7.63 and 6.28 (each 1H, d, *J*= 9.5 Hz) assigned to H-4 and H-3, while the signals at δ_H 7.37 (1H, d, *J*= 8.6 Hz), 7.03 (1H, d, *J*= 2.5 Hz) and 7.19 (1H, dd, *J*= 2.5, 8.6 Hz) were assigned to H-5, H-8 and H-6 of the coumarin moiety, respectively. The methylene protons (H-11`) showed a different splitting than those reported in the literature, whereas, one of them appeared as double doublet signal at δ_H 4.28 (1H, dd, *J*= 6.65, 10.2 Hz), and the second



Scheme 1. Proposed biosynthetic route of Ferusinol **1** (coum= coumarin moiety).

Table 1. ^{13}C-NMR data of Ferusinol **1*** and Samarcandin **2*** in $CDCl_3$ (125 MHz)

Position	1*	2*
	δ_C (mult.)	δ_C (mult.)
1		
2	162.2 (s)	162.8
3	113.0 (d)	112.9
4	143.4 (d)	143.8
5	128.7 (d)	129.3
6	113.0 (d)	113.4
7	161.2 (s)	160.9
8	101.1 (d)	101.9
4a	112.4 (s)	112.6
8a	155.9 (s)	156.5
1`	32.3 (t)	44.9
2`	34.4 (t)	28.2
3`	47.6 (d)	77.8
4`	144.1 (s)	38.7
5`	48.4 (d)	55.3
6`	29.1 (t)	20.7
7`	70.2 (d)	38.3 (t)
8`	35.1 (d)	71.4 (s)
9`	57.3 (d)	60.7
10`	44.3 (s)	39.4
11`	69.8 (t)	66.8
12`	23.0 (q)	28.7
13`	24.6 (q)	28.0
14`	116.9 (t)	25.1 (q)
15`	17.9 (q)	16.3

*Assignments by ^{1}H-NMR, ^{13}C-NMR and NOE experiments. s= quaternary, d= methine, t= methylene, q= methyl carbons. compacted with compactional pressure of 74-372 Mpa.

appeared as a doublet signal at δ_H 4.66 (1H, d, *J*= 6.65 Hz). The two protons showed a ^{1}H-^{1}H COSY correlation with a double doublet signal at δ_H 2.00 (1H, dd, *J*= 4.9, 10.2 Hz, H-9`). An additional oxygenated proton (H-3`) was found as double doublet at δ_H 3.42 (1H, J= 11, 2 Hz). The methyl signals appeared at δ_H 1.39 (s), 1.20 (s), 1.04 (s), and 1.02 (s) were assigned to H-12`, H-13`, H-14` and H-15`, respectively. The ^{13}C-NMR and DEPT experiments (Table 1) showed signals for 24 carbon signals, four quartets at δ_C 28.7, 28.0, 25.1 and 16.3 that were attributed to C-12`, C-13`, C-14` and C-15`, respectively, five triplet (four aliphatic methylenes at δ_C 45.0, 28.0, 20.7 and 38.3 being typical for C-1`, C-2`, C-6` and C-7`, respectively and one primary alcoholic carbon at δ_C 66.8 characteristic for C-11`, eight doublets at $\delta_C$ 77.8 (H-3`), 60.7 (H-9`), 55.3 (H-5`) five of which were due to the umbelliferone moiety at δ_C 112.9 (C-3), 143.8 (C-4), 129.3 (C-5), 113.4 (C-6) and 102.0 (C-8). The downfield signal at δ_C 162.796 was assigned to the carbonyl carbon of the coumarin moiety (C-2). Assignments of all protonated carbons were made by the analysis of the HMQC and HMBC. These data suggested that **2** was derived from sesquiterpene and umbelliferone components. The stereochemistry of **2** was deduced from NOE experiments; irradiation of H-3` resulted in enhancements of H-14` and H-5` signal, whereas irradiation of H-9` resulted in enhancement of Me-12`. Additionally, irradiation of H-15` showed a clear enhancement of Me-13`, indicating that α-orientation of H-3`, H-14` and β-orientation of H-9`, H-12`, H-13` and H-15`. EIMS of compound **2** showed a molecular ion peak at *m/z* 400 consistent with the molecular formula $C_{24}H_{32}O_3$. The general feature of the data was in agreement with the samarcandin group (8-10). This is the first report of H-11` protons as one doublet and one double doublet, which suggest a new samarcandin diastreomer .

Compounds **1** and **2** were screend for their possible in vitro antibacterial activity against an assortment of two Gram-positive bacteria (*B. cereus, S. aureus*) and Gram-negative bacteria (*Serratia* sp., *Pseudomonos* sp., *E. coli*) using ampicillin and amoxillin as reference standards. The minimum inhibitory concentrations (MICs, µg/ml) were determined using standard agar dilution method (13). The MIC values are summarized in Table 2.

From the obtained data, it is clear that ferusinol possesses high activity against both Gram-positive strains, particularly *B. cereus* and Gram-negative ones. On the contrary, samarcandin diasteriomer showed higher activity against Gram-negative strains but it was inactive at tested concentrations against Gram-positive strains as shown in Table 2. Our results are in agreement with previous reports (14). Antibiotics such as ampicillin and amoxillin are bacterial cell wall synthesis inhibitors. Ferusinol may also inhibit cell wall synthesis in both Gram-positive and Gram-negative strains. On

Table 2. Antimicrobial activities of compounds **1** and **2** (dry DMSO as solvent).

Test organism	Ferusinol [c]	Samarcandin diastereomer [c]	Ampicillin [d]	Amoxillin [d]
Gram-positive strains				
Bacillus cereus	10[a] 18[b]	N[a] N[b]	10	N
Staphylococcus aureus	N[a] 5[b]	N[a] N[b]	8	N
Gram-negative strains				
Serratia sp.	10[a] 18[b]	14[a] 19[b]	11	13
Pseudomonas sp.	11[a] 18[b]	14[a] 18[b]	11	13
Escherichia coli	10[a] 17[b]	13[a] 19[b]	11	13

[a] Values show the zone of inhibition in mm.; Concentration. of the samples was 200 μg/ml.
[b] Values show the zone of inhibition in mm.; Concentration. of the samples was 400 μg/ml.
[c] Data are the mean of five measurements with neglegible standard errors.
[d] Values show the zone of inhibition in mm.; Concentration of the reference antibiotics was 200 μg/ml.
N=No effect

the other hand, samarcandin diastreomer may have similar mechanism of action, but on Gram-positive strains only. In other words, ferusinol and samarcandin diastereomer might act similar to penicillins against bacteria.

Acknowledgement

The authors thanks the Department of Botany, Faculty of Science, Beni Suef University for deposition of the voucher specimen of the plant.

References

(1) Boulus L. *Medicinal Plants of North Africa.* Algonae, MI (1998)183
(2) Appendino G, Spagliardi P, Cravotto G, Pocock V, Milligan S. Daucane phytoestrogens: a structure-activity study. *J. Nat. Prod.* (2002) 65: 1612-1615
(3) Chaudhury RR.The quest for a herbal contraceptive. *Natl. Med. J. India.* (1993) 6:199-201
(4) Gonzalez AG and Barrera JB. Chemistry and the sources of mono- and bicyclic sesquiterpenes from *Ferula* species. *Progress in the Chemistry of Organic Natural Products* (1995) 64: 1
(5) Ahmed AA and Mahmud AA. Jasonol a rare tricyclic eudesmane sesquiterpene and six other new sesquiterpenoids from *Jasonia candicans*. *Tetrahedron* (1998) 54: 8141
(6) Ahmed AA. Sesquiterpene coumarins and sesquiterpenes from *Ferula sinaica*. *Phytochem.* (1999) 50:109
(7) Appendino G, Tagliapietra S, Nano GM and Jakupovic J. Sesquiterpene coumarin ethers from asafetida. *Phytochem.* (1994) 35: 183
(8) Appendino G, Ozen CH, Mario G and Sisero M. Sesquiterpene coumarin ethers from the genus *Heptaptera*. *Phytochem.* (1992) 31: 4223
(9) Nassar IM, Abu-Mustafa AE and Ahmed AA. Sesquiterpene coumarins from *Ferula assafoetida* L. *Pharmazie* (1995) 50: 766
(10) Zedan ZI and Omar MA. Coumarins and other constituents from *Ferula sinaica* Boiss. growing in Egypt. *Bull. Pharm. Sci., Assuit University* (1996) 19: 15
(11) Ahmed AA. New sesquiterpenes from *Ferula sinaica. J. Nat. Prod.* (1990) 53: 483
(12) Ahmed AA. Daucanes and other constituents from *Ferula sinaica*. *Phytochem.* (1991) 30: 1207
(13) Steers F, Foltz FL and Graves BS. *Antibiot. Chemother.* (1959) 9: 307
(14) Joklik KW, Willett PH and Amos BD. *Zinsser Microbiology*. 20th ed. (1992) 155

This article is available online at http://www.ijpr-online.com