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Sesquiterpenoids from *Ferula kuhistanica*

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Abstract

Methanol extracts of the air-dried roots and stems of *Ferula kuhistanica* afforded seven daucane-type sesquiterpenes, called kuhistanicaol A–G, together with 13 known daucane esters. Their structures were established on the basis of spectroscopic evidence and the results of chemical reactions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Ferula kuhistanica*; Umbelliferae; Sesquiterpenes; Daucane esters; Kuhistanicaol A–G

1. Introduction

The exclusively Old World genus *Ferula* belongs to the family Umbelliferae, which consists of some 130 species distributed throughout the Mediterranean area and Central Asia. The chemistry of this genus has been studied by many groups. The widespread sesquiterpene compounds in this genus are characteristic daucanes, humulanes, himachalanes, germacranes, eudesmanes, and guainanes (Gonzalez and Barrera, 1995). Several species of this genus have been used in folk medicines (Uphof, 1968). As a part of our studies on Turkish folk medicinal plants (Sezik et al., 1997), we have investigated the constituents of *Ferula kuhistanica* and describe here the isolation and characterization of 20 compounds **1**–**20**. This includes five new daucane esters, kuhistanicaol A (**1**), B (**5**), C (**7**), D (**12**) and G (**17**), together with eight known daucane esters, jaeschkeanadiol *p*-hydroxybenzoate (ferutinin) (**6**) (Singh et al., 1988), jaeschkeanadiol vanillate (**9**) (Miski et al., 1983), lapidol vanillate (**10**) (Gonzalez et al., 1988), 5α-*p*-hydroxybenzoyloxydauc-2-ene-1-one (**11**) (Ahmed, 1991), lancerotriol 6-vanillate (**14**) (Miski and Jakupovic, 1990), lancerotriol *p*-hydroxybenzoate (**16**) (Fraga et al., 1985), 4β,8α-dihydroxy-6α-vanilloyloxydauc-9-ene (**18**) (Miski and Jakupovic, 1990) and 4β,8β,9α-trihydroxy-6α-*p*-hydroxybenzoyloxydaucane (**20**) (Ahmed, 1990) from the roots of *F. kuhistanica*. Two new daucane esters, kuhistanicaol E (**13**) and F (**15**) along with five known compounds, 5α-vanillate of 2,3-epoxy-jaeschkeanadiol (**2**) (Garg and Agrawal, 1988), 8,9-epoxy-ferutinin (**3**) (Razdan et al., 1989), 2,3-epoxy-jaeschkeanadiol *p*-methoxybenzoate (**4**) (Miski and Jakupovic, 1990), jaeschkeanadiol *p*-methoxybenzoate (Ferutidin) (**8**) (Miski and Mabry, 1985) and 4β,8β-dihydroxy-6α-vanilloyloxydauc-9-ene (**19**) (Miski and Jakupovic, 1990) were also isolated from the stems of the same plant.

2. Results and discussion

Methanol extracts of the air-dried roots of *F. kuhistanica* were partitioned between H_2O and EtOAc, with

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the EtOAc extracts separated by column chromatography (CC) to afford five new compounds **1**, **5**, **7**, **12** and **17**, together with eight known compounds **6**, **9**–**11**, **14**, **16**, **18** and **20**. These were identified by comparison of their physical and spectral data with those in the literature. The stems of *F. kuhistanica* were extracted in the same way to afford the two new compounds **13** and **15**, as well as the known compounds **2**–**4**, **8** and **19**, which were also identified by comparison of their physicochemical and spectral data.

Kuhistanicaol A (**1**) displayed absorption bands of hydroxy (3409 cm^{-1}), ketone (1717 cm^{-1}) and ester (1609 cm^{-1}) groups in its IR spectrum, while its UV spectrum showed the presence of an aromatic ring (292, 263 and 220 nm). The ^{13}C NMR spectrum of **1** had signals for two carbonyls (δ_C 166.2 and 171.2), a benzene ring (δ_C 111.9, 114.5, 122.1, 124.5, 146.5 and 150.8), six methyls, three methylenes, five methines, three of which bore an oxygen atom (δ_C 78.7, 69.6 and 60.4), and three quaternary carbons, two of which were linked to an oxygen (δ_C 82.1 and 56.2). The ^1H NMR spectrum, analyzed using 2D COSY techniques, exhibited an angular methyl [δ_H 1.31 (3H, *s*)], an isopropyl group [δ_H 0.84, 0.97 (each 3H, *d*, $J = 6.8$ Hz)], an acetoxyl [δ_H 2.10 (3H, *s*)], a methoxyl [δ_H 3.94 (3H, *s*)] and a methyl attached to a carbon with an oxygen function [δ_H 1.50 (3H, *s*)]. These findings were consistent with a molecular formula of $C_{25}H_{34}O_8$, which was supported by HR-EIMS data. In the mass spectrum, the fragment peak at *m*/*z* 419 was assigned to loss of C_3H_7 from the molecular ion. This is a typical loss in daucane-type sesquiterpenes (Ahmed, 1990). Based on these findings, **1** was deduced to be a daucane-type sesquiterpene. The ^{13}C NMR spectral data (Table 1) of **1** were very similar to those of **2**, except for the resonances of an acetoxyl group and a methine bearing the acetoxyl group in **1**, which is a methylene in **2**. It was assumed that **1** and **2** had the same framework as well as similar aromatic ring substituents. To confirm the location of the *O*-acetoxyl group, the 2D NMR spectra of **1** were examined. In the HMBC spectrum, the methine signal at δ_H 4.40 bearing the acetoxyl group showed long-range correlations with the carbon signals at δ_C 14.3 (C-15), 38.6 (C-10) and 171.2, and the proton signals at δ_H 1.60 and 2.45 (3-H_2) had long-range correlations with the carbon signals at δ_C 45.9 (C-1), 78.7 (C-2) and 82.1 (C-4). Thus, the acetoxyl group was attributed to C-2. In addition, based on correlations of the 15-H_3 and 6-H in the NOESY spectrum, the relative stereochemistry of the vanillate group (Van) was determined to be 6α and the correlation of 2-H and 5-H revealed that 2-H had an α-configuration. Thus, the acetoxyl group was determined to be 2β. Therefore, the structure of **1** was as shown.

Table 1
^{13}C NMR spectral data for compounds **1**, **2**, **5**–**7**[a]

C	1	2	5	6	7
1	45.9	44.4	44.9	44.0	44.0
2	78.7	31.7	42.7	41.3	41.4
3	37.2	40.5	32.8	31.6	31.6
4	82.1	86.0	87.6	86.5	86.4
5	58.0	60.8	60.4	60.1	60.2
6	69.6	70.2	71.5	71.2	71.4
7	44.3	44.3	31.2	41.4	37.5
8	56.2	56.1	142.0	133.6	136.8
9	60.4	60.9	157.9	125.3	127.0
10	38.6	41.2	43.6	41.0	40.7
11	37.0	37.2	37.8	37.1	37.3
12	17.3	17.3	18.0	17.6	17.5
13	18.5	18.5	19.0	18.6	18.6
14	23.3	23.3	195.5	26.4	68.6
15	14.3	19.3	21.1	20.3	20.1
1′	166.2	166.0	167.8	167.6	166.5
2′	122.1	122.1	123.1	121.6	122.7
3′	124.5	124.2	132.9	132.1	131.8
4′	114.5	114.3	116.3	115.6	113.9
5′	150.8	150.4	163.6	161.6	163.6
6′	146.5	146.3	116.3	115.6	113.9
7′	111.9	111.7	132.9	132.1	131.8
OCH_3	56.2	55.9	–	–	55.5
OAc	171.2	–	–	–	–
	21.3	–	–	–	–

[a] Spectra of compounds **1**, **2**, **6** and **7** were recorded in $CDCl_3$, and that of compound **5** was obtained in CD_3OD.

The ^1H NMR spectrum of kuhistanicaol B (**5**) exhibited one angular methyl, an isopropyl group, an aldehyde (δ_H 9.39, 1H, *s*, 14-H), and a *p*-hydroxybenzoyl group (*p*-HyBz). The ^{13}C NMR spectral data (Table 1) of **5** were very similar to those of **6**, except that it showed an aldehyde at δ_C 195.5 instead of the olefinic methyl at δ_C 26.4 in **6**, as well as a downfield shift of C-8 and C-9 and an upfield shift of C-7 in **5**. In addition, the ^1H NMR spectral data of **5** exhibited an olefinic proton and a proton geminal to an ester group at δ_H 7.00 and 5.19, which were found at δ_H 5.55 and 5.27, respectively, for **6**. In the HMBC spectrum, the aldehyde proton (δ_H 9.39) had a long-range correlation with the carbon signals at δ_C 142.0 (C-8). Thus, the aldehyde group was considered to be located at C-14. This assignment was in agreement with the NOESY spectrum, in which the 9-H was correlated with 14-H and 10-H_2. Hence, the structure of compound **5** was determined to be as shown.

The ^1H and ^{13}C NMR spectral data for kuhistanicaol C (**7**) were compared with those of the closely related compound **8**, which has been reported from the same genus (Diaz et al., 1984). The presence of two protons at δ_H 4.08 in the ^1H NMR spectrum and a methylene at δ_C 68.6 in the ^{13}C NMR spectrum indicated the existence of a hydroxymethyl in **7**. The absence of an olefinic methyl suggested that **7** had a hydroxymethyl instead of the methyl in **8**. A strong NOE was observed between 14-H_2 and 9-H, which is

consistent with its location on the hydroxy group. In addition, the correlations of 15-H_3, 6-H and 7-Hβ in the NOESY spectrum revealed the α-configuration of the *p*-anisate group. Therefore, compound **7** was determined to be as shown.

The ^{13}C NMR spectral data of kuhistanicaol D (**12**) were very similar to those of **9**, except for the chemical shifts of the C-15 and C-1 carbons, and the methine (δ_C 79.4) bearing an oxygen group instead of a methylene in **9**. Based on the MS results, **12** was confirmed to have one more hydroxyl group than **9**, which could be connected to C-2, C-3, C-7 or C-10. In the HMBC spectrum, the C-14-H_3 protons showed long-range correlations with the carbon signals at δ_C 41.4 (C-7), 130.7 (C-8) and 132.2 (C-9) and the C-15-H_3 protons had long-range correlations with the carbon signals at δ_C 49.7 (C-1), 37.7 (C-2), 57.7 (C-5) and 79.4 (C-10), clearly showing the presence of the hydroxyl group at C-10. The ^1H NMR spectrum showed that a broad singlet at δ_H 5.43, which could be attributed to the H-9 vinylic proton, interacts with a broad singlet at δ_H 4.10 (10-H). Inspection of Dreiding models of **12** confirmed a small coupling between 9-H and 10-H which was only possible when the proton at C-10 had the α-configuration (Miski and Mabry, 1985). In addition, due to the correlations of the 5-H, 7-Hα and 10-H protons in the NOESY spectrum, the relative stereochemistry of the hydroxyl group was 10β. The structure of **12** was also confirmed chemically. Treatment of **12** with Jones reagent gave compound **10**. Thus, the structure of compound **12** was determined to be as shown.

The structure of kuhistanicaol E (**13**) was deduced from its ^1H NMR spectral data, which were very similar to that of **14**. In the HMBC spectrum, 6-H showed long-range correlations with the carbon signals at δ_C 86.6 (C-4), 54.0 (C-5), 128.1 (C-7), 136.9 (C-8) and 166.6 (C-1′), which confirmed the location of the vanillate group at C-6. The other cross peaks observed at 7-H/C-5, C-6, C-8, C-9 and C-14, as well as 9-H/C-1, C-7, C-8 and C-10, revealed that **13** had the same framework as **14**. These facts indicated that the main difference between the two compounds was the configuration of the hydroxyl group at C-9. When **13** and **14** were oxidized with Jones reagent, both gave the same compound **21**, which was identical to the natural compound isolated from *F. orientalis* (Miski et al., 1987). From the ^1H NMR spectral data of **13** and **14**, the stereochemistry of both hydroxyl groups was also inferred. Thus, of the different conformations that **13** and **14** can adopt, we chose the one which explains the coupling constant of zero between H-6 and H-7, observed in the ^1H NMR spectra of both epimers. This corresponds to a chair conformation, with an equatorial hydroxy (α) for **14** and an axial hydroxy (β) for **13** (Garg and Agrawal, 1988). Thus, the structure of **13** was determined as shown.

The ^1H and ^{13}C NMR spectral data (Table 2) of kuhistanicaol F (**15**) were very similar to those of **13**, except for a *p*-anisate group in **15** instead of the vanillate group in **13**. The structure of **15** was confirmed by interpretation of its 2D NMR spectral data.

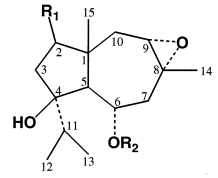
The ^1H and ^{13}C NMR spectral data (Table 2) of kuhistanicaol G (**17**) were compared with those of the closely related compound **18**. The ester group was confirmed to be at C-6 since the 6-H proton showed a long-range correlation with the carbonyl carbon signals at δ_C 168.3. In addition, due to the correlations of 6-H, C-15-H_3 and C-14-H_3 protons shown in the NOESY spectrum of **17**, the relative stereochemistries of the ester and hydroxyl group were confirmed to be 6α and 8α, respectively. The presence of two proton doublets at δ_H 7.92 and 6.81 in the ^1H NMR spectrum of **17** and three aromatic protons at δ_H 7.62 (1H, *dd*, $J = 1.6$, 8.3 Hz), 7.56 (1H, *d*, $J = 1.6$ Hz) and 6.95 (1H, *d*, $J = 8.3$ Hz) in the spectrum of **18**, respectively, indicated the presence of a *p*-hydroxybenzoyl group in **17** instead of the vanillate group in **18**. The MS, ^{13}C and 2D NMR spectral data all agreed with the proposed structure.

Although many sesquiterpenoids and coumarins have been isolated from the genus *Ferula*, it was found that although *F. kuhistanica* contains many sesquiterpenoids, no coumarins were isolated from this species in the present study.

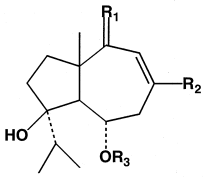
Table 2
^{13}C NMR spectral data for compounds **12**–**15**, **17** and **18**[a]

C	12	13	14	15	17	18
1	49.7	42.3	42.6	42.2	46.8	46.7
2	37.7	42.0	42.1	42.0	42.3	42.2
3	31.3	31.9	31.9	31.8	32.0	32.0
4	86.6	86.6	86.7	86.5	87.0	87.1
5	57.7	54.0	55.0	54.0	52.7	52.7
6	70.4	72.9	73.2	72.7	73.1	73.4
7	41.4	128.1	126.4	128.0	45.2	45.1
8	130.7	136.9	139.2	136.9	71.6	71.7
9	132.2	83.3	70.0	83.2	133.4	133.3
10	79.4	44.5	50.7	44.5	142.2	142.2
11	37.4	37.0	37.2	37.0	37.4	37.4
12	17.6	17.5	17.6	17.4	17.8	17.9
13	18.6	18.4	18.6	18.4	18.9	19.0
14	26.4	24.2	24.1	24.2	34.2	34.1
15	14.7	19.9	19.2	19.9	21.1	21.1
1′	166.3	166.6	166.6	166.7	168.3	168.3
2′	122.4	122.2	122.5	122.5	123.4	123.6
3′	124.3	124.5	124.6	131.9	133.0	125.2
4′	114.3	114.3	111.4	113.8	116.0	115.8
5′	150.4	150.5	150.5	163.7	163.3	152.5
6′	146.4	146.4	146.4	113.8	116.0	148.6
7′	111.9	112.0	112.1	131.9	133.0	113.9
OCH_3	56.1	56.1	56.3	55.5	–	56.5

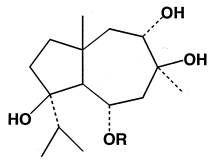
[a] Spectra of compounds **12**–**15** were recorded in $CDCl_3$, and those of **17** and **18** were recorded in CD_3OD.



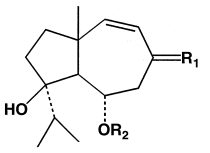
	R_1	R_2
1	OAc	Van
2	H_2	Van
3	H_2	*p*-HyBz
4	H_2	*p*-Anis



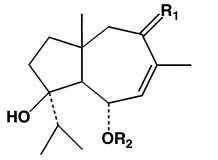
	R_1	R_2	R_3
5	H_2	CHO	*p*-HyBz
6	H_2	Me	*p*-HyBz
7	H_2	CH_2OH	*p*-Anis
8	H_2	Me	*p*-Anis
9	H_2	Me	Van
10	O	Me	Van
11	O	Me	*p*-HyBz
12	β-OH, H	Me	Van



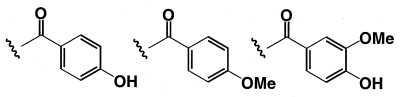
20 R=*p*-HyBz



	R_1	R_2
17	α-OH, β-Me	*p*-HyBz
18	α-OH, β-Me	Van
19	β-OH, α-Me	Van



	R_1	R_2
13	β-OH, H	Van
14	α-OH, H	Van
15	β-OH, H	*p*-Anis
16	α-OH, H	*p*-HyBz
21	O	Van



p-HyBz *p*-Anis Van

17

3. Experiments

3.1. General

^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz with TMS as internal standard; MS: JEOL JMSD-300 instrument; CC: silica gel, Sephadex LH-20 (Pharmacia), Toyo Pearl HW-40 (Tosoh); HPLC: GPC (Asahipak GS-310 2G, MeOH; Shodex H-2001, 2002, $CHCl_3$), silica gel (Si60, Hibar TR250-25, Merck), ODS (RP-18, Hibar RT250-25, Merck).

3.2. Plant material

The roots and stems of *F. kuhistanica* were collected in July 1997 from Uzbekistan. Herbarium specimens were deposited in the herbarium of the Academy of Sciences, Institute of Botany, Uzbekistan.

3.3. Extraction and isolation

The roots of *F. kuhistanica* (2.25 kg) were crushed and extracted three times with MeOH at 60°C. The MeOH extracts were concentrated in vacuo to give a residue, which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (247 g), which was subjected to a silica gel column, eluted with solvents of increasing polarity (hexane–EtOAc; $CHCl_3$–MeOH) to give 21 frs. Fr. 4 (75 g) was next applied to a silica gel column with $CHCl_3$–MeOH as eluent to give 6 frs. (4.1–4.6), with fr. 4.2 (16.4 g) being loaded onto a Toyo Pearl HW-40 column eluted with $CHCl_3$–MeOH (2:1) to give 5 frs. (4.2.1–4.2.5). Fr. 4.2.3 (713 mg) was next subjected to silica gel CC with hexane–EtOAc as eluent to give **9** (33.0 mg). Fr. 4.3 (2.28 g) was applied to a silica gel column eluted with $CHCl_3$–MeOH to give **6** (711 mg). Fr. 11 (7.6 g) was next subjected to a silica gel chromatography with $CHCl_3$–MeOH as eluent to give 17 frs. (11.1–11.17), with fr. 11.2 (493 mg) applied to a Toyo Pearl HW-40 column eluted with $CHCl_3$–MeOH to give 4 frs. (11.2.1–11.2.4). Fr. 11.2.3 (90.5 mg) was subjected to HPLC (GPC, MeOH) separation to give 6 frs. (11.2.3.1–11.2.3.6); fr. 11.2.3.6 (17.4 mg) was further purified by preparative TLC (Hex:EtOAc = 1:1) to give **1** (14.7 mg). Fr. 11.6 was subjected to Sephadex LH-20 chromatography with MeOH as eluent to give 6 frs. (11.6.1–11.6.6); fr. 11.6.5 (93.8 mg) was purified by HPLC (silica, Hex:EtOAc = 2:3) to give **7** (10.7 mg). Fr. 11.6.5.5 (18.5 mg) was purified by GPC (MeOH) to give **18** (11.4 mg). Fr. 11.6.6 (49.7 mg) was purified by HPLC (silica, Hex:EtOAc = 2:3; GPC, MeOH) to give **10** (8.9 mg). Fr. 11.8 (1 g) was applied to a Sephadex LH-20 column with MeOH as eluent to give 5 frs. (11.8.1–11.8.5), with fr. 11.8.3 (599 mg) further purified by GPC (MeOH) to give 4 frs. (11.8.3.1–11.8.3.4). Fr. 11.8.3.1 (351 mg) was purified by HPLC (silica gel, Hex:EtOAc = 1:1) to give **12** (16.1 mg) and **14** (6.2 mg). Fr. 11.8.3.1.8 was purified by GPC (MeOH) to give **5** (8.2 mg) and **17** (10.9 mg). Fr. 8.3.2 (153 mg) was applied to an HPLC column (silica, Hex:EtOAc = 1:1; GPC, MeOH and ODS) to give **11** (45.1 mg). Fr. 11.10 (783 mg) was separated on a Sephadex LH-20 column with MeOH as eluent to give 7 frs. (11.10.1–11.10.7). Fr. 11.10.3 was purified by HPLC (ODS, GPC) to give **16** (57.0 mg). Fr. 14 (9 g) was subjected to MPLC (silica, $CHCl_3$–MeOH) to give 15 frs. (14.1–14.15). Fr. 14.8 (833 mg) was applied to a Sephadex LH-20 column to give 5 frs. (14.8.1–14.8.5); fr. 14.8.3 was purifed by HPLC (silica gel, Hex:EtOAc) to give **20** (13.7 mg).

The EtOAc layer of the stems was concentrated to give a residue (60 g), which was subjected to silica gel chromatography with solvents of increasing polarity to give 14 frs. Fr. 2 (2.8 g) was purified further by silica gel, HPLC (GPC, MeOH; silica gel, $CHCl_3$:MeOH = 99:1) to afford **13** (41.7 mg), whereas fr. 2.2 (9.2 g) was subjected to silica gel chromatography and then to

HPLC (silica gel, $CHCl_3$:MeOH = 99:1) to give **4** (14.0 mg) and **19** (12.4 mg). Fr. 5 (0.7 g) was purified by GPC ($CHCl_3$) to give **3** (32.8 mg). Fr. 5.1 (52 mg) was subjected to HPLC (GPC, $CHCl_3$; silica gel, $CHCl_3$:MeOH = 99:1) to give **2** (19.4 mg), whereas fr. 1 (3.4 g) was applied to a silica gel column eluted with Hex:$CHCl_3$ to give 16 frs. (1.1–1.16), with fr. 1.14 (937 mg) being applied to a silica gel column eluted with Hex:EtOAc to give **8** (65.7 mg) and **15** (109.0 mg).

3.4. Kuhistanicaol A (*1*)

Amorphous. $[\alpha]_D^{25}$ +82.6° (MeOH, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3409, 2971, 2358, 1717, 1609, 1280, 1121, 963, 762; UV λ_{max}^{MeOH} nm (log ε): 292 (3.8), 263 (4.1), 219 (4.3), 205 (4.3); ^1H NMR spectral data δ_H ($CDCl_3$): 0.84 (3H, *d*, *J* = 6.8 Hz, 12-H), 0.97 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.23 (1H, *dd*, *J* = 7.1, 13.9 Hz, 10-Hα), 1.31 (3H, *s*, 15-H), 1.50 (3H, *s*, 14-H), 1.60 (1H, *dd*, *J* = 9.3, 13.6 Hz, 3-Hα), 1.91 (1H, *m*, 7-Hα), 1.93 (1H, *d*, *J* = 10.7 Hz, 5-H), 1.97 (1H, *m*, 11-H), 2.10 (3H, *s*, 2-AcO), 2.29 (1H, *br d*, *J* = ca. 14 Hz, 7-Hβ), 2.31 (1H, *dd*, *J* = 7.1, 13.9 Hz, 10-Hβ), 2.45 (1H, *dd*, *J* = 9.3, 13.6 Hz, 3-Hβ), 2.84 (1H, *dd*, *J* = 7.1, 7.1 Hz, 9-H), 3.94 (3H, *s*, OCH_3), 4.40 (1H, *dd*, *J* = 9.3, 9.3 Hz, 2-H), 5.52 (1H, *br t*, *J* = ca. 10 Hz, 6-H), 6.96 (1H, *d*, *J* = 8.4 Hz, 4′-H), 7.51 (1H, *d*, *J* = 1.6 Hz, 7′-H), 7.57 (1H, *dd*, *J* = 1.6, 8.4 Hz, 3′-H); ^{13}C NMR spectral data ($CDCl_3$): Table 1; HR-EIMS *m*/*z* (rel. int.): 462.2229 $[M]^+$, $C_{25}H_{34}O_8$, requires 462.2254; EIMS *m*/*z* (rel. int.): 43 (39), 151 (100), 168 (37), 191 (28), 235 (32), 419 (16), 462 $[M]^+$ (2).

3.5. Kuhistanicaol B (*5*)

Amorphous. $[\alpha]_D^{25}$ +123.6° (MeOH, *c* 0.5); IR ν_{max}^{KBr} cm^{-1}: 3390, 2966, 1687, 1609, 1278, 1165, 1099, 772; UV λ_{max}^{MeOH} nm (log ε): 255 (4.2), 209 (4.2); ^1H NMR spectral data δ_H (CD_3OD): 0.86 (3H, *d*, *J* = 6.8 Hz, 12-H), 1.02 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.11 (3H, *s*, 15-H), 1.43 (1H, *m*, 2-Hα), 1.59 (1H, *m*, 3-Hα), 1.66 (1H, *m*, 2-Hβ), 2.01 (1H, *m*, 3-Hβ), 2.14 (1H, *sept*, *J* = 6.8 Hz, 11-H), 2.21 (1H, *d*, *J* = 10.5 Hz, 5-H), 2.27 (1H, *m*, 7-Hα), 2.41 (1H, *dd*, *J* = 3.5, 15.3 Hz, 10-Hα), 2.61 (1H, *dd*, *J* = 8.5, 15.3 Hz, 10-Hβ), 3.21 (1H, *dd*, *J* = 3.1, 14.5 Hz, 7-Hβ), 5.19 (1H, *ddd*, *J* = 3.1, 10.3, 10.5 Hz, 6-H), 6.83 (2H, *d*, *J* = 8.7 Hz, 4′,6′-H), 7.00 (1H, *br s*, 9-H), 7.86 (2H, *d*, *J* = 8.7 Hz, 3′,7′-H), 9.39 (1H, *s*, 14-CHO); ^{13}C NMR spectral data (CD_3OD): Table 1; HR-FABMS *m*/*z* (rel. int.): 395.1825 $[M + Na]^+$ $C_{22}H_{28}O_5Na$, requires 395.1834; EIMS *m*/*z* (rel. int.): 43 (51), 93 (39), 107 (24), 121 (100), 139 (27), 191 (53), 234 (31), 329 $[M - 43]^+$ (38).

3.6. Kuhistanicaol C (*7*)

Amorphous. $[\alpha]_D^{25}$ +29.3° (MeOH, *c* 0.45); IR ν_{max}^{KBr} cm^{-1}: 3424, 2965, 1709, 1607, 1218, 1100, 1030, 759; UV λ_{max}^{MeOH} nm (log ε): 257 (3.9), 205 (4.0); ^1H NMR spectral data δ_H ($CDCl_3$): 0.86 (3H, *d*, *J* = 6.4 Hz, 12-H), 0.97 (3H, *d*, *J* = 6.4 Hz, 13-H), 1.11 (3H, *s*, 15-H), 1.32 (1H, *m*, 2-H), 1.60 (2H, *m*, 2, 3-H), 1.95 (1H, *sept*, *J* = 6.4 Hz, 11-H), 1.97 (1H, *m*, 3-H), 2.05 (1H, *m*, 10-H), 2.10 (1H, *m*, 5-H), 2.18 (1H, *m*, 10-H), 2.50 (2H, *m*, 7-H_2), 3.87 (3H, *s*, OCH_3), 4.08 (2H, *br s*, 14-H), 5.24 (1H, *br t*, *J* = ca. 8 Hz, 6-H), 5.82 (1H, *br s*, 9-H), 6.93 (2H, *d*, *J* = 8.5 Hz, 4′,6′-H), 7.97 (2H, *d*, *J* = 8.5 Hz, 3′,7′-H); ^{13}C NMR spectral data ($CDCl_3$): Table 1; HR-FABMS *m*/*z* (rel. int.): 411.2178 $[M + Na]^+$ $C_{23}H_{32}O_5Na$, requires 411.2147; EIMS *m*/*z* (rel. int.): 43 (36), 55 (13), 77 (16), 83 (34), 119 (14), 135 (100), 152 (23), 175 (17), 193 (8), 345 $[M - 43]^+$ (6).

3.7. Kuhistanicaol D (*12*)

Amorphous. $[\alpha]_D^{25}$ −26.3° (MeOH, *c* 1.2); IR ν_{max}^{KBr} cm^{-1}: 3426, 2968, 2362, 1693, 1599, 1284, 1033, 765; UV λ_{max}^{MeOH} nm (log ε): 291 (3.6), 261 (4.0), 218 (4.1), 207 (4.2); ^1H NMR spectral data δ_H ($CDCl_3$): 0.87 (3H, *d*, *J* = 6.8 Hz, 12-H), 0.98 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.29 (3H, *s*, 15-H), 1.30 (1H, *m*, 2-H), 1.65 (H, *m*, 3-H), 1.80 (1H, *m*, 2-H), 1.84 (3H, *s*, 14-H), 2.00 (H, *m*, 3-H), 2.01 (1H, *d*, *J* = 10.7 Hz, 5-H), 2.05 (1H, *sept*, *J* = 6.8 Hz, 11-H), 2.29 (1H, *dd*, *J* = 2.8, 14.0 Hz, 7-Hα), 2.43 (1H, *br t*, *J* = ca. 14 Hz, 7-Hβ), 3.95 (3H, *s*, OCH_3), 4.10 (1H, *br s*, 10-H), 5.26 (1H, *ddd*, *J* = 2.8, 10.7, 13.5 Hz, 6-H), 5.43 (1H, *br s*, 9-H), 6.95 (1H, *d*, *J* = 8.3 Hz, 4′-H), 7.54 (1H, *d*, *J* = 1.7 Hz, 7′-H), 7.59 (1H, *dd*, *J* = 1.7, 8.3 Hz, 3′-H); ^{13}C NMR spectral data ($CDCl_3$): Table 2; HR-FABMS *m*/*z* (rel. int.): 427.2078 $[M + Na]^+$, $C_{23}H_{32}O_6Na$, requires 427.2097; EIMS *m*/*z* (rel. int.): 43 (26), 71 (13), 121 (10), 151 (100), 168 (27), 193 (9), 235 (10), 361 $[M - 43]^+$ (8).

3.8. Chromium trioxide oxidation of *12*

A solution (0.1 ml) of Jones reagent (Bowers et al., 1953) was cautiously added to a stirred solution of **12** (5.0 mg) in acetone (2 ml) and water (1 ml) at 0°C over 30 min and allowed to reach room temperature over 60 min. About half of the acetone (1 ml) was then removed under reduced pressure. Water (100 ml) was added and then extracted with $CHCl_3$ (100 ml). The $CHCl_3$ extract was next washed with saturated sodium chloride, dried (Na_2SO_4), and on removal of solvent yielded **10** (3.5 mg, 70%).

3.9. Kuhistanicaol E (**13**)

Amorphous. $[\alpha]_D^{25}$ +17.2° (MeOH, *c* 1.2); IR ν_{max}^{KBr} cm^{-1}: 3423, 2965, 1690, 1516, 1285, 1032, 763; UV λ_{max}^{MeOH} nm (log ε): 293 (3.7), 263 (4.0), 221 (4.0); ^1H NMR spectral data δ_H ($CDCl_3$): 0.85 (3H, *d*, *J* = 6.8 Hz, 12-H), 0.93 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.18 (3H, *s*, 15-H), 1.36 (1H, *m*, 2-H), 1.63 (1H, *m*, 3-H), 1.64 (1H, *m*, 2-H), 1.78 (1H, *sept*, *J* = 6.8 Hz, H-11), 1.92 (3H, *s*, 14-H), 1.95 (1H, *m*, 3-H), 1.99 (1H, *m*, 10-Hβ), 2.11 (1H, *dd*, *J* = 5.2, 13.7 Hz, 10-Hα), 2.38 (1H, *d*, *J* = 10.9 Hz, 5-H), 3.94 (3H, *s*, OCH_3), 4.48 (1H, *br s*, 9-H), 5.54 (1H, *br s*, 7-H), 5.86 (1H, *br d*, *J* = ca. 10 Hz, 6-H), 6.95 (1H, *d*, *J* = 8.3 Hz, 4′-H), 7.55 (1H, *d*, *J* = 1.7 Hz, 7′-H), 7.64 (1H, *dd*, *J* = 1.7, 8.3 Hz, 3′-H); ^{13}C NMR spectral data ($CDCl_3$): Table 2; HR-FABMS *m*/*z* (rel. int.): 427.2097 $[M + Na]^+$, $C_{23}H_{32}O_6Na$, requires 427.2091; EIMS *m*/*z* (rel. int.): 43 (36), 71 (16), 77 (14), 123 (20), 151 (100), 168 (48), 191 (14), 402 (19), 404 $[M]^+$ (5).

3.10. Chromium trioxide oxidation of **13**

Treatment of **13** (26 mg), in the same manner as described for **12**, yielded **21** (10 mg, 39%).

3.11. Kuhistanicaol F (**15**)

Amorphous. $[\alpha]_D^{25}$ +87.1° (MeOH, *c* 1.1); IR ν_{max}^{KBr} cm^{-1}: 3416, 2964, 1693, 1607, 1512, 1279, 1259, 1169, 1104, 1031, 757; UV λ_{max}^{MeOH} nm (log ε): 255 (4.1), 212 (4.0); ^1H NMR spectral data δ_H ($CDCl_3$): 0.85 (3H, *d*, *J* = 6.8 Hz, 12-H), 0.92 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.18 (3H, *s*, 15-H), 1.32 (1H, *m*, 2-H), 1.62 (2H, *m*, 2, 3-H), 1.74 (1H, *sept*, *J* = 6.8 Hz, H-11), 1.92 (3H, *s*, 14-H), 1.96 (1H, *m*, 3-H), 1.99 (1H, *m*, 10-Hβ), 2.11 (1H, *dd*, *J* = 5.2, 13.7 Hz, 10-Hα), 2.39 (1H, *d*, *J* = 10.9 Hz, 5-H), 3.87 (3H, *s*, OCH_3), 4.49 (1H, *br s*, 9-H), 5.55 (1H, *br s*, 7-H), 5.86 (1H, *br d*, *J* = ca. 10 Hz, 6-H), 6.94 (2H, *d*, *J* = 8.9 Hz, 4′,6′-H), 8.00 (2H, *d*, *J* = 8.9 Hz, 3′,7′-H); ^{13}C NMR spectral data ($CDCl_3$): Table 2; HR-FABMS *m*/*z* (rel. int.): 411.2120 $[M + Na]^+$, $C_{23}H_{32}O_5$,Na, requires 411.2147; EIMS *m*/*z* (rel. int.): 43 (23), 55 (9), 71 (11), 77 (12), 135 (100), 148 (20), 152 (19), 191 (25), 343 (2), 361 $[M - 43]^+$ (2), 387 (7), 388 (2).

3.12. Kuhistanicaol G (**17**)

Amorphous. $[\alpha]_D^{25}$ −68.9° (MeOH *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3514, 3191, 2971, 1684, 1610, 1289, 1168, 1066; UV λ_{max}^{MeOH} nm (log ε): 258 (4.1), 205 (4.2), 193 (3.7); ^1H NMR spectral data δ_H (CD_3OD): 0.85 (3H, *d*, *J* = 6.8 Hz, 12-H), 0.97 (3H, *d*, *J* = 6.8 Hz, 13-H), 1.24 (3H, *s*, 15-H), 1.29 (3H, *s*, 14-H), 1.47 (1H, *m*, 2-H), 1.55 (1H, *m*, 3-H), 1.63 (1H, *m*, H-2), 1.89 (1H, *sept*, *J* = 6.8 Hz, 11-H), 1.99 (1H, *m*, 3-H), 2.10 (1H, *br d*, *J* = ca. 16 Hz, 7-H), 2.32 (1H, *dd*, *J* = 5.6, 16.1 Hz, 7-H), 3.07 (1H, *d*, *J* = 11.1 Hz, 5-H), 5.43 (1H, *d*, *J* = 12.5 Hz, 9-H), 5.64 (1H, *d*, *J* = 12.5 Hz, 10-H), 5.65 (1H, *br dd*, *J* = ca.6, 11 Hz, 6-H), 6.81 (2H, *d*, *J* = 8.7 Hz, 4′,6′-H), 7.92 (2H, *d*, *J* = 8.7 Hz, 3′,7′-H); ^{13}C NMR spectral data (CD_3OD): Table 2; HR-FABMS *m*/*z* (rel. int.): 397.2008 $[M + Na]^+$, $C_{22}H_{30}O_5Na$, requires 397.1991; EIMS *m*/*z* (rel. int.): 31 (85), 43 (39), 71 (18), 93 (26), 121 (75), 132 (30), 175 (100), 236 (22), 313 (10), 331 $[M - 43]^+$ (20).

References

Ahmed, A.A., 1990. New sesquiterpenes from *Ferula sinica*. J. Nat. Prod. 53, 483.

Ahmed, A.A., 1991. Daucanes and other constituents from *Ferula sinica*. Phytochemistry 30, 1207.

Bowers, A., Halsall, T.G., Jones, E.R.H., Lemin, A.J., 1953. J. Chem. Soc., 2548.

Diaz, J.G., Fraga, B.M., Gonzalez, A.G., Hernandez, M.G., Perales, A., 1984. Eight carotane sesquiterpenes from *Ferula linkii*. Phytochemistry 23, 2541.

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

Garg, S.N., Agrawal, S.K., 1988. Further new sesquiterpenes from *Ferula jasechkeana*. J. Nat. Prod. 51, 771.

Gonzalez, A.G., Barrera, J.B., 1995. Chemistry and sources of mono- and bicyclic sesquiterpenes from *Ferula* species. Prog. Chem. Org. Nat. Prod. 64, 1.

Gonzalez, A.G., Bermejo, J., Diaz, J.G., Arancibia, L., 1988. Humulenes ad other constituents of *Ferula latipinna*. J. Nat. Prod. 51, 1140.

Miski, M., Jakupovic, J., 1990. Daucane esters from *Ferula rigidula*. Phytochemistry 29, 173.

Miski, M., Mabry, T.J., 1985. Daucane esters from *Ferula communis* subsp. *communis*. Phytochemistry 24, 1735.

Miski, M., Mabry, T.J., Saya, O., 1987. New daucane and germacrane esters from *Ferula orientalis* var. *orientalis*. J. Nat. Prod. 50, 829.

Miski, M., Ulubelen, A., Mabry, T.J., 1983. Six sesquiterpene alcohol esters from *Ferula elaeochytris*. Phytochemistry 22, 2231.

Razdan, T.K., Qadri, B., Qurishi, M.A., Khuroo, M.A., Kachroo, P.K., 1989. Sesquiterpene esters and sesquiterpene-coumarin ethers from *Ferula jaeskeana*. Phytochemistry 28, 3389.

Sezik, E., Yesilada, E., Tabata, M., Honda, G., Takaishi, Y., Fujita, T., Tanaka, T., Takede, Y., 1997. Traditional medicine in Turkey VIII. Folk medicine in East Anatoria: Erzurum, Erzincan, Agri, Kars, Igdia provices. Economic Botany 51, 195.

Singh, M.M., Agnihotri, A., Garg, S.N., Agarwal, S.K., Gupta, D.N., Keshri, G., Kamboj, V.P., 1988. Antifertility and hormonal properties of contain carotane sesquiterpenes of *Ferula jaeschkeana*. Planta Med. 54, 492.

Uphof, J.C.TH., 1968. Dictionary of Economic Plants, 2nd ed. Verlag von J. Cramer.