

## COUMARINS AND OTHER CONSTITUENTS FROM *FERULA SINAICA* BOISS GROWING IN EGYPT

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تم في هذا البحث استكمال دراسة محتوى الكلوروفورم لنبات الفريولا سينايا حيث تم فصل والتعرف على مركب سيسكيتربينى-كومارينى جديد ومركب من نوع الفينيل بروبانويد جديد هو ٥،٢ ثنائى ميثوكسى-٤،٣ إيثيلين ثنائى أوكسى بروبانوفينون. هذا بالإضافة إلى خمسة مركبات معروفة هي ٥-ميثوكسى-٤،٣-إيثيلين ثنائى أوكسى بروبانوفينون ، فيسيلول ، ايزوسمركاندين ، مارمارسين ، لابيقرين. وقد تم التعرف على المركبات المفصولة باستخدام طرق التقنية الحديثة متمثلة فى الأشعة دون الحمراء والرنين النووى المغناطيسى الهيدورجيني والكربونى بأنواعه ومطياف الكتلة.

*Reinvestigation of the constituents of the dried roots of Ferula sinaica Boiss resulted in the isolation of a new sesquiterpene-coumarin together with three known sesquiterpene-coumarins, two phenylpropanoids one of them is new and one carotane sesquiterpene. The identification of the isolated compounds was carried out using spectral analysis including IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, 2D <sup>1</sup>H-<sup>1</sup>H COSY and MS.*

### INTRODUCTION

*Ferula sinaica* Boiss is a wild desert plant belonging to the Family *Umbelliferae* (*Apiaceae*). The genus *Ferula* represented in Egypt by only two species viz *F. sinaica* Boiss and *F. marmarica* Asch and Taub<sup>1</sup>. The genus *Ferula* has many folkloric uses, as carminative, intestinal antiseptic, laxative, antispasmodic, stimulant, emmenagogue, anthelmintic, for hypochondriasis, expectorant, antirheumatic, for scorpion stings, in hysteria, tumours, boils, swellings, nervous system disorders and as flavouring for foods<sup>2-12</sup>.

The phytochemical study on *F. sinaica* Boiss resulted in the isolation and identification of sesquiterpenes and coumarins<sup>13-17</sup>. In our previous study on this plant, ten carotane sesquiterpenes were isolated<sup>18</sup>. The present communication describes the isolation and identification of four sesquiterpene coumarins, one carotane sesquiterpene and two phenylpropanoides from the dried roots of the titled plant.

### EXPERIMENTAL

#### I- Plant material

The fresh roots of *F. sinaica* Boiss were collected from Sainai around the Arish-Ismallia road during flowering in March 1992. The plant was authenticated by Prof. Dr. A. Faid, professor of plant taxonomy, Faculty of Science, Assiut University. The fresh roots were cutted into slices, air-dried, powdered and sieved.

#### II- Instruments

- 1- Digital melting point, electrothermal 9100, all mp are uncorrected.
- 2- IR spectrophotometer, Pye Unicam sp 1000, using KBr.
- 3- NMR Spectrophotometer, Brucker 400 A (400 MHz for <sup>1</sup>H- and 100 MHz for <sup>13</sup>C-NMR), using CDCl<sub>3</sub> as a solvent and TMS as an internal standard.
- 4- MS, Hitachi M-80 spectrophotometer 70 e.v. (Japan).
- 5- Moderate pressure liquid chromatography (CIG column system, 22mm i.d. x 30 cm, Kusano Sci Co.) prepacked with RP-18

(R 18-37, Fuji Gel, Hanbai, LTD,  
Tokyo, Japan).

#### Extraction and isolation

About 3.8 Kg of the air-dried powdered roots of *Ferula sinaica* Boiss were extracted with chloroform as mentioned<sup>18</sup>. 10 g of the dried chloroformic extract was fractionated over silica gel column (430 g) using gradient elution with benzene-methanol mixture. Fractions 100 ml each were collected, concentrated and monitored by TLC. 10% H<sub>2</sub>SO<sub>4</sub> was used for location of the spots over the chromatoplates.

Group (1), (frs 3-10) eluted with benzene alone upon repeated column chromatography using CIG column system and RP-18 as adsorbant, with MeOH-H<sub>2</sub>O (80:20 and 75:25), fractions 10 ml each were collected.

Fractions 6-10 yielded compound 1, further purification of fractions (13-15) yielded compound 2, while fractions 19-24 yielded compound 5.

Group (2), (frs 13-18) eluted with 1% methanol in benzene afforded a mixture of coumarins, upon repeated purification using CIG column system over prepacked RP-18 column with MeOH-H<sub>2</sub>O (85:15 and 80:20), fractions each 10 ml were collected.

Fractions 4-9 eluted with MeOH-H<sub>2</sub>O (85:15) upon repeated crystallization from methanol afforded compound 3, fractions 11-16 eluted with MeOH-H<sub>2</sub>O (80:20) yielded compound 4 while fractions 19-22 yielded compound 6.

Group (3), (frs 20-24) eluted with 2% methanol in benzene. On repeated column chromatography over silica gel with hexane-ethyl acetate (85:15), fractions 25 ml were collected. Fractions (10-14) afforded compounds 7 while fractions (18-20) yielded compound 8. Compound 8 was isolated in a small amount beyond the need for complete analysis.

**Compound (1)**, obtained as colourless gum (33 mg). IR  $\nu_{\max}^{\text{KBr}}$  3010, 2960, 2850, 1672, 1605, 1510, 1250, 1210, 1070 and 1010 cm<sup>-1</sup>. 400 MHz <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  7.01, (1H, s, H-

6), 6.04 (2H, s, O-CH<sub>2</sub>-O), 3.97 (3H, s, OCH<sub>3</sub>), 3.86 (3H, s, OCH<sub>3</sub>), 2.93 (2H, q, J= 7.2 Hz) and 1.15 (3H, t, J= 7.1 Hz). 100 MHz <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  200.94 (s, C=O), 140.34 (s, C-2), 139.90 (s, C-5), 139.29 (s, C-4), 138.96 (s, C-3), 125.06 (s, C-1), 108.35 (d, C-6), 102.43 (t, OCH<sub>2</sub>O), 60.17 (q, OCH<sub>3</sub>), 56.59 (q, OCH<sub>3</sub>), 36.60 (t, CH<sub>2</sub>-CH<sub>3</sub>), 8.55 (q, CH<sub>2</sub>-CH<sub>3</sub>). EIMS, m/z (% rel.) 238 (18), 223 (2), 209 (28), 207 (23), 195 (48), 181 (16), 180 (4) and 167 (18).

**Compound (2)**, This compound was identified as 5-methoxy-3,4-methylenedioxypropinophenone isolated from *F. sinaica* grown in Saudi Arabia by comparing its spectral data with those reported<sup>16</sup>. This is the first report for the isolation of this compound from *F. sinaica* Boiss growing in Egypt.

**Compound (3)**: Fine needles (46 mg), m.p. 199-201 °C (methanol), IR  $\nu_{\max}^{\text{KBr}}$  3450, 3010, 2980, 2870, 1685, 1610, 1595, 1482, 1255, 1205, 1150 cm<sup>-1</sup>. EIMS m/z 400 [M<sup>+</sup>], 383, 345, 317, 302, 273, 203, 175, 162, 134, 121, 105, 91, 77, 55 and 43. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) coumarin moiety  $\delta$  7.61 (1H, d, J=9.5 Hz, H-4), 7.34 (1H, d, J=8.6 Hz, H-5), 6.89 (1H, d, J=2.4 Hz, H-8), 6.83 (1H, dd, J=2.4 and 8.6 Hz, H-6), 6.23, (1H, d, J=9.5 Hz, H-3), terpenoidal moiety  $\delta$  4.36 (1H, dd, J= 10.0 and 5.5 Hz, H-11'A), 4.17 (1H, dd, J=10.0 and 5.5 Hz, H-11'B), 3.25 (1H, dd, J= 11.0 and 5.0 Hz, H-3'), other protons appeared between  $\delta$  2.00 and 0.90. The methyl signals appeared at  $\delta$  1.23, 1.05, 0.99 and 0.81 (3H, s, each). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>) see Table 1.

**Compound 4**: Very fine needles (32 mg), m.p. 112-114 °C (methanol-chloroform 1:4). The physical and spectral data of compound (4) are similar to those reported for Feselol<sup>4</sup>, which was isolated from this plant<sup>14</sup>.

**Compound 5**: (28 mg), this compound was identified as Isosamarcandin previously isolated from this plant<sup>14</sup>.

Table 1: <sup>13</sup>C-NMR of compound (3).

Carbon No.	δ (ppm)	Carbon No.	δ (ppm)
2	161.71	1'	38.07
3	113.22	2'	27.09
4	143.32	3'	78.44
5	128.72	4'	38.86
6	113.14	5'	54.80
7	155.89	6'	20.01
8	101.66	7'	44.07
9	161.13	8'	72.53
10	122.69	9'	54.32
		10'	37.83
		11'	66.59
		12'	15.48
		13'	28.22
		14'	24.73
		15'	16.14

**Compound 6:** (40 mg) this compound was identified as Marmarin by comparing its physical and spectral data with those reported<sup>33</sup>. This is the first report for the isolation of this compound from *F. sinaica* Boiss.

**Compound 7:** (28 mg), it was identified as Lapiferin previously isolated from *F. linkii* by comparing its spectral data with those reported<sup>34</sup>. This is the first report on the isolation of this compound from the titled plant.

## RESULTS AND DISCUSSION

Preliminary phytochemical screening of the dried roots of *F. sinaica* Boiss revealed the presence of terpenes, sterols, coumarins, lactones and/or esters and flavonoids. Column chromatography of the chloroform fraction resulted in the isolation and identification of two phenylpropanoids (1,2), four terpenoidal-coumarins (3-6) and one carotane sesquiterpene (7).

**Compound (1):** The IR spectrum of compound (1) showed the presence of aromatic system (3010, 1605 and 1510 cm<sup>-1</sup>) and conjugated ketone group (1672 cm<sup>-1</sup>), but it does

not show any hydroxyl absorption band. MS showed M<sup>+</sup> at m/z 238 consistent with the molecular formula C<sub>12</sub>H<sub>14</sub>O<sub>5</sub>, other fragmentation peaks at m/z 223, 209 and 207 for the loss of CH<sub>3</sub>, CH<sub>2</sub>-CH<sub>3</sub> and OCH<sub>3</sub> respectively.

400 MHz <sup>1</sup>H-NMR (CDCl<sub>3</sub>) showed the presence of signals characteristic for propanone at δ 1.15 (3H, t, J= 7.1 Hz) and 2.94 (2H, q, J= 7.2 Hz), two methoxy signals at δ 3.97 and 3.86 (3H, s, each) and signal for methylenedioxy at δ 6.04 (2H, s) in addition to an aromatic proton at δ 7.04 (1H, s), which indicate the presence of penta substituted benzene ring. The appearance of the aromatic proton at δ 7.04 indicate that this aromatic proton present between a carbonyl and oxygen function group<sup>10,19</sup>.

100 MHz <sup>13</sup>C-NMR aided with DEPT spectra (CDCl<sub>3</sub>) showed the presence of twelve carbon signals i.e. six >C<, one >CH-, one >CH<sub>2</sub>, one O-CH<sub>2</sub>-O, one -CH<sub>3</sub> and two -OCH<sub>3</sub>. The signals at δ 200.94 (s), 36.60 (t) and 8.55 (q) were assigned to the propanone group, while the signals at δ 102.43, 60.17 and 56.59 were interpreted to the methylenedioxy and two methoxy groups. The other carbons were assigned as cited in experimental. From all the above mentioned data compound 1, was identified as 2,5-dimethoxy-3,4-methylenedioxypropinophenone. The 2-hydroxy-5-methoxy-3,4 methylene- dioxypropinophenone was isolated from *F. latipinna*<sup>20</sup> but this is the first report on the isolation of compound (1) from natural source.

**Compound (3):** Obtained as needle crystals (methanol). It is soluble in hot alkali and reprecipitate upon acidification. It showed IR absorptions for hydroxy group at 3450 cm<sup>-1</sup>, α-pyrone at 1685 cm<sup>-1</sup>, aromatic system at 1610 and 1595 cm<sup>-1</sup> and C-O-C ether linkage at 1225, 1205 and 1150 cm<sup>-1</sup>. Its MS showed M<sup>+</sup> at m/z 400 consistent with the molecular formula C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>. Moreover, fragmentation peaks were observed at m/z 383 (M<sup>+</sup>-OH), 382 (M<sup>+</sup>-H<sub>2</sub>O), 221 [(M<sup>+</sup>-H<sub>2</sub>O)-hydroxycoumarin], 203 [(M<sup>+</sup>-2H<sub>2</sub>O)-hydroxy- coumarin], 162 (for 4-hydroxy coumarin), and at 134 and 107 (for the successive loss of CO<sub>2</sub>), a phenomenon characteristic for coumarins<sup>21</sup>

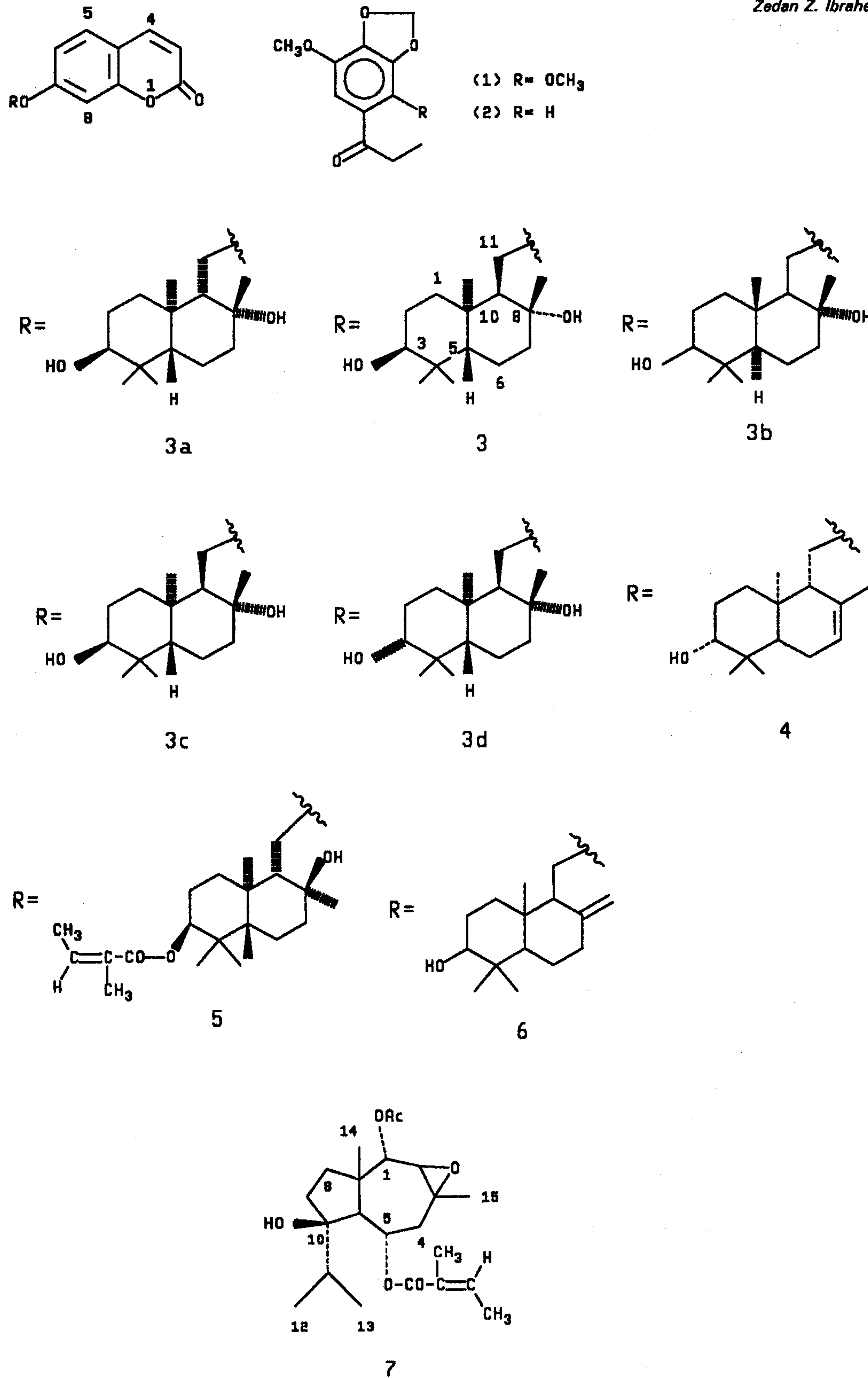


Fig. 1: Compounds isolated from *F. sinaica* Boiss.

<sup>1</sup>H-NMR aided with 2D <sup>1</sup>H-<sup>1</sup>H COSY showed two signals at  $\delta$  7.61 and 6.23 (each 1H, d,  $J=9.5$  Hz) assigned to H-4 and H-3 while the signals at  $\delta$  7.34 (1H, d,  $J=8.6$  Hz), 6.89 (1H, d,  $J=2.4$  Hz) and 6.83 (1H, dd,  $J=2.4$  and 8.6 Hz) were assigned to H-5, H-8 and H-6 of the coumarin moiety, respectively. The pair of doublet at  $\delta$  4.17 and 4.36 (1H, dd,  $J=10$  and 5.5 Hz, each) and the signal at  $\delta$  3.25 (1H, dd,  $J=11.0$  and 5.0 Hz) were assigned to C-11' methylene protons and H-3' of the terpenoidal moiety. The methyl signals appeared at  $\delta$  1.23, 1.05, 0.99 and 0.81 indicate that compound (3) has  $\alpha$ -CH<sub>3</sub> at C-10' and  $\beta$ -H at C-5'<sup>22</sup>.

The <sup>1</sup>H-NMR of compound (3) is similar to those reported for feshurin (3a) isolated from *F. schtschurowskiana*<sup>23</sup>, Compound (3b) isolated from *F. galbaniflua* Boiss<sup>24</sup>, fepaldin (3c) isolated from *F. pallida*<sup>25</sup> and ferukrin isolated from *F. krylovii* (3d)<sup>26</sup>. All of these compounds have the same molecular formula C<sub>24</sub>H<sub>32</sub>O<sub>5</sub> and the same coumarin part and differ in the configuration at C-3', C-5', C-8', C-9' and C-10' of the terpenoid moiety. Compound (3) differ from (3a) in the configuration at C-9', so, the methylene protons of C-11' in compound (3) appeared as a part of ABX system at  $\delta_A$  4.36  $\delta_B$  4.17 ( $J_{AB}=10.0$ Hz,  $J_{AX}=J_{BX}=5.5$  Hz) while in compound (3a) and similar C-11' equatorial methylene as (3b) and (3c) appeared as a doublet around  $\delta$  4.17 ( $J=5.5$  Hz)<sup>22,27</sup>. The axial position of the -CH<sub>2</sub>O- is clear from the chemical shift of the corresponding CH<sub>2</sub>O-, the differences in the chemical shift ( $\Delta AB=0.19$  ppm) and this is a typical for axial -CH<sub>2</sub>O-Ar moiety, for the corresponding equatorial,  $\Delta\delta$  is generally  $< 0.1$  ppm<sup>28-30</sup>. Also compound (3) differ from (3b) in the configuration of the C-3' hydroxyl group and differ from compound (3c) in the configuration of the CH<sub>3</sub> and -OH groups at C-8' (Fig. 1). Compound (3) differ from (3d) in the configuration at C-3'. The hydroxyl group at C-3' in (3) was deduced to be  $\beta$ -equatorial<sup>22</sup>.

The <sup>13</sup>C-NMR aided with DEPT experiments (Table 1) showed 24 carbon signals, i.e. 4 quartet, 5 triplet, 8 doublet and 7 singlet carbon signals and this further confirmed the terpenoidal-coumarin structure of compound (3) and support the molecular formula (C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>). The chemical shifts of the coumarin moiety are

in a good agreement with those reported<sup>31,32</sup>

For the terpenoidal moiety, the signal at  $\delta$  78.44 was assigned to C-3' carrying the hydroxyl group which is probably equatorial (the axial hydroxylic function at C-3' is upfield shifted by *ca* 2.8 ppm)<sup>22</sup>. Also, the appearance of C-15' at  $\delta$  16.10 and C-5' at  $\delta$  54.80 as in compound (3a) indicate that compound (3) has  $\alpha$ -CH<sub>3</sub> at C-10' and  $\beta$ -H at C-5', while compound (3b) has  $\beta$ -CH<sub>3</sub> at C-10' and  $\alpha$ -H at C-5'.

From all the above mentioned data, and by comparing the data of compound (3) with those reported for compounds (3a-3d), it is clear that compound (3) is an epimer of feshurin (1b) at C-9'.

The known compounds were identified as 5-methoxy-3,4-methylenedioxypropinophenone (2)<sup>(16)</sup>, feselol (4)<sup>(4)</sup>, isosamarcandin (5)<sup>(14)</sup>, marmarin (6)<sup>(33)</sup> and lapiferin (7)<sup>(34)</sup> by comparing their physical and spectral data with those reported

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