



# Sesquiterpene coumarins and sesquiterpenes from *Ferula sinaica*

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## Abstract

Further study on the methylene chloride extract of roots of *Ferula sinaica* has resulted in the isolation of six sesquiterpene coumarins, of which two were new, six sesquiterpenes, including a new one, and a known monoterpene. The structure of the new compounds was established by high-field NMR analysis. © 1998 Published by Elsevier Science Ltd. All rights reserved.

**Keywords:** *Ferula sinaica*; Apiaceae; Roots; Sesquiterpene coumarins; Sesquiterpenes; Monoterpene

## 1. Introduction

Members of the genus *Ferula* are widespread throughout central Asia and Afghanistan. The gum resins which are obtained by incision of roots of several species are used as spices and drugs in many countries and the resins have been reported to cure stomachic, vermifuge and carminative troubles (Boulus, 1983). Moreover, some of the isolated compounds have antifertility activities (Gunther, 1959). Recently, from roots of *F. sinaica* several daucanes, sesquiterpene coumarins and monoterpenes have been reported (Ahmed, 1990, 1991). Additionally, investigation of leaves of this species gave a new glucose derivative (Ahmed et al., 1996).

A continued study of the extract of *F. sinaica* has resulted in the isolation of seven sesquiterpene coumarins, six sesquiterpenes and a monoterpene.

## 2. Results and discussion

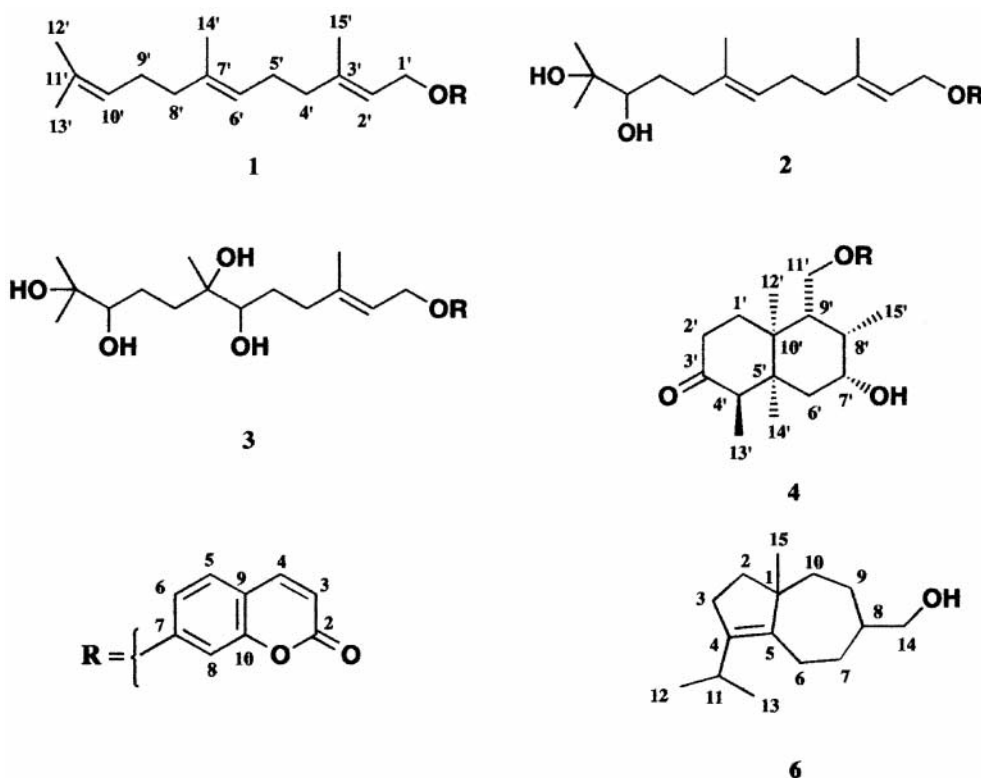
Reinvestigation of roots of *F. sinaica* allowed the isolation of two new sesquiterpene coumarins, **3** and **4**, and a new sesquiterpene, **5**, together with the already known, samarcandin (Kir'yalov & Movchan, 1968), samarcandone (Kir'yalov & Movchan, 1968), isosamarcandin (Saidkhodzhev & Malikov, 1978), *p*-ani-

sate, and the vanillate of jaeschkeanadiol (Miski & Jakupovic, 1990), the *p*-hydroxybenzoate of lancero-triol (Fraga, Gonzalez, Gonzalez, Hernandez, & Larruga, 1985), 4,8-dihydroxy-6-vanilloxyloxydauc-9-ene (Miski & Jakupovic, 1990), fercomin (Miski & Tom, 1986) and bornane (Bohlmann, Jakupovic, Dutta, & Goodman, 1980).

The structure of **2** was deduced from its mass spectrum, <sup>1</sup>H and <sup>13</sup>C spectral data. The EI mass spectrum showed a [M]<sup>+</sup> at *m/z* 400, in accordance with C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>. Comparison of <sup>1</sup>H and <sup>13</sup>C NMR data with those of umbelliprenin **1**, which has been reported from *F. assafoetida* (Nassar, Abu Mostafa, & Ahmed, 1995), suggested that **2** was a 10',11'-dihydroxy derivative of **1**. Most likely, **2** was formed by oxidation of the Δ<sup>10'</sup> double bond of **1** to form the epoxide, followed by hydrolysis. Previously, compound **2** has been reported and given the name karatavicinol (Kir'yalov & Bagirov, 1969; Appendino, Jakupovic, Aloatti, & Ballero, 1997); because no high-field <sup>1</sup>H and <sup>13</sup>C NMR data are reported in the literature, these data are included in Tables 1 and 2, respectively.

The <sup>1</sup>H (Table 1) and <sup>13</sup>C NMR (Table 2) data of **3** were very similar to those of **1** and **2**, and confirmed the hydroxylation of a second double bond. This was clear from the presence of four oxygen-bearing carbons at δ 72.1, 76.1, 84.4 and 86.5, together with two protons at δ 3.53 and 3.80, and three methyl groups at δ 1.10, 1.15 and 1.25. Furthermore, two signals, an olefinic methyl and a double bond proton, were found at δ 1.77 and 5.50, respectively. Comparison between

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the  $^1\text{H}$  NMR of **3** and **1** and **2**, located the double bond at  $\text{C}_{2'}\text{--C}_{3'}$ . The other signals were typical of a 7-hydroxycoumarin, at  $\delta$  6.23, H-3, 7.62, H-4, 7.30, H-5, 6.84, H-6 and 6.83, H-8. APT experiments allowed the assignment of the multiplicity of all carbons. The

structure of **3** was supported by the EI mass spectrum which gave a  $[\text{M}]^+$  at  $m/z$  434 ( $\text{C}_{24}\text{H}_{34}\text{O}_7$ ).

The structure of **4** was proved to be a bicyclic sesquiterpene coumarin from elemental analysis,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, HMQC and HMBC. In the IR spectrum, absorption bands at 3480, 1750 and  $1730\text{ cm}^{-1}$  were in accordance with the presence of a hydroxyl and two carbonyl functions. The  $^1\text{H}$  NMR of **4** showed four three-proton signals, two being due to tertiary methyl groups at  $\delta$  0.68 and 1.01, and two doublets at  $\delta$  1.03 and 1.08 in accordance with two secondary methyl groups. Moreover, a down-field signal appeared at  $\delta$  3.60, which showed correlation with the carbon signal at  $\delta$  48.4 in the HMQC. This quartet exhibited a cross-peak with a three-proton doublet at  $\delta$  1.03 in the  $^1\text{H}\text{--}^1\text{H}$  COSY. Additionally, the down-field chemical shift,  $\delta$  3.60, was consistent with the presence of a carbonyl carbon function as a neighbor to this proton. This was established from the correlation between the carbonyl carbon at  $\delta$  214.1 with the signals at  $\delta$  3.60, H-4', 2.60, H-2', 1.97, H-1', and the three-proton signal at  $\delta$  1.03, H-13', in the HMBC (Table 3). The remaining correlation supported the structure of **4** (Table 3). The location of a hydroxyl function and a secondary methyl at C-7 and C-8 could be proved by  $^1\text{H}\text{--}^1\text{H}$  COSY. The relative stereochemistry of the asymmetrical centers of **4** were confirmed by NOESY; H-11' showed correlation with H-15', H-7' with H-13', H-4' with H-2 $\alpha$ , at  $\delta$  2.60 and H-15' with H-6 $\alpha$ , at  $\delta$  1.88. The EI mass spectrum of **4** revealed  $[\text{M}]^+$  at  $m/z$

Table 1  
 $^1\text{H}$  NMR of compounds **2–4** (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ -values)

Protons	2	3	4
H-3	6.21 d (9.5)	6.23	6.22
H-4	7.62 d (9.5)	7.62	7.62
H-5	7.30 d (8.5)	7.35	7.36
H-6	6.80 dd (8.5, 2.5)	6.84	6.81
H-8	6.75 d (2.5)	6.83	6.80
H-1'	4.57 d (6)	4.60	1.97 ddd (15, 5, 3), 1.78 ddd (15, 15, 5)
H-2'	5.45 br t (6)	5.50	2.60 ddd (15, 15, 5), 1.98 ddd (15, 6, 3)
H-4'			3.60 q (6.5)
H-6'	5.14 br t (6)	3.80 t	1.88 dd (15.5, 3.5), 1.54 dd (15.5, 3.5)
H-7'	—	—	3.93 ddd (3.5, 3.5, 3.5)
H-8'	—	—	2.09 m
H-9'	—	—	2.74 ddd (10, 4, 3.5)
H-10'	3.35 dd (10, 2)	3.53	—
H-11'	—	—	4.16 dd (10, 4), 4.10 dd (10, 3.5)
H-12'	1.16 s	1.10	1.01 s
H-13'	1.19 s	1.15	1.03 d (6.5)
H-14'	1.73 br s	1.25 s	0.68 s
H-15'	1.60 br s	1.77	1.08 d (7)

Table 2  
<sup>13</sup>C NMR of compounds 2–4 (100 MHz, CDCl<sub>3</sub>, δ-values)<sup>a</sup>

Carbons	2	3	4 <sup>b</sup>	Carbons	2	3	4 <sup>b</sup>
1'	17.0 q	17.9	33.2 t	2	162.0 s	162.5	162.7
2'	118.3	118.8	37.9 t	3	112.4 <sup>c</sup> d	114.0 <sup>d</sup>	112.9
3'	144.0 s	143.0	214.1 s	4	144.0 d	144.0	143.3
4'	36.7 t	36.3	48.4 d	5	128.9 d	129.9	128.8
5'	30.0 t	32.0	44.9 s	6	113.0 <sup>c</sup> d	114.0 <sup>d</sup>	113.0
6'	124.1 d	84.4	37.7 t	7	161.3 s	162.0	161.9
7'	135.6 s	86.5	72.1 d	8	101.5 d	101.9	101.3
8'	39.2 t	30.0	34.3 d	9	156.0 s	156.0	156.4
9'	26.2 t	27.5	37.7 d	10	112.5 s	112.8	112.8
10'	78.0 d	76.1	40.2 s				
11'	73.0 s	72.1	67.9 t				
12'	23.8 q	24.0	19.0 q				
13'	17.0 q	17.9	18.2 q				
14'	16.3 q	26.0	8.8 q				
15'	26.1 q	28.0	16.4 q				

<sup>a</sup>Multiplicities deduced from DEPT.

<sup>b</sup>Assignments confirmed by HMQC and HMBC.

<sup>c,d</sup>Signals may be interchangeable.

398, C<sub>24</sub>H<sub>30</sub>O<sub>5</sub>. Compound 4 seems to be a rare sesquiterpene coumarin in which one of the geminal methyls at C-4' has been shifted to C-5'. Two similar compounds have been reported from *F. assafoetida* and *F. microcarpa* (Nabier & Malikov, 1983; Hofer, Wihalm, & Greger, 1984).

The <sup>1</sup>H and <sup>13</sup>C NMR of compound 5 indicated the presence of a sesquiterpene skeleton. DEPT experiments exhibited 15 non-equivalent carbons, which could be classified as three quaternary carbons, of which two were olefinic, a primary alcoholic, six aliphatic methylenes, two aliphatic methine and three methyls. Furthermore, the <sup>1</sup>H NMR showed the primary alcoholic protons as a part of an ABX system at δ 3.40 and 3.37. Spin-decoupling of these protons collapsed a multiplet at δ 1.51, which was assigned for H-8. Moreover, irradiation of the methyl doublets at either δ 1.05 or 0.92 collapsed the downfield signals at δ 2.63, and, thus, they were assigned to H-11. The down-field shift of H-11, with no further coupling, confirmed the presence of a Δ<sup>4</sup>-double bond. Further spin-decoupling allowed the assignments of all signals (see Section 3). The EI mass spectrum showed a [M]<sup>+</sup>

at *m/z* 222 (C<sub>15</sub>H<sub>26</sub>O), followed by elimination of a methyl group at *m/z* 207 and further loss of an *iso*-propyl group at *m/z* 179. Few sesquiterpenes with a Δ<sup>4</sup>-double bond have been reported from the genus *Ferula* (Fraga et al., 1985; Miski & Mabry, 1986).

### 3. Experimental

#### 3.1. Plant material

Roots of *F. sinaica* Bioss were collected from the North Sinai peninsula, in April 1990. A voucher specimen (A. Ahmed 110) is deposited in the Department of Botany, El-Minia University.

#### 3.2. Extraction

Dried and coarsely powdered roots (3 kg) were extracted with CH<sub>2</sub>Cl<sub>2</sub> at room temp for 24 h and gave 300 g of a viscous oil. The extract was flash chromatographed over a silica gel column (1 kg), eluting with *n*-hexane with increasing amounts of CH<sub>2</sub>Cl<sub>2</sub>. Fr. A (*n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>, 1:1) was further chromatographed over Sephadex LH-20 (*n*-hexane–EtOAc, 1:1) to give 9 mg of samarcandone, 3 mg of isosamarcandin, 12 mg of the *p*-anisate of jaeschkeanadiol and 2 mg of bornane. Fr. B (*n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>, 1:3, 2 l) was further chromatographed over Sephadex LH-20 (*n*-hexane–CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 5:3:0.5) to afford 6 mg of samarcandin (70 mg), 6 mg of the vanillate of jaeschkeanadiol, 16 mg of the *p*-hydroxybenzoate of lancero-triol, 3 mg of fercomin and 1.5 mg of compound 4. Fr. C (CH<sub>2</sub>Cl<sub>2</sub>) was separated by TLC (silica gel,

Table 3  
 HMBC of compound 4

Carbon irradiated	Proton correlated
C-3'	H-4', H-1', H-2', H-13'
C-10'	H-14', H-11', H-6'
C-4'	H-14', H-13', H-6'
C-7'	H-15', H-6'
C-8'	H-15', H-11', H-6'
C-5'	H-14', H-13', H-12', H-7', H-4'

$\text{CH}_2\text{Cl}_2$ –MeOH, 7.0:0.5) to give 4 mg of 4 $\beta$ ,8 $\alpha$ -dihydroxy-6 $\alpha$ -vanilloyloxydauc-9-ene and 17 mg of compound **6**. The polar fr. D ( $\text{CH}_2\text{Cl}_2$ –MeOH, 10:1), was separated by TLC (silica gel,  $\text{CH}_2\text{Cl}_2$ –MeOH, 7.0:0.5) to give 10 mg of karatavicol **2** and 15 mg of 6',7'-dihydroxykaratavicol **3**. Known compounds were identified by comparison of their IR, MS and  $^1\text{H}$  NMR with those reported in the lit.

### 3.3. Karatavicol (**2**)

IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3500, 3050, 2900, 1630.  $[\alpha]_{\text{D}}^{24^\circ\text{C}}$ : +2.7° ( $c$  0.6,  $\text{CHCl}_3$ ). EIMS  $m/z$  (rel. int.): 400  $[\text{M}]^+$  (30), 382  $[\text{M}-\text{H}_2\text{O}]^+$  (21), 364  $[\text{M}-2\text{H}_2\text{O}]^+$  (50), 239  $[\text{M}-\text{coumarin}]^+$  (60), 162  $[\text{M}-\text{sesquiterpene}]^+$  (100).

### 3.4. 6,7-Dihydroxykaratavicol (**3**)

IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3550, 3530, 2850.  $[\alpha]_{\text{D}}^{24^\circ\text{C}}$ : +4.1° ( $c$  1.1,  $\text{CHCl}_3$ ). EIMS  $m/z$  (rel. int.): 434  $[\text{M}]^+$  (3), 416  $[\text{M}-\text{H}_2\text{O}]^+$  (20), 398  $[\text{M}-2\text{H}_2\text{O}]^+$  (35), 273  $[\text{M}-\text{coumarin}]^+$  (60), 162  $[\text{M}-\text{sesquiterpene}]^+$  (100).

### 3.5. Compound (**4**)

IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3480, 2790, 1760. EIMS  $m/z$  (rel. int.): 398  $[\text{M}]^+$  (5), 380  $[\text{M}-2\text{H}_2\text{O}]^+$  (12), 237  $[\text{M}-\text{coumarin}]^+$  (50), 162  $[\text{M}-\text{sesquiterpene}]^+$  (100).

### 3.6. 14-Hydroxy-dauc-4-ene (**5**)

IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  ( $\text{cm}^{-1}$ ): 3520, 1710, 1600, 1260.  $[\alpha]_{\text{D}}^{24^\circ\text{C}}$ : +3.7° ( $c$  1.6,  $\text{CHCl}_3$ ). EIMS  $m/z$  (rel. int.): 222  $[\text{M}]^+$  (20), 207  $[\text{M}-\text{CH}_3]^+$  (50), 179  $[\text{M}-\text{iso-propyl}]^+$  (100), 161  $[\text{M}-\text{H}_2\text{O}]^+$  (35).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.40 (1H, dd,  $J = 6$  and 10 Hz, H-14), 3.37

(1H, dd,  $J = 7$  and 10 Hz, H-14'), 2.63 (1H, qq,  $J = 7$  and 7 Hz, H-11), 2.43 (1H, ddd,  $J = 3$ , 5 and 14 Hz, H-6), 2.17 (1H, m, H-3'), 2.10 (1H, m, H-3'), 1.87 (2H, m, H-6) 1.51 (1H, m, H-8), 1.05 (3H, d,  $J = 7$  Hz, H-12), 0.92 (3H, d,  $J = 7$  Hz, H-13).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (C-1–C-15): 49.9 (s), 33.9\* (t), 39.2\* (t), 141.5 (s), 140.5 (s), 36.2 (t), 27.1 (t), 46.4 (d), 26.3 (t), 23.1 (t), 26.7 (d), 25.5 (q), 21.9 (q), 68.9, 21.1 (q).

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