Sesquiterpene Coumarins from the Roots of *Ferula sinkiangensis* and *Ferula teterrima*

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Three new natural sesquiterpene coumarins, isofeterin (1), lehmannolol (3), sinkianone (4), and one known compound, lehmannolone (2), were isolated from the roots of *Ferula teterrima* and *Ferula sinkiangensis*. Their chemical structures were established on the basis of spectroscopic analysis, including X-ray crystallography and CD spectrum measurements for determining the absolute configuration of compound 2.

Key words sesquiterpene coumarin; *Ferula sinkiangensis*; *Ferula teterrima*

The chemical constituents of plants in the genus *Ferula* (Umbelliferea) have been studied by many groups. The compounds commonly found in this genus are sesquiterpenes[1,2] and sesquiterpene coumarins.[1,3] There were reports about sesquiterpene coumarins found in the roots of *F. teterrima*,[4,5] and in previous chemical investigations on *F. sinkiangensis,* polysulfanes were identified by GC-MS.[6] *F. sinkiangensis* and *F. teterrima* mainly grow in Xinjiang Uygur Autonomous Region of China, and have been used in traditional medicine for treatment of rheumatoid arthritis and stomach disease. As part of our studies of the genus *Ferula*, we investigated on the constituents of the roots of *F. sinkiangensis* and *F. teterrima*. These studies have led to the isolation of four natural sesquiterpene coumarins, isofeterin (**1**), lehmannolone (**2**), lehmannolol (**3**), and sinkianone (**4**). In this report, the isolation and structure elucidation of compounds **1**—**4** are presented.

Results and Discussion

The roots of *F. sinkiangensis* were extracted with 95% ethanol, and the extract was separated by solvent partitions to give petroleum ether-, EtOAc-, *n*-BuOH-, and water-soluble fractions. The EtOAc fraction was subjected to repeated column chromatography to afford compounds **2**—**4**. The roots of *F. teterrima* were treated in the same way, and the EtOAc fraction was subjected to repeated column chromatography to afford compound **1**.

Compound **1** was obtained as a white amorphous powder. Its HR-EI-MS spectrum exhibited the $[M]^+$ at *m*/*z* 440.2193, corresponding to the molecular formula $C_{26}H_{32}O_6$. The IR spectrum indicated the presence of carbonyl and hydroxyl groups (1712, 1728, 3433 cm^{-1}). The ^{13}C-NMR spectrum of **1** displayed 26 carbon signals (Table 2). Nine of which were typical for an umbelliferone skeleton and the other 17 carbons were associated to an acetyl group and a sesquiterpene unit. This suggested that **1** was derived from sesquiterpene and umbelliferone structures.[7] The carbon signals were classified referring to the DEPT together with HSQC and ^1H-NMR spectra as four methyl groups at δ_C 31.3, 22.6, 16.9, and 21.9, three aliphatic methylene units at δ_C 31.9, 25.3, and 43.3, an olefinic methylene at δ_C 110.3, a primary oxygenated carbon at δ_C 65.7 characteristic for C-11′, two secondary oxygenated carbons at δ_C 77.0 and 71.7, seven methenyl carbons of which five being to the umbelliferone moiety, and eight quaternary carbons. In the HMBC spectrum, the oxygenated methylene protons H-11′a (δ_H 4.16, dd, J=9.5, 7.5 Hz) and H-11′b (δ_H 4.24, dd, J=9.5, 4.5 Hz) showed long-range coupling to C-7 at δ_C 162.0 of the umbelliferone skeleton. In the ^1H–^1H COSY spectrum, the oxygenated proton H-6′ (δ_H 5.15, td, J=11.0, 5.0 Hz) showed correlations to H-5′ (δ_H 1.97, d, J=11.5 Hz), H-7′ax (δ_H 2.18, dd, J=12.0, 11.0 Hz) and H-7′eq (δ_H 2.76, dd, J=13.0, 5.0 Hz). Together with the ^1H–^{13}C long-range correlation between H-6′ at δ_H 5.15 and C-1″ at δ_C 170.1, it was possible to confirm the attachment of the acetyl group to C-6′. The location of the hydroxyl group at C-3′ and the double bond at C-8′ and C-12′ was determined by HMBC correlation: Me-13′ (δ_H 1.12, 3H, s), Me-14′ (δ_H 0.94, 3H, s), H-2′ax (δ_H 1.94, ov.), and H-2′eq (δ_H 1.69, m) were correlated with C-3′ (δ_C 77.0); H-12′a (δ_H 4.67, s) and H-12′b (δ_H 4.99, s) were associated to C-7′ (δ_C 43.3) and C-9′ (δ_C 53.9), respectively. The observed NOE correlations of Me-15′ with Me-14′ and H-6′, H-9′ with H-5′ as well as H-3′ with Me-13′ and Me-14′ confirmed the axial orientation of Me-14′, Me-15′, H-5′, H-9′, H-6′, and 3′-OH (Fig. 2). Thus the structure of **1** was determined to be as shown in Fig. 1.

Compound **2** was obtained as colorless crystals. Its HR-EI-MS spectrum exhibited the $[M]^+$ at *m*/*z* 382.2124, corre-

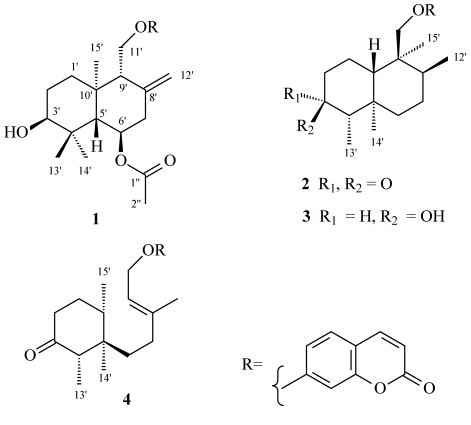

Fig. 1. Structures of Compounds **1**—**4**

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sponding to the molecular formula $C_{24}H_{30}O_4$. The IR spectrum showed absorptions of two carbonyl functions at 1730 and 1707 cm^{-1}. The ^{13}C-NMR spectrum of **2** displayed 24 carbon signals typical for a sesquiterpene coumarin (Table 2). The locations of Me-12′, Me-13′, Me-14′, Me-15′ and the ketone carbonyl were proven by HMBC correlations. The relative configuration of **2** was established by NOESY experiment. The ^1H- and ^{13}C-NMR data were in consistent with those of lehmannolone.[8] Recrystallization of **2** from EtOAc afforded colorless crystals, and unequivocal evidence for the assigned structure was obtained from an X-ray diffraction analysis (Fig. 4).

The absolute configuration of compound **2** was derived from the CD spectrum based on the octant rule and comparison with the CD spectra of drimachone.[9] The CD spectra of **2** in EtOH exhibited a positive Cotton effect at 288 nm with $\Delta\varepsilon = +7.7$, which indicated that the absolute configuration of **2** was 5′*R*, 10′*S*. Thus the complete structure of **2** was determined to be as shown in Fig. 1. Compound **2** had ever been isolated from *F. lehmannii*,[8] with no data of optical rotation reported.

Compound **3** was isolated as an amorphous powder. Its molecular formula $C_{24}H_{32}O_4$ was established by HR-EI-MS (*m*/*z* 384.2308 $[M]^+$). The IR spectrum indicated the presence of hydroxyl (3444 cm^{-1}) and carbonyl groups (1730 cm^{-1}). The ^1H- and ^{13}C-NMR spectral data of **3** showed close resemblance to that of **2**. The main difference was the absence of the signal for the ketone carbonyl at C-3′ and the appearance of one oxygenated methine signal at δ_C 71.7 and δ_H 3.39 (1H, td, J=10.5, 5.0 Hz). Differences in shifts compared to the signals from compound **2** at C-1′ (−2.5), C-2′ (−5.0), C-4′ (−5.2), and C-5′ (−3.8) were observed. The NOE correlations between Me-14′ and H-3′, Me-14′ and Me-15′, Me-12′ and H-10′, H-10′ and H-4′ allowed the definition of the structure of **3** to be as shown in Fig. 1.

Compound **4**, obtained as an amorphous powder, had the molecular formula $C_{24}H_{30}O_4$ based on HR-EI-MS (*m*/*z* 382.2168 $[M]^+$). The IR spectrum showed absorptions of two carbonyl functions at 1734 and 1712 cm^{-1}. ^1H- and ^{13}C-NMR spectral data indicated that compound **4** was also a

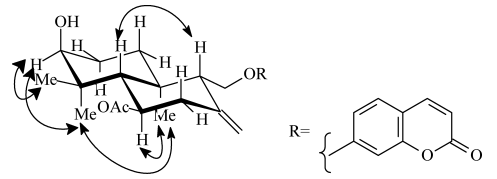

Fig. 2. Key NOEs Observed in **1**

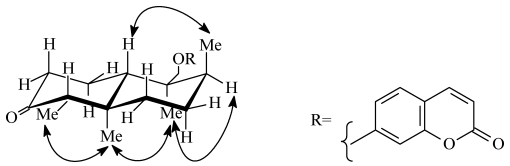

Fig. 3. Key NOEs Observed in **2**

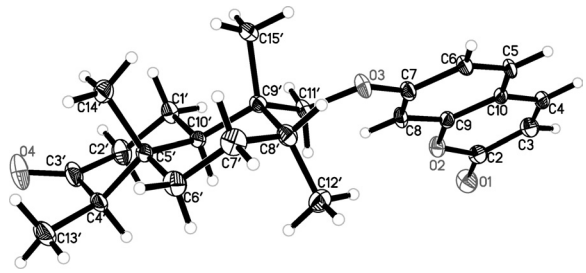

Fig. 4. Diagram of **2** by X-Ray

Table 1. ^1H-NMR (500 MHz) Data of **1**—**4** (J in Hz, Recorded in $CDCl_3$)

H	1	2	3	4
3	6.24 (d, 9.5)	6.25 (d, 9.5)	6.23 (d, 9.5)	6.25 (d, 9.5)
4	7.62 (d, 9.5)	7.63 (d, 9.5)	7.62 (d, 9.5)	7.64 (d, 9.5)
5	7.35 (d, 8.0)	7.37 (d, 8.5)	7.35 (d, 8.5)	7.37 (d, 8.5)
6	6.83 (dd, 8.0, 2.5)	6.86 (dd, 8.5, 2.5)	6.83 (dd, 8.5, 2.0)	6.85 (dd, 8.5, 2.0)
8	6.82 (d, 2.5)	6.81 (d, 2.0)	6.79 (d, 2.0)	6.82 (d, 2.0)
1′ax	1.94 (ov.)[a]	1.83 (m)	1.47 (ov.)	1.64 (m)
1′eq	1.47 (m)	1.81 (m)	2.03 (m)	1.86 (m)
2′ax	1.94 (ov.)	2.35 (ov.)	1.30 (ov.)	2.34 (m)
2′eq	1.69 (m)	2.45 (m)	2.10 (m)	2.36 (m)
3′	3.39 (br s)		3.39 (td, 10.5, 5.0)	
4′		2.35 (ov.)	1.13 (m)	2.48 (q, 7.0)
5′	1.97 (d, 11.5)			
6′ax	5.15 (td, 11.0, 5.0)	1.53 (td, 13.0, 3.5)	1.30 (ov.)	1.50 (td, 14.0, 3.5)
6′eq		1.45 (dt, 13.5, 3.5)	1.47 (ov.)	1.43 (td, 13.0, 3.0))
7′ax	2.18 (dd, 12.0, 11.0)	1.97 (tt, 13.5, 4.0)	1.95 (tt, 13.5, 4.0)	1.96 (td, 13.0, 4.5)
7′eq	2.76 (dd, 13.0, 5.0)	1.41 (m)	1.33 (m)	2.11 (td, 13.5, 4.5)
8′		1.90 (m)	1.83 (m)	
9′	2.40 (br s)			5.50 (t, 6.5)
10′		2.10 (dd, 11.5, 4.5)	1.47 (ov.)	2.02 (m)
11′a	4.16 (dd, 9.5, 7.5)	3.76 (d, 9.0)	3.66 (d, 8.5)	4.60 (2H, d, 6.5)
11′b	4.24 (dd, 9.5, 4.5)	3.79 (d, 9.0)	3.72 (d, 9.0)	
12′	4.67 (s), 4.99 (s)	1.03 (d, 6.5)	0.94 (d, 7.0)	1.80 (s)
13′	1.12 (s)	0.93 (d, 7.0)	0.94 (d, 7.0)	0.94 (d, 7.0)
14′	0.94 (s)	0.82 (s)	0.86 (s)	0.61 (s)
15′	0.94 (s)	1.12 (s)	1.06 (s)	0.90 (d, 7.0)
2″	2.06 (s)			

a) ov.=overlapped signals.

Table 2. ^{13}C-NMR Data for **1**—**4** (125 MHz, in $CDCl_3$)

C	1	2	3	4
2	161.2	161.0	161.2	161.5
3	113.1	113.1	112.9	113.3
4	143.4	143.3	143.4	143.7
5	128.7	128.7	128.6	129.0
6	113.1	112.9	113.0	113.4
7	162.0	162.2	162.5	162.3
8	101.3	101.4	101.4	101.8
9	155.9	155.9	155.9	156.1
10	112.5	112.6	112.4	112.7
1′	31.9	23.5	21.0	31.1
2′	25.3	41.4	36.4	41.8
3′	77.0	212.1	71.7	213.8
4′	37.7	58.1	52.9	50.7
5′	51.2	41.7	37.9	43.6
6′	71.7	32.3	32.4	35.6
7′	43.3	25.4	25.1	32.7
8′	142.6	35.7	35.3	142.6
9′	53.9	39.5	39.1	118.8
10′	38.7	44.0	44.6	36.4
11′	65.7	75.9	76.1	65.7
12′	110.3	14.7	14.8	17.2
13′	31.3	6.8	9.9	7.8
14′	22.6	14.4	14.2	15.6
15′	16.9	19.9	19.9	15.3
1″	170.1			
2″	21.9			

sesquiterpene coumarin (Tables 1, 2). The ^{1}H-NMR spectrum displayed two secondary methyl groups Me-13′ (δ_H 0.94, 3H, d, J=7.0 Hz) and Me-15′ (δ_H 0.90, 3H, d, J=7.0 Hz), two tertiary methyl groups Me-12′ (δ_H 1.80, 3H, s) and Me-14′ (δ_H 0.61, 3H, s), an oxygenated methylene unit H-11′ (δ_H 4.60, 2H, d, J=6.5 Hz), an olefinic proton H-9′ (δ_H 5.50, d, J=6.5 Hz), as well as another ten aliphatic protons from the sesquiterpene unit and five aromatic protons from the umbelliferone moiety. The ^{13}C-NMR spectrum indicated the presence of a ketone carbonyl C-3′ at δ_C 213.8 and two olefinic carbons C-8′ and C-9′ at δ_C 142.6 and 118.8. The HMBC correlations of Me-14′ (δ_H 0.61, 3H, s) with C-10′ (δ_C 36.4), C-4′ (δ_C 50.7), C-6′ (δ_C 35.6), C-5′ (δ_C 43.6) and Me-13′ (δ_H 0.94, 3H, d, J=7.0 Hz) with C-4′ (δ_C 50.7), C-3′ (δ_C 213.8) and C-5′ (δ_C 43.6); Me-15′ (δ_H 0.90, 3H, d, J=7.0 Hz) with C-5′ (δ_C 43.6), C-10′ (δ_C 36.4), C-1′ (δ_C 31.1), as well as Me-12′ (δ_H 1.80, 3H, s) with C-8′ (δ_C 142.6), C-9′ (δ_C 118.8) and C-7′ (δ_C 32.7) established the attachment of Me-14′ to C-5′, Me-13′ to C-4′, Me-15′ to C-10′ and Me-12′ to C-8′. The HMBC correlations of the olefinic proton H-9′ (δ_H 5.50, 1H, d, J=6.5 Hz) with C-7′ (δ_C 32.7), C-11′ (δ_C 65.7), and C-12′ (δ_C 17.2) suggested that the double bond is located at C-8′ and C-9′. NOE correlations between Me-14′ and H-1′ax, H-2′ax and H-10′, H-2′ax and H-4′ confirmed the relative configuration. Thus the structure of **4** was determined to be as shown in Fig. 1. Compound **4** was identified as a side product in the synthesis of farnesiferol C.[10] Here it was isolated from nature for the first time and named sinkianone.

Experimental

General Experimental Procedures Melting points were measured using a XT_4-100x microscopic apparatus and are uncorrected. Optical rotations were measured with a PEModel 343 digital polarimeter. CD spectra were measured on a JASCO J-715 circular dichroism spectrometer. IR spectra were determined using an IMPACT-400 FTIR spectrometer. The ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz) spectra as well as 2D NMR spectra were measured on INOVA-500. MS spectra were recorded on a Autospec-UItima ETOF instrument. Silica gel (200—300 mesh, Qingdao Haiyang Chemical Group Co., Qingdao, P.R. China) was used for column chromatography (CC). X-Ray crystallography was performed by using R-AXIS-IV.

Plant Material The roots of the title plants were collected in Xin jiang Uygur Autonomous Region, People's Republic of China, in May 2005 for *F. sinkisngensis* and June 2002 for *F. teterrima*. The former was identified by Prof. Song Jing (Xin Jiang Yi Li Prefecture Institute for Drug Control) and the later by Dr. Guo-Qiang Li (Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College). Two voucher specimens (No. 337-05 and No. 337-06) were deposited in the Department of Natural Medicinal Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Extraction and Isolation The air-dried and powdered roots (2.7 kg) of *F. sinkiangensis* were refluxed with 95% EtOH three times for 2 h. The combined EtOH extracts were evaporated under reduced pressure to yield a viscous residue (350 g), which was dissolved in 80% aq. EtOH (*ca.* 3000 ml), followed by extraction with petroleum ether (60—90 °C) (1500 ml×3). Evaporation of the aq. layer under reduced pressure yielded a brown residue which was dissolved in water (*ca.* 3000 ml), and then extracted with EtOAc (1500 ml×3). The combined EtOAc layer was washed with aq. 5% $NaHCO_3$ (2000 ml×3), then with water (2000 ml×2) and dried with anhydrous Na_2SO_4. After removal of the organic solvent under reduced pressure, a brown residue (125 g) was obtained. The residue was chromatographed over silica gel (200—300 mesh, 950 g) eluted with petroleum ether (60—90 °C)–EtOAc of increasing polarity to obtain 140 fractions (500 ml for each). Compound **4** (780 mg) was identified in fractions 25—28. Fractions 29—46 were combined and chromatographed on silica gel column, eluted with petroleum ether–EtOAc (4 : 1), to give compound **2** (150 mg). Fractions 69—79 were combined and chromatographed to give compound **3** (30 mg).

The roots of *F. teterrima* (8 kg) were extracted with 95% EtOH under reflux. The extracts were treated in the same way to give an EtOAc-soluble fraction (300 g), which was subjected to silica gel column, eluted with petroleum ether–EtOAc of increasing polarity to obtain 200 fractions (500 ml for each). Fractions 130—136 were combined and chromatographed on silica gel column, eluted with petroleum ether–EtOAc (4 : 1), to give compound **1** (20 mg).

Isofeterin (**1**): Amorphous powder, $[\alpha]_D^{18}$ −43.0° (c=0.27, $CHCl_3$). IR (KBr) cm^{-1}: 3554, 3433, 2943, 2877, 1728, 1712, 1620, 1614, 1267, 1232. ^{1}H- and ^{13}C-NMR see Tables 1 and 2. EI-MS m/z (%): 440 (M^+) (10), 422 (37), 201 (100), 187 (43), 162 (48), 119 (54). HR-EI-MS m/z: 440.2193 (Calcd for $C_{26}H_{32}O_6$: 440.2199).

Lehmannolone (**2**): Colorless crystal (EtOAc), mp 197—198 °C, $[\alpha]_D^{18}$ +38.3° (c=0.35, $CHCl_3$). CD (c=3×10^{-4} g/ml, EtOH): λ_{max} ($\Delta\varepsilon$)=288 (+7.7). IR (KBr) cm^{-1}: 2952, 1730, 1707, 1610, 1120. ^{1}H- and ^{13}C-NMR see Tables 1 and 2. EI-MS m/z (%): 382 (M^+) (42), 163 (69), 162 (100), 95 (50), 83 (62), 69 (85), 55 (82). HR-EI-MS m/z 382.2124 (Calcd for $C_{24}H_{30}O_4$: 382.2144).

X-Ray Crystal Structure Determination of **2**: Crystal data: $C_{24}H_{30}O_4$, 382.48, orthorhombic, space group $P2_12_12_1$, a=7.6202(15) Å, b=14.261(3) Å, c=18.865(4) Å, $\alpha=\gamma=\beta$=90°, V=2050.1(7) Å^3, Z=4, D_{calc}=1.239 g/cm^3, λ=0.71073 Å, μ(Mo$K\alpha$)=0.083 mm^{-1}, F(000)=824, T=291(2) K. Of the 6561 reflections that were collected, 2161 were unique (R_{int}=0.0428). The structure was solved by direct methods with SHELXS-97 and refined by full-matrix least-squares on F^2. Final refinement: data/restraints/parameters=2161/0/254; R_1=0.0760 (all data), wR_2=0.1284 (all data). Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 294963. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Lehmannolol (**3**): Amorphous powder, $[\alpha]_D^{18}$ −10.4° (c=0.41, $CHCl_3$). IR (KBr) cm^{-1}: 3444, 2931, 1730, 1612, 1122. ^{1}H- and ^{13}C-NMR see Tables 1 and 2. EI-MS m/z (%): 384 (M^+) (8), 366 (29), 205 (36), 203 (56), 163 (40), 162 (49), 109 (34), 95 (100). HR-EI-MS m/z 384.2308 (Calcd for $C_{24}H_{32}O_4$: 384.2300).

Sinkianone (**4**): Amorphous powder, $[\alpha]_D^{18}$ ±0° (c=0.76, $CHCl_3$). IR (KBr) cm^{-1}: 2966, 2939, 1734, 1712, 1610, 1120. ^{1}H- and ^{13}C-NMR see Tables 1 and 2. EI-MS m/z (%): 382 (M^+) (15), 221 (53), 163 (85), 162 (100), 139 (50), 97 (76), 81 (87), 55 (77). HR-EI-MS m/z 382.2168 (Calcd for $C_{24}H_{30}O_4$: 382.2144).

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