Ferulsinaic acid, a sesquiterpene coumarin with a rare carbon skeleton from *Ferula* species

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Abstract

Fractionation of methylene chloride extracts of the resin of *Ferula vesceritensis* and *F. sinaica* afforded three new sesquiterpene coumarins and a new glucose derivative. One of them was a sesquiterpene with a rare carbon skeleton. The structures of these compounds were determined by extensive NMR studies, including DEPT, COSY, NOE, HMQC, and HMBC.

Keywords: *Ferula vesceritensis*; *Ferula sinaica*; Apiaceae; sesquiterpene coumarins; glucose derivative.

1. Introduction

The exclusively old-world genus *Ferula* belonging to the family Apiaceae has some 130 species distributed throughout the Mediterranean area and Central Asia. These plants are often used as spices and in the preparation of local drugs. The resins are reported to be used for stomachic disorders such as a febrifuge and carminative agent (Boulus, 1983). Some species are used in traditional medicine for the treatment of skin infections (Appendino et al., 2002) and hysteria (Boulus, 1983). Previous work on members of this genus revealed that the main constituents are sesquiterpenes and sesquiterpene coumarins (Gonzalez and Barrera, 1995; Appendino et al., 1997; Kojima et al., 1999, 2000; Chen et al., 2000; Su et al., 2000; Murray, 1989; Ahmed, 1999; Nagatsu et al., 2002; El-Razek et al., 2003). Some compounds isolated from *Ferula* species (e.g. *F. communis* L.) show poisonous effects due to prenylcoumarins, which mainly affect sheep and goats, cattle, and horses (Rubiolo et al., 2006). For *F. sinaica*, extracts inhibited the spontaneous movements of rabbit jejunum and guinea pig ileum and acetylcholine-induced contractions. Extracts also inhibited the contractions of rabbit tracheal smooth muscle induced by acetylcholine stimulation and the contractions of guinea pig tracheal smooth muscle induced by histamine stimulation (Aqel et al., 1991).

The biological importance of members of this genus prompted us to investigate the roots of *F. vesceritensis* Coss et Dur, previously not chemically investigated, to afford two new sesquiterpene coumarins (**1** and **2**). Also, re-investigation of the roots of *F. sinaica* L. yielded a new sesquiterpene coumarin **3** (named as ferulsinaic acid), an enantiomer **4** of samarcandone and a glucose derivative (**5**), in addition to the known compounds coladin (**6**) and coladonin (**7**) (Ban'kovskii et al., 1970; Appendino et al., 1997), feselol (**8**) (Ahmed 1990), lancerodiol *p*-hydroxybenzoate (**9**) (Fraga et al., 1985) and jaeschkeanin (**10**) (Diab et al., 2001).

2. Results and Discussion

Compound **1** was assigned a molecular formula of $C_{24}H_{30}O_5$ by HRFABMS (*m*/*z* 399.2165). The structure of **1** was established from analysis of its ^{1}H NMR (Table 1) and ^{13}C NMR spectra (Table 2). In the ^{1}H NMR spectrum, umbelliferone protons were found at δ_H 6.18 (H-3), 7.60 (H-4), 7.31 (H-5), 6.77 (H-6) and 6.75 (H-8). The sesquiterpene moiety showed an olefinic proton at δ_H 5.44 (H-7′), olefinic methyl at δ_H 1.60, (H-12′), a sharp singlet signal that integrated as six protons at δ_H 0.87 (H-14′, H-15′), an oxygenated methine proton at δ_H 3.59, and two oxygenated methylene groups at δ_H 3.94 (H-11′a), 4.10 (H-11′b), 3.30 and 3.56. Compound **1** displayed 24 carbon signals, with nine of these typical for the umbelliferone skeleton and the other 15 assigned to the sesquiterpene moiety. DEPT experiments classified the protonated carbon signals to three methyls at δ_C 15.3, 11.2 and 21.4, three aliphatic methylenes at δ_C 26.3, 23.2 and 37.4, two primary alcoholic carbons at δ_C 66.8 and 70.2, and seven methines, five of them in the umbelliferone moiety, at δ_C 113.2 (C-3), 143.6 (C-4), 128.7 (C-5), 112.2 (C-6), 101.3 (C-8), 43.4 (C-5′) and 53.5 (C-9′). The presence of only three tertiary methyl groups in **1** at δ_H 1.60 (H-12′), 0.87 (H-14′) and 0.87 (H-15′), in addition to the presence of a primary alcoholic proton at δ_H 3.30 and 3.56, suggests that the fourth tertiary methyl is hydroxylated. The position of the hydroxylated methyl group was determined on the basis of HMQC and HMBC spectra. In the HMBC experiment, the two proton signals at δ_H 3.56 (H-13′a) and 3.30 (H-13′b) showed clear correlations with the carbon signals at δ_C 75.8 (C-3′), 43.4 (C-5′) and 11.2 (C-14′), while the carbon signal at δ_C 70.2 (C-13′) showed a correlation with the proton signal at δ_H 3.60 (H-3′). Other important correlations were observed between the carbon signals at δ_C 37.4 (C-1′), 53.5 (C-9′) and 35.7 (C-10′) and the proton signal at δ_H 0.87 (H-15′); the carbon signals at δ_C 123.2 (C-7′), 132.3 (C-8′) and 53.5 (C-9′) showed correlation with δ_H 1.60 (H-12′); and the carbon signal at δ_C 21.4 (C-12′) showed correlation with the proton signals at δ_H 2.01 (H-9′) and 5.44 (H-7′). Therefore, the hydroxylated methyl was placed at C-4′. Acetylation of **1** afforded the diacetyl derivative (**1a**), for which the

^{1}H NMR spectrum showed two new acetyl signals at δ_H 2.03 and 2.05, supported by HRFABMS, which showed an ion peak at 483.2377. In addition, downfield shifts were observed in the ^{1}H NMR spectrum of **1a**: H-3′ to δ_H 4.82 compared to δ_H 3.60 in **1a**, and H-13′ to 3.74/3.81 compared to 3.30/3.56 in **1**. The other proton and carbon signals were closed to those of **1** (Tables 1 and 2). NOE correlations were observed between H-3′/H-13′, H-5′/H-9′, H-5′/H-13′, H-11′/H-15′, and H-14′/H-15′ (Fig. 1), indicating the β-orientation of H-3′, H-5′, H-9′ and H-13′, and the α-orientation of H-11′, H-14′ and H-15′ in **1**. Therefore, compound **1** was identified as 13-hydroxyfeselol, a new natural compound. This is the first report of a sesquiterpene coumarin ether of the hydroxymethyl type (Appendino et al., 1992) from the genus *Ferula*.

The IR spectrum of compound **2** showed absorption bands for two carbonyl groups at 1736 and 1712 cm^{-1}. FABMS showed an $[M+H]^+$ ion peak at *m*/*z* 465, which, together with ^{1}H, ^{13}C NMR and DEPT spectral data (Tables 1 and 2), suggests a molecular formula of $C_{29}H_{36}O_5$ for **2**, supported by the HRFABMS ion peak at *m*/*z* 465.2646 ($[M+H]^+$). The ^{1}H and ^{1}H-^{1}H COSY of **2** showed the presence of an umbelliferone skeleton, including signals at δ_H 6.17 (H-3), 7.56 (H-4), 7.28 (H-5), 6.79 (H-6) and 6.74 (H-8). In addition, the sesquiterpene moiety was determined from the exomethylene protons at δ_H 4.85 (H-12′a) and 4.46 (H-12′b), the primary alcoholic protons at δ_H 4.14 (H-11′a) and 4.09 (H-11′b), a secondary alcoholic proton at δ_H 4.56 (H-3′) and three methyl groups at δ_H 0.86 (H-13′), 0.87 (H-14′) and 0.81 (H-15′). The ^{1}H NMR spectrum also exhibited signals typical for an angelate moiety at δ_H 5.98 (H-3″), 1.93 (H-4″) and 1.78 (H-5″). Table 1 lists the other protons were assigned by ^{1}H-^{1}H COSY. The ^{13}C NMR spectrum of **2** showed 29 carbon signals that were classified by DEPT and HMQC as: carbonyl esters at δ_C 167.7 and 161.2, an oxygenated methylene at δ_C 65.7, and five methyls, five methylenes, nine methines and seven quaternary carbons. In the HMBC spectrum, the secondary alcoholic proton at δ_H 4.56 (H-3′) showed

long-range correlations with the carbon signals at δ_C 167.7 (C-1″), 23.1 (C-2′) and 28.9 (C-13′), which clearly places the angelate moiety at C-3′. The stereochemistry of **2** was deduced from comparison of its coupling constants and chemical shifts with those of coladonin (Appendino et al., 1997) and from NOE experiments (Fig. 2). Irradiation of the signal at δ_H 4.56 (H-3′) enhanced the signal at δ_H 0.86 (H-13′) and 1.39 (H-5′). Therefore, the structure of **2** was determined to be 3-angeloxycoladonin, a new natural compound.

Ferulsinaic acid (**3**) was assigned the molecular formula $C_{24}H_{30}O_5$ on the basis of positive HRFABMS $[M+H]^+$ at *m*/*z* 399.2167 (calc. 399.2172) and IR absorption bands at 2963, 1726 (C=O, coumarin), 1711 (COOH) and 1614 cm^{-1}. The structure of **3** was established from analysis of its NMR spectral data (Table 1). The ^1H NMR spectrum showed umbelliferous protons at δ_H 6.24 (H-3), 7.63 (H-4), 7.36 (H-5), 6.83 (H-6) and 6.82 (H-8). The proton sequences of the sesquiterpene moiety were established from ^1H-^1H COSY: the downfield olefinic proton at δ_H 5.12 (H-5′) showed correlations with the signal at δ_H 2.51 (H-6′) and a weak correlations with the two methyl signals at δ_H 1.63 (H-13′) and 1.72 (H-14′). In addition, the signal at δ_H 2.51 (H-6′) showed further correlations with the two proton signals at δ_H 1.19 (H-7′α) and 1.93 (H-7′β). The multiplet signal at δ_H 1.83 (H-8′) exhibited correlations with the signals at δ_H 1.19 (H-7′α), 1.93 (H-7′β), 1.75 (H-9′) and the methyl doublet at δ_H 1.14 (H-12′). The methine signal at δ_H 1.75 (H-9′), in turn, correlated with the oxymethylene signals at δ_H 3.97 and 4.00 (H-11′), thereby giving the partial structure A, $(CH_3)_2C$=CH–CH(R)–CH_2–$CH(CH_3)$–CH(R′)–CH_2O–. Furthermore, two methylene groups were detected at δ_H 1.70 (H-1′) and 2.37 (H-2′) and coupled with each other in ^1H-^1H COSY, leading to identification of fragment B as –CH_2–CH_2–. Compound **3** displayed 24 carbon signals in its ^{13}C NMR spectrum, with nine of these typical for the

coumarin skeleton and the other 15 assigned to the sesquiterpene moiety. Assignment of all protonated carbons was made by analysis of HMQC and DEPT data. DEPT experiments classified the carbon signals as four methyls at δ_C 18.1, 20.8, 21.0 and 26.2, three aliphatic methylenes at δ_C 29.2, 33.0 and 40.0, a primary alcoholic carbon at δ_C 69.5 characteristic for C-11′, one carboxylic carbon at δ_C 179.9, and nine methines at δ_C 36.5, 49.3, 53.2, 101.2, 112.6, 113.2, 125.4, 128.7 and 143.4. The sequences and connectivity of the two fragments A and B were established by HMBC correlations between the proton signal at δ_H 1.70 (H-1′) and the carbon signals at δ_C 179.9 (C-3′), 21.0 (C-15′) and 53.2 (C-9′), between the proton signal at δ_H 1.14 (H-12′) and the carbon signals at δ_C 40.0 (C-7′) and 36.5 (C-8′), and between the proton signal at δ_H 4.00 (H-11′) and the carbon signals at δ_C 47.0 (C-10′) and 53.2 (C-9′). These findings suggest the presence of a rearranged 3,4-seco-drimane moiety. The stereochemistry of **3** was established from the NOESY spectrum, as shown in Fig. 3. The signal at δ_H 0.92 (H-15′) exhibited NOESY correlations with the signals at δ_H 4.00/3.97 (H-11′), 1.83 (H-8′) and 2.51 (H-6′), indicating the α-orientation of these protons. In addition, the signal at δ_H 1.75 (H-9′) showed NOESY correlations with the signals at δ_H 1.14 (H-12′) and 1.93 (H-7′β), indicating the β-orientation of H-9′, H-12′ and H-7′β. The biosynthesis of **3** may proceed from feselol (**8**) (Ibraheim and Abdallah, 1996; Ahmed, 1990), similar to the formation of galbanic acid (Bagirov et al., 1979), as shown in Fig. 4. Although the suggested biosynthesis of galbanic acid involves a methyl transformation (C-15′) from C-10′ to C-9′, ferulsinaic acid does not follow the same route. The structure of ferulsinaic acid (**3**) is of particular interest since it is the first member of a new rearranged class of sesquiterpene coumarins from the genus *Ferula*.

HRFABMS of compound **4** showed a pseudomolecular ion peak $[M+H]^+$ at *m*/*z* 399.2180, in accordance with the molecular formula $C_{24}H_{30}O_5$. The structure of **4** was

determined from analysis of its ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2). In the ^{1}H NMR spectrum, umbelliferone protons were found at δ_H 6.26 (H-3), 7.63 (H-4), 7.36 (H-5), 6.85 (H-6) and 6.91 (H-8). The sesquiterpene moiety showed four sharp singlet signals, each integrated for three protons, at δ_H 1.13 (H-13′), 1.07 (H-14′), 1.06 (H-15′) and 1.29 (H-12′), and an oxygenated methylene group at δ_H 4.42 (H-11′a) and 4.21 (H-11′b). Compound **4** displayed 24 carbon signals, nine of them typical for the umbelliferone skeleton and the other 15 assigned to the sesquiterpene moiety, with one carbon signal apparent at δ_C 216.3 (keto group). DEPT experiments classified the protonated carbon signals into four methyls at δ_C 26.7, 24.5, 21.2 and 15.6, four aliphatic methylenes at δ_C 43.3, 38.5, 33.8 and 21.4, one primary alcoholic carbon at δ_C 66.4, and seven methines, five of them in the umbelliferone moiety, at δ_C 113.4 (C-3), 143.3 (C-4), 128.8 (C-5), 113.1 (C-6), 101.6 (C-8), 54.7 (C-5′) and 58.4 (C-9′). The placement of the keto group of the sesquiterpene moiety at C-3′ was deduced from HMBC experiments. The carbonyl carbon at δ_C 216.3 showed correlation with the proton signal at δ_H 2.03 (H-1′a) and the methyl signals at δ_H 1.13 (H-13′) and 1.07 (H-14′). The stereochemistry of **4** was deduced from NOE experiments; irradiation of H-11′ enhanced H-9′, H-12′ and H-15′, while irradiation of H-12′ showed effects on H-15′ and H-7′ (Fig. 5). These data suggest that the structure of **4** is samarcandone (Kir'yalov and Movchan, 1968). However, samarcandone showed an optical rotation of +25°, while compound **4** gave an optical rotation of –15.0° (*c* 0.4, $CHCl_3$). Therefore, compound **4** is an enantiomer of samarcandone.

Compound **5** was isolated in the form of its tetraacetyl derivative (**5a**) with optical rotation of $[\alpha]_D^{25}$=–57° (*c* 0.35, $CHCl_3$). The molecular formula of **5a** was established as $C_{15}H_{22}O_{10}$ on the basis of HRFABMS, which exhibited an ion peak $[M-(CH_3O)]^+$ at *m*/*z* 331.1033. The ^{1}H NMR spectrum gave signals in accordance with

the presence of a β-glucopyranoside. An anomeric proton appeared as a doublet at δ_H 4.44 (J=8.0 Hz) and showed ^{1}H-^{1}H COSY coupling with a signal at δ_H 4.99 (dd, J=8.0, 9.5 Hz, H-2). The other protons could be assigned based on the same experiment: the signal at δ_H 5.22 (t, J=9.5 Hz, H-3) showed coupling with two signals at δ_H 4.99 (H-2) and δ_H 5.10 (t, J=9.5 Hz, H-4). The two doublets at δ_H 4.17 (dd, J=12.5, 2.5 Hz, H-6b) and δ_H 4.28 (dd, J=12.5, 5.0 Hz, H-6a) showed coupling to each other and to a complex signal at δ_H 3.71 (ddd, J=9.5, 5.0, 2.5 Hz, H-5). The carbon signals were determined on the basis of HMQC. Placement of the methoxy group (δ_H 3.43) at C-1 was established from the HMBC experiment: its proton signal showed a cross-peak with the anomeric carbon at δ_C 101.6. Therefore, compound **5** was identified as 1-methoxy-β-L-glucopyranoside.

The known compounds coladin (**6**) and coladonin (**7**) (Ban'kovskii et al., 1970; Appendino et al., 1997), feselol (**8**) (Ahmed, 1990), lancerodiol *p*-hydroxybenzoate (**9**) (Fraga et al., 1985) and jaeschkeanin (**10**) (Diab et al., 2001) were isolated and identified by comparison of their spectral data with the literature.

Thus, phytochemical investigation showed that *F. vesceritensis* mainly contains sesquiterpene coumarin compounds, indicating that it might be very closely related to *F. sinaica* in terms of chemotaxonomy.

3. Experimental

3.1. General

^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and 2D spectra were recorded on a JEOL Lambda 500 spectrometer, with TMS as an internal standard. FABMS and HRFABMS were recorded on a JEOL SX102A mass spectrometer. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer.

3.2. Plant material

F. vesceritensis was collected during the flowering stage in March 2003 near Biskra, approximately 300 miles southeast of Algiers, Algeria by Amar Zellagui, Department of Chemistry, Constantine University, where a voucher specimen has been deposited (AM #112). Roots of *F. sinaica* were collected from North Sinai Peninsula, El-arish, Egypt, in March 1997 by one of the authors (AAA). A voucher specimen (AAA 110) is deposited in the Department of Botany, El-Minia University, Egypt.

3.3. Extraction and isolation

Root of *F. vesceritensis* (800 g) was crushed and extracted with CH_2Cl_2-MeOH (1:1) at room temperature. The extract was concentrated *in vacuo* to obtain a residue of 30 g. The residue was fractionated on a silica gel column (6×120 cm) eluted with hexane (3 L), followed by a gradient of hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 15% MeOH (2 L of each solvent mixture). The hexane–CH_2Cl_2 (3:1) fraction was subjected to a silica gel column (2×60 cm) and eluted with hexane–CH_2Cl_2–MeOH to give three sub-fractions. Sub-fraction 1 was further purified on a silica gel column (2×40 cm) eluted with hexane-EtOAc (6:1) to afford **2** (10 mg). Sub-fraction 2 was further purified on a silica gel column (2×40 cm) eluted with hexane–EtOAc (4:1), and then further separated by TLC to afford **6** (5 mg) and **7** (3 mg). Sub-fraction 3 was further purified on a silica gel column (2×40 cm) eluted with hexane–EtOAc (3:1) to afford **1** (27 mg).

Air-dried roots (1.7 kg) of *F. sinaica* were ground and extracted with CH_2Cl_2 at room temperature. The extract was concentrated *in vacuo* to obtain a residue of 55 g. The residue was fractionated by column chromatography (6×120 cm) on a silica gel column eluted with hexane (3 L), followed by gradient elution with hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and finally with CH_2Cl_2–MeOH (85:15). The hexane–CH_2Cl_2 extract (1:3, 7 g) was purified by HPLC (MeOH–H_2O, 73:27) to afford **3** (5 mg), **4** (12 mg), and a mixture of two compounds that were separated by TLC (ether–hexane, 5:1) to

yield **6** (40 mg), **7** (25 mg) and **8** (35 mg). The CH_2Cl_2 (100%) fraction (14 g) was subjected to a Sephadex LH-20 column (2×60 cm) eluted with hexane–CH_2Cl_2–MeOH (7:4:0.5) to afford **9** and **10**. The CH_2Cl_2–MeOH (85:15) fraction gave an amount of crude **5**, which was converted to tetraacetate **5a** and purified.

13-Hydroxyfeselol (**1**)

Yellowish oil; $[\alpha]_D^{25}$ –27.5° (*c* 0.02, MeOH); IR (ν_{max}^{KBr} cm^{-1}) 3458, 2934, 1736, 1612; FABMS *m*/*z* 399 ($[M+H]^+$), 381 ($[M+H–H_2O]^+$); HRFABMS *m*/*z* 399.2165 ($[M+H]^+$) (calc. for $C_{24}H_{31}O_5$, 399.2172); ^1H and ^{13}C NMR, see Tables 1 and 2.

13-Hydroxyfeselol diacetate (**1a**)

Yellowish oil; FABMS *m*/*z* 483 ($[M+H]^+$); HRFABMS *m*/*z* 483.2377 ($[M+H]^+$) (calc. for $C_{28}H_{35}O_7$, 483.2383); ^1H and ^{13}C NMR, see Tables 1 and 2.

3-Angeloxycoladin (**2**)

Yellow material; IR (ν_{max}^{KBr} cm^{-1}) 3458, 2934, 1736, 1712, 1620; FABMS *m*/*z* 465 ($[M+H]^+$), 365 ($[M+H\text{-angelate}]^+$); HRFABMS *m*/*z* 465.2646 ($[M+H]^+$) (calc. for $C_{29}H_{37}O_5$, 465.2641); ^1H and ^{13}C NMR, see Tables 1 and 2.

Ferulsinaic acid (**3**)

White amorphous powder; $[\alpha]_D^{25}$ –4.5° (*c* 0.67, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}) 2963, 1726, 1711, 1614; HRFABMS *m*/*z* 399.2167 ($[M+H]^+$) (calc. for $C_{24}H_{31}O_5$, 399.2172); ^1H and ^{13}C NMR, see Tables 1 and 2.

Epiferukrinone (**4**)

White powder; $[\alpha]_D^{25}$ –15° (*c* 0.4, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}) 3500, 2963, 1726,

1617; HRFABMS *m*/*z* 399.2180 ([M+H]$^+$) (calc. for $C_{24}H_{31}O_5$, 339.2172); ^{1}H and ^{13}C NMR, see Tables 1 and 2.

1-Methoxy-β-L-glucopyranoside tetraacetate (**5a**)

White powder; $[\alpha]_D^{25}$ –57° (*c* 0.35, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}) 1747; HRFABMS *m*/*z* 331.1033 ([M+H-CH_3OH]$^+$) (calc. for $C_{14}H_{19}O_9$, 331.1029); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.20 (1H, t, *J*=9.5 Hz, H-3), 5.10 (1H, t, *J*=9.5 Hz, H-4), 4.98 (1H, dd, *J*=9.5, 8.0 Hz, H-2), 4.38 (1H, d, *J*=8.0 Hz, H-1), 4.28 (1H, dd, *J*=12.5, 5.0 Hz, H-6a), 4.18 (1H, dd, *J*=12.5, 3.0 Hz, H-6b), 3.71 (1H, ddd, *J*=9.5, 5.0, 3.0 Hz, H-5), 3.43 (3H, s, OCH3), 1.82–2.06 (12H, s, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 101.6 (C-1), 71.2 (C-2), 71.8 (C-3), 68.4 (C-4), 72.9 (C-5), 61.9 (C-6), 56.9 (OCH_3), 20.5-20.6 (OAc), 169.3–170.6 (C=O).

References

Ahmed, A. A., 1990. New sesquiterpenes from *Ferula sinaica*. Journal of Natural Products 53, 483–486.

Ahmed, A. A., 1999. Sesquiterpene coumarins and sesquiterpenes from *Ferula sinaica*. Phytochemistry 50, 109–112.

Appendino, G., Ozen, H., Tagliapietra, S., Cisero, M., 1992. Coumarins from *Heptaptera anisoptera*. Phytochemistry 31, 3211–3213.

Appendino, G., Jakupovic, J., Alloatti, S., Ballero, M., 1997. Ducane esters from *Ferula arrigonii*. Phytochemistry 45, 1639–1643.

Appendino, G., Spagliardi, P., Cravotto, G., Pocock, V., Milligan, S., 2002. Daucane phytoestrogens: A structure–activity study. Journal of Natural Products 65, 1612–1615.

Aqel, M. B., Al-Khalil, S., Afifi, F., Al-Eisawi, D., 1991. Relaxant effects of *Ferula sinaica* root extract on rabbit and guinea pig smooth muscle. Journal of Ethnopharmacology 31, 373–381.

Bagirov, V. Yu., Sheichenko, V. I., Savina, A. A., Veselovskaya, N. V., 1979. Dehydration in terpenoid coumarins. Khimiya Prirodnykh Soedinenii 5, 722–723.

Ban'kovskii, A. I., Ermatov, N. E., Perel'son, M. E., Bubeva-Ivanova, L., Pavlova, N. St., 1970. Structure of the coumarins coladine and coladonine. II. Khimiya Prirodnykh Soedinenii 6, 173–180.

Boulus, L., 1983. Medicinal plants of North Africa. Algonae, MI, p. 183.

Chen, B., Teranishi, R., Kawazoe, K., Takaishi, Y., Honda, G., Itoh, M., Takeda, Y., Kodzhimatov, O. K., 2000. Sesquiterpenoids from *Ferula kuhistanica*. Phytochemistry 54, 717–754.

Diab, Y., Dolmazon, R., Bessiere, J.-M., 2001. Daucane aryl esters composition from the Lebanese *Ferula hermonis* Boiss (zallooh root). Flavour and Fragrance Journal 16, 120–122.

El-Razek, M. H., Ohta, S., Hirata, T., 2003. Terpenoid coumarins of the genus *Ferula*. Heterocycles 60, 689–716.

Fraga, B. M., Gonzalez, A. G., Gonzalez, P., Hernandez, M. G., Larruga, C. L., 1985. Carotane sesquiterpenes from *Ferula lancerottensis*. Phytochemistry 24, 501–504.

Gonzalez, A. G., Barrera, J. B., 1995. Chemistry and the sources of mono and bicyclic sesquiterpenes from *Ferula* species. Progress in the Chemistry Organic Natural Products 64, 1–92.

Ibraheim, Z. Z., Abdallah, O. M., 1996. Coumarins and other constituents from *Ferula sinaica* Boiss growing in Egypt. Bulletin of Pharmaceutical Sciences, Assiut University 19, 15–20.

Kir'yalov, N. P., Movchan, S. D., 1968. Structure of samarkandin and samarkandone, coumarin compounds of *Ferula samarkandica*. Khimiya Prirodnykh Soedinenii 4, 73–77.

Kojima, K., Isakam, K., Ondognii, P., Zevgeegiino, O., Davgiin, K., Mizukami, H., Ogihiara, Y., 1999. Sesquiterpenoid derivatives from *Ferula feruloides* III. Chemical and Pharmaceutical Bulletin 47, 1145–1147.

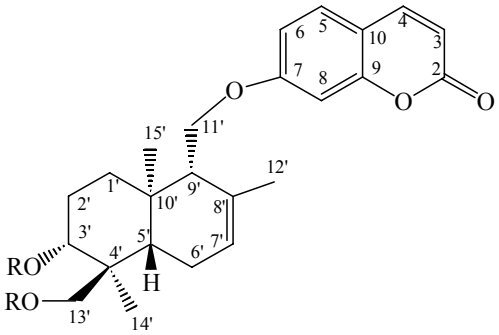
Kojima, K., Isakam, K., Ondognii, P., Zevgeegiino, O., Gombosurengyin, P., Davgiin, K., Mizukami, H., Ogihiara, Y., 2000. Sesquiterpenoid derivatives from *Ferula feruloides* IV. Chemical and Pharmaceutical Bulletin 48, 353–356.

Murray, R. D. H., 1989. Coumarins. Natural Product Reports 6, 591–624.

Nagatsu, A., Isaka, K., Kojima, K., Ondognii, P., Zevgeegiin, O., Gombosurengyin, P., Davgiin, K., Irfan, B., Iqubal, C. M., Ogihara, Y., 2002. New sesquiterpenes from *Ferula ferulaeoides.* Chemical and Pharmaceutical Bulletin 50, 675–677.

Rubiolo, P., Matteodo, M., Riccio, G., Ballero, M., Christen, P., Fleury-Souverain, S., Veuthey, J. L., Bicchi, C., 2006. Analytical discrimination of poisonous and nonpoisonous chemotypes of giant fennel (*Ferula communis* L.) through their biologically active and volatile fractions. Journal of Agricultural and Food Chemistry 54, 7556–7563.

Su, B.-N., Takaishi, Y., Honda, G., Itoh, M., Takeda, Y., Kodzhimatov, O. K., Ashurmetov, O., 2000. Sesquiterpene coumarins and related derivatives from *Ferula pallida*. Journal of Natural Products 63, 436–440.



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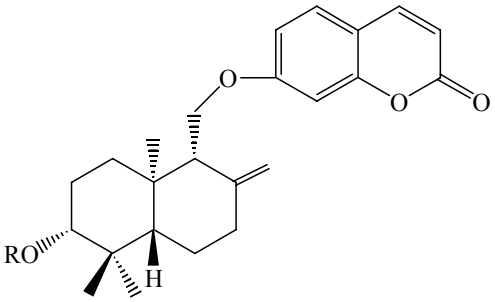

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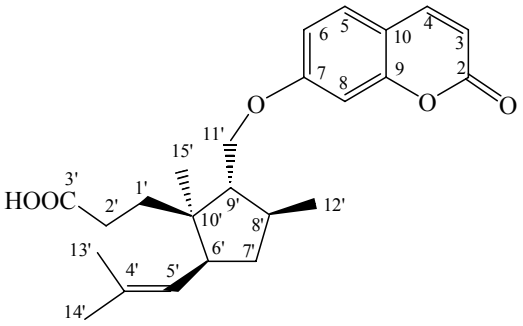

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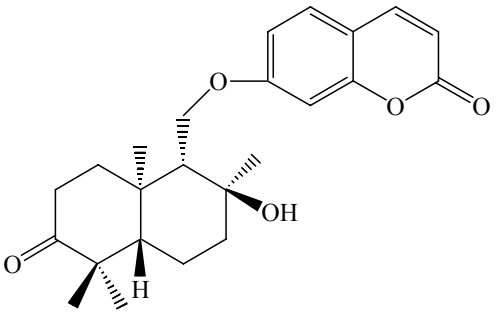

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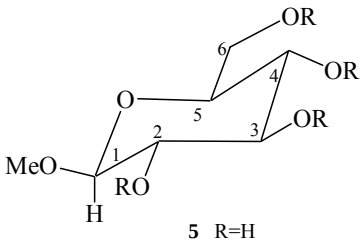

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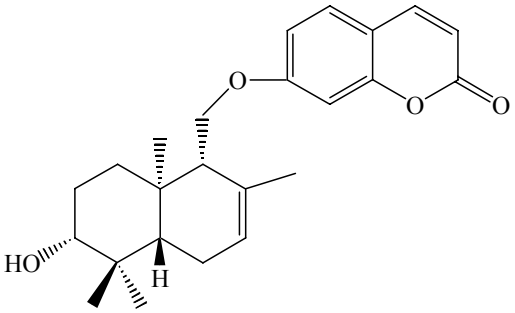

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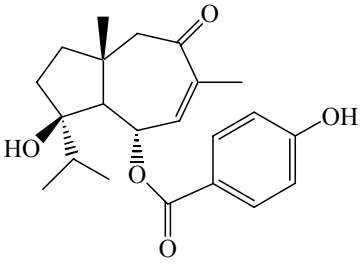

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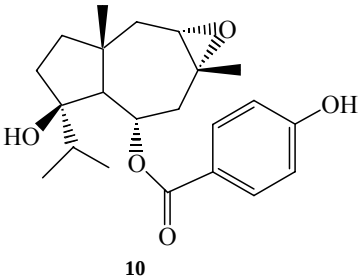

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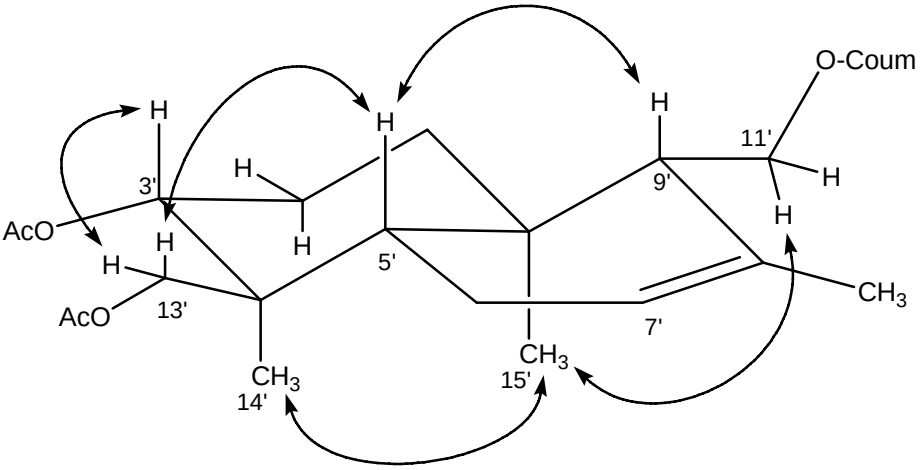

Fig 1. Selective NOE correlations of **1a**.

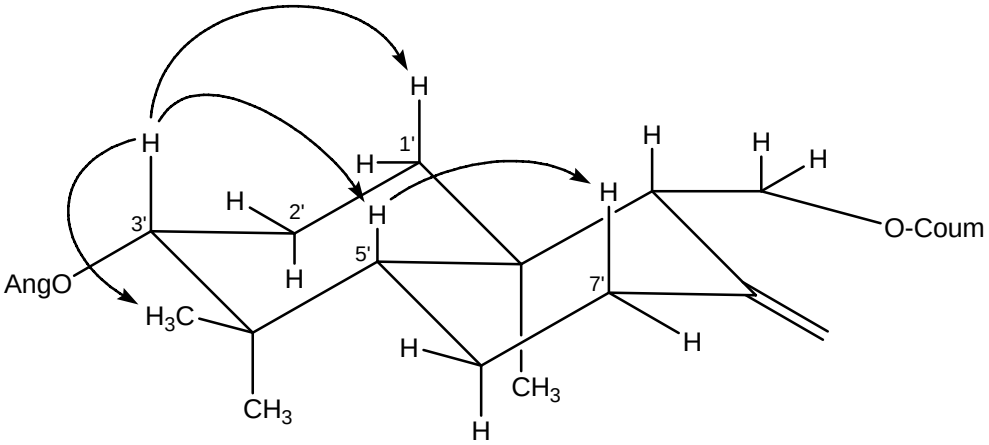

Fig 2. Selective NOE correlations of **2**.

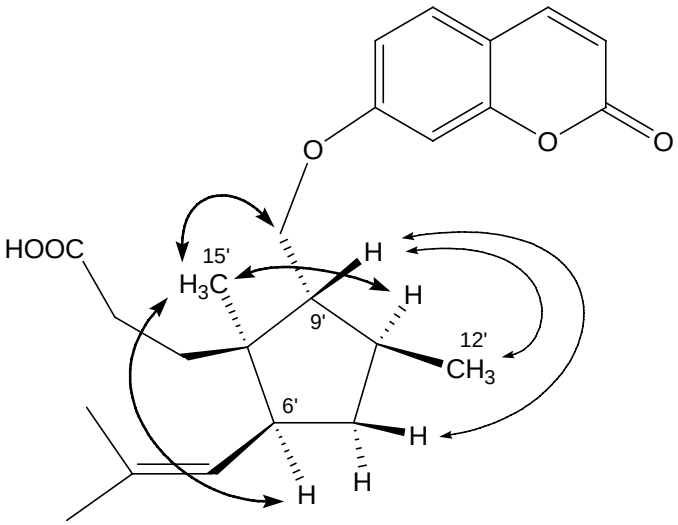

Fig 3. Selective NOE correlations of **3**.

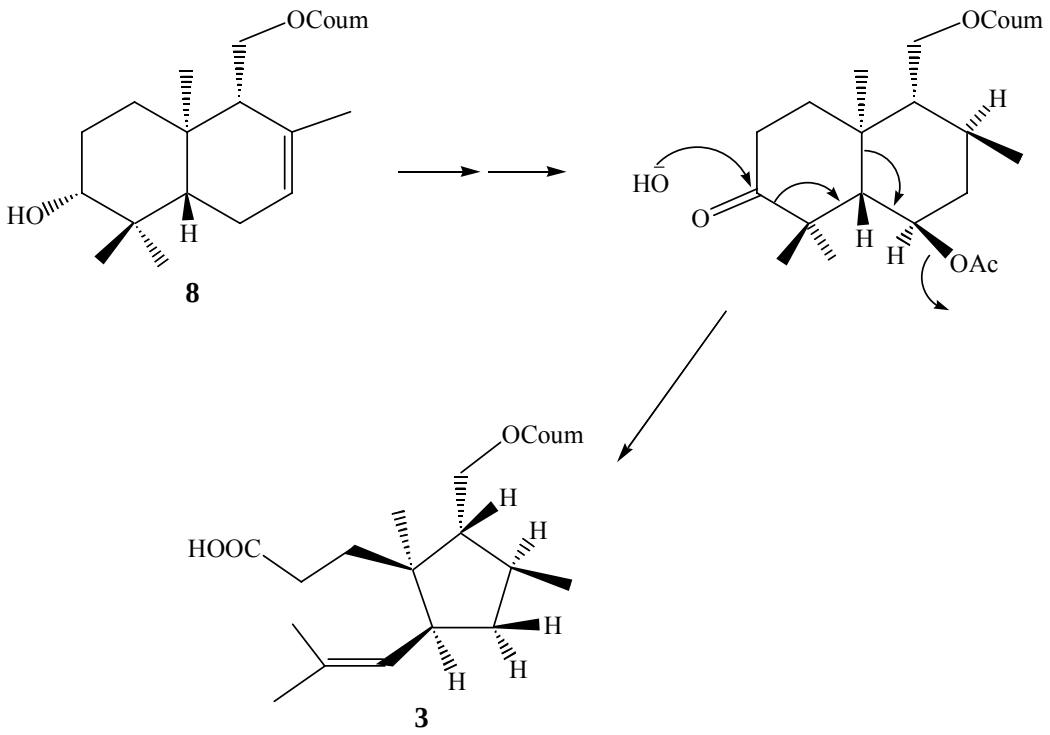

Fig 4. Possible biogenetic pathway of **3**.

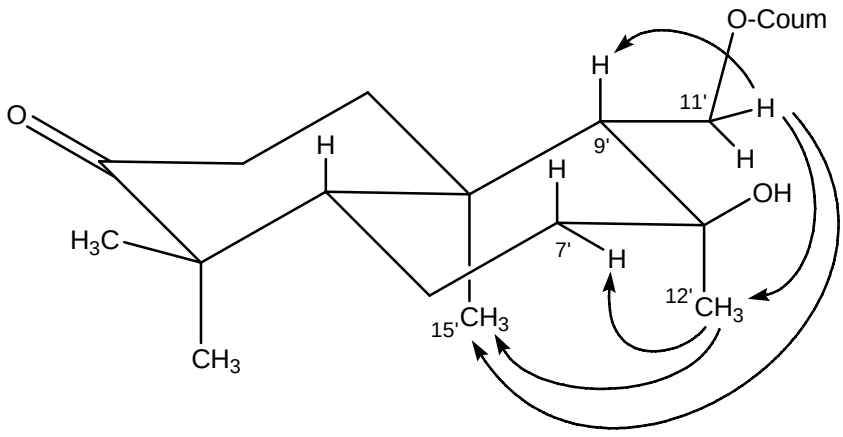

Fig 5. Selective NOE correlations of **4**.

Table 1

^{1}HNMR data for **1-4** [500 MHz, $CDCl_3$, δ_H/ppm, mult (*J*/Hz)]

Position	**1**	**1a**	**2**	**3**	**4**
3	6.18 d (9.5)	6.25 d (9.5)	6.17 d (9.5)	6.24 d (9.5)	6.26 d (9.5)
4	7.60 d (9.5)	7.63 d (9.5)	7.56 d (9.5)	7.63 d (9.5)	7.63 d (9.5)
5	7.31 d (8.5)	7.36 d (8.5)	7.28 d (8.5)	7.36 d (8.5)	7.36 d (8.5)
6	6.77 dd (8.5, 2.5)	6.82 dd (8.5, 2.5)	6.79 dd (8.5, 2.5)	6.83 dd (8.5, 2.5)	6.85 dd (8.5, 2.5)
8	6.75 d (2.5)	6.81 br s	6.74 brd (2.5)	6.82 d (2.5)	6.91 brd (2.5)
1′	1.95 m	2.03 m	1.81 m	1.70 m	2.03 ddd (12.5, 5.5,
	1.24m	1.45 m	1.57 dt (14.5, 4.5)		3.5)
					1.74 ddd (12.5, 10.0,
					6.8)
2′	1.54 m	1.80 m	1.70 m	2.37 m	2.56 ddd (17.5, 10.0,
		1.72 m			3.5)
					2.48 ddd (17.5, 6.8,
					5.5)
3′	3.60 dd (11.0, 7.0)	4.82 dd (11.5, 4.5)	4.56 dd (12.5, 4.5)	---	---
5′	1.36 dd (12.5, 5.0)	1.75 dd (12.5, 5.0)	1.39 dd (12.5, 2.5)	5.12 brd (10.0)	1.57 m
6′	1.98 m	2.05 m	1.77 m	2.51 td (10.0, 10.0,	1.68 dq (13.5, 3.5)
	1.81 m	1.95 m	1.48 m	6.5)	1.48 qd (13.5, 3.5)
7′	5.44 br s	5.53 m	2.47 ddd (13.5, 4.0, 2.5)	1.19 dt (12.5, 10.0)	1.59 m
			2.13 dt (13.5, 4.5)	1.93 dt (12.5, 6.5)	1.98 dt (13.5, 3.5)
8′	---	---	---	1.83 m	---
9′	2.01 dd (5.5, 3.5)	2.31 m	2.17 dd (5.5, 3.0)	1.75 ddd, (9.5, 7.5,	1.86 dd (6.0, 5.0)
				5.0)	
11′	4.10 dd (10.0, 3.5)	4.17 dd (10.0, 3.5)	4.14 dd (9.5, 3.0)	3.97 dd (9.5, 5.0)	4.42 dd (10.0, 5.0)
	3.94 dd(10.0, 5.5)	4.04 dd (10.0, 6.0)	4.09 dd (9.5, 5.5)	4.00 dd (9.5, 7.5)	4.21 dd (10.0, 6.0)
12′	1.60 br s	1.71 br s	4.85 br s	1.14 d (6.5)	1.29 s
			4.46 br s		
13′	3.56 d (10.5)	3.81 d (11.5)	0.86 s	1.63 br s	1.13 s
	3.30 d (10.5)	3.74 d (11.5)			
14′	0.87 s	0.95 s	0.87 s	1.72 br s	1.07 s
15′	0.87 s	0.97 s	0.81 s	0.92 s	1.06 s
3″	---	---	5.98 qq (7.0, 1.5)	---	---
4″	---	---	1.93 d (7.0)	---	---
5″	---	---	1.78 d (1.5)	---	---
OAc	---	2.03 s; 2.05 s	---	---	---

Table 2

^{13}C NMR data for **1-4** (125 MHz, $CDCl_3$, δ_C/ppm)*

Position	**1**	**1a**	**2**	**3**	**4**
2	161.9 s	160.9 s	161.2 s	161.2 s	161.5 s
3	113.2 d	113.0 d	113.0 d	113.2 d	113.4 d
4	143.6 d	143.2 d	143.4 d	143.4 d	143.3 d
5	128.7 d	128.6 d	128.4 d	128.7 d	128.8 d
6	112.2 d	112.9 d	113.1 d	112.6 d	113.1 d
7	161.6 s	161.6 s	162.2 s	162.1 s	161.1 s
8	101.3 d	101.3 d	101.3 d	101.2 d	101.6 d
9	155.7 s	155.7 s	155.9 s	156.0 s	155.9 s
10	112.7 s	112.5 s	112.5 s	112.5 s	112.8 s
1′	37.4 t	37.1 t	35.8 t	33.0 t	38.5 t
2′	26.3 t	22.9 t	23. 1 t	29.2 t	33.8 t
3′	75.8 d	74.1 d	80.1 d	179.9 s	216.3 s
4′	41.8 s	40.6 s	38.7 s	132.1 s	47.4 s
5′	43.4 d	42.4 d	49.6 d	125.4 d	54.7 d
6′	23.2 t	23.3 t	24.2 t	49.3 d	21.4 t
7′	123.2 d	122.9 d	37.3 t	40.0 t	43.3 t
8′	132.3 s	132.2 s	113.1 s	36.5 d	72.3 s
9′	53.5 d	53.6 d	54.7 d	53.2 d	58.4 d
10′	35.7 s	35.6 s	38.1 s	47.0 s	37.5 s
11′	66.8 t	66.8 t	65.7 t	69.5 t	66.4 t
12′	21.4 q	21.7 q	107.8 t	20.8 q	24.5 q
13′	70.2 t	65.0 t	28.9 q	18.1 q	26.7 q
14′	11.2 q	13.1 q	20.4 q	26.2 q	21.2 q
15′	15.3 q	15.6 q	15.7 q	21.0 q	15.6 q
1″		170.3 s [b)]	167.7 s [c)]		
2″		170.8 s [b)]	128.3 s [c)]		
3″		21.1 s [b)]	137.5 d [c)]		
4″		21.4 s [b)]	25.6 q [c)]		
5″			30.2 q [c)]		

* Multiplicity was determined by DEPT experiments (s=quaternary, d=methine, t=methylene, q=methyl). b) Signals due to acetyl groups. c) Signals due to an angelic group.