Two Novel Sesquiterpenoids from the Roots of *Ferula ferulaeoides* (Steud.) Korov.

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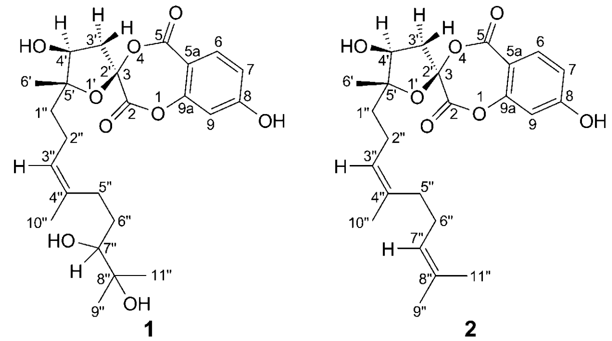
Two new sesquiterpenoids, ferulactones A and B (**1** and **2**, resp.), have been isolated from the roots of *Ferula ferulaeoides* (Steud.) Korov. Their structures and relative configurations were established by analysis of spectroscopic data. Their absolute configuration was assigned by application of the CD technique.

Introduction. – The genus *Ferula* from the family of Umbelliferae has been used as the source of medicinal plants with a rich history in central Asia. Over 150 species are distributed in the world, mostly in the Mediterranean sea region and central Asia region, and among them 26 species were found in China [1]. The plants from the genus *Ferula* have been reported to exhibit a variety of biological effects, such as antispasmodic and hypotensive effect [2], anticonvulsant activity [3], antibacterial activity [4], reproductive toxicity, and infertility effect [5]. *Ferula ferulaeoides* (Steud.) Korov. is a traditional ethnic medicine, and the gum obtained from the aerial parts has been used for detoxication, and for anti-inflammatory helminthicide, anti-convulsion, anti-epileptic, and antifertility treatments [6]. To study the bioactive components of a different part of the plant, the 95% EtOH extract of the roots from *F. ferulaeoides* was isolated. Here, we describe the isolation and structural elucidation of two novel sesquiterpenoids, ferulactones A and B (**1** and **2**, resp.) (see *Fig. 1*) from the roots of *F. ferulaeoides.*

Results and Discussion. – Compound **1** was obtained as a cinnamon amorphous solid and its molecular formula, $C_{24}H_{32}O_9$, was deduced from the HR-ESI-MS ($[M-H]^+$ at *m/z* 463.1994), indicating the presence of nine degrees of unsaturation. Twenty-four C-atom signals comprising four Me, five CH_2, six CH, and nine quaternary C-atoms were evident from the ^{13}C-NMR and HSQC spectra. In addition, two O-bearing CH groups (δ(C) 65.4 (C(4')), 77.1 (C(7''))), three O-bearing quaternary C-atoms (δ(C) 92.1 (C(2')), 85.7 (C(5')), and 71.6 (C(8''))), two CO C-atoms (δ(C) 161.3 (C(2)) and 176.2 (C(5))), and one C=C bond (δ(C) 123.0 (C(3'')) and 135.7 (C(4''))) could be discerned by analysis of the HSQC spectrum (*Table 1*). In the ^{1}H-NMR spectrum of **1** three aromatic H-atoms showing up as an *ABX* system (δ(H) 7.81 (*d*, $J = 9.0$, H−C(6)),



1 **2**

Fig. 1. *Structures of compounds* **1** *and* **2**

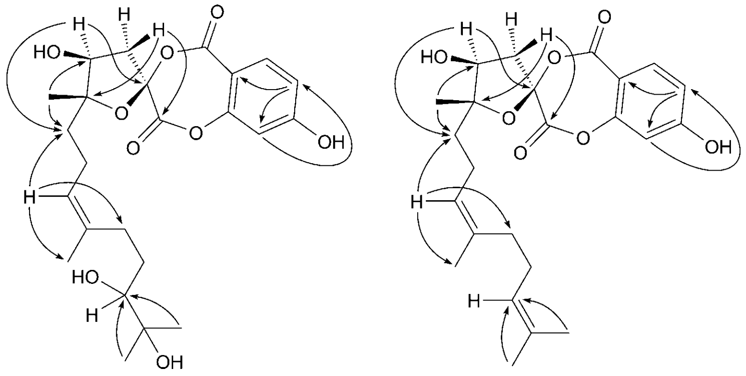
6.86 (*d*, $J=9.0$, H–C(7)), and 6.78 (br. *s*, H–C(9))), indicated the presence of a trisubstituted phenyl group.

Table. *^{1}H- (600 MHz) and ^{13}C-NMR Data* (150 MHz) *of* **1**[a]) *and ^{1}H-* (600 MHz) *and ^{13}C-NMR Data* (150 MHz) of **2**[b]). δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(2)	161.3		162.4	
C(3) = C(2′)	92.1		93.4	
C(5)	176.2		177.6	
C(5a)	114.5		115.9	
H–C(6)	126.4	7.81 (*d*, $J=9.0$)	127.5	8.39 (*d*, $J=9.0$)
H–C(7)	114.3	6.86 (*d*, $J=9.0$)	115.1	7.18 (*dd*, $J=9.0$, $J=1.8$)
C(8)	161.9		163.6	
H–C(9)	102.0	6.78 (br. *s*)	103.0	7.06 (*d*, $J=1.8$)
C(9b)	154.4		155.7	
CH_2(3′)	24.3	2.35 (*dd*, $J=16.2$, 9.6), 2.64 (*dd*, $J=16.2$, 6.0)	25.7	3.07 (*dd*, $J=16.0$, 6.0), 3.30 (*dd*, $J=16.0$, 7.2)
H–C(4′)	65.4	3.78 (br. *s*)	66.9	4.20 (*dd*, $J=7.2$, 6.0)
C(5′)	85.7		86.3	
Me(6′)	18.4	1.27 (*s*)	18.7	1.57 (*s*)
CH_2(1″)	36.9	1.64–1.65 (*m*)	38.1	1.94–1.95 (*m*), 2.02–2.03 (*m*)
CH_2(2″)	21.1	2.07–2.08 (*m*), 2.12–2.16 (*m*)	22.0	2.30–2.32 (*m*), 2.39–2.40 (*m*)
H–C(3″)	123.0	5.11–5.12 (*m*)	124.3	5.24–5.26 (*m*)
C(4″)	135.7		135.7	
CH_2(5″)	36.6	1.86–1.87 (*m*), 2.15–2.16 (*m*)	40.0	2.04–2.05 (*m*), 2.14–2.15 (*m*)
CH_2(6″)	29.4	1.17–1.18 (*m*), 1.58–1.60 (*m*)	27.1	2.07–2.08 (*m*), 2.16–2.17 (*m*)
H–C(7″)	77.1	3.01–3.05 (*m*)	124.9	5.16–5.18 (*m*)
C(8″)	71.6		131.4	
Me(9″)	26.3	1.03 (*s*)	25.8	1.65 (*s*)
Me(10″)	16.0	1.57 (*s*)	16.1	1.61 (*s*)
Me(11″)	24.6	0.97 (*s*)	17.8	1.59 (*s*)
3 OH		5.34 (br. *s*), 4.27 (br. *s*), 4.02 (br. *s*)		

[a]) Measured in (D_6)DMSO. [b]) Measured in (D_5)pyridine.

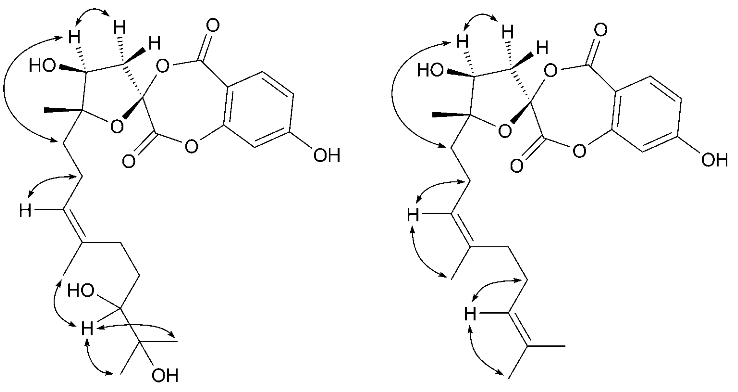
Analysis of the ^{1}H- and ^{13}C-NMR, HSQC, HMBC (*Fig. 2*), and ^{1}H,^{1}H-COSY spectra enabled us to allot the H-atoms to their bonded C-atoms, and suggested that **1** is a sesquiterpenoid. At the same time, it was demonstrated that the Me(10'') group (δ(C) 16.0, δ(H) 1.57) was connected to C(4'') (δ(C) 135.7), which is part of a C=C bond, and the CH$_2$(5'') group (δ(C) 36.6, δ(H) 1.86–1.87 and 2.15–2.16) was connected to the same C-atom (δ(C) 135.7, C(4'')), and another C=C bond C-atom (H–C(3'', δ(C) 123.0, δ(H) 5.11–5.12) was connected to the CH$_2$(2'') group (δ(C) 21.1, δ(H) 2.07–2.08 and 2.12–2.16) by means of ^{1}H,^{1}H-COSY and HMBC spectra. The Me(6') group (δ(C) 18.4, δ(H) 1.27), the CH$_2$(1'') group (δ(C) 36.9, δ(H) 1.64–1.65), and the O-bearing H–C(4') group (δ(C) 65.4, δ(H) 3.78) were all connected to an O-bearing quaternary C-atom (δ(C) 85.7, C(5')). The CH$_2$(3') group (δ(C) 24.3, δ(H) 2.35 and 2.64) was connected to the O-bearing H–C(4') group (δ(C) 65.4, δ(H) 3.78). Me(9'') (δ(C) 26.3, δ(H) 1.03), Me(11'') (δ(C) 24.6, δ(H) 0.97), and the O-bearing H–C(7'') group (δ(C) 77.1, δ(H) 3.01–3.05) were confirmed to be linked to the O-bearing quaternary C-atom C(8'') (δ(C) 71.6), and H–C(7'') was also linked to the CH$_2$(6'') group (δ(C) 29.4, δ(H) 1.17–1.18 and 1.58–1.60). The ^{1}H,^{1}H-COSY spectra demonstrated that the CH$_2$(2'') group (δ(C) 21.1, δ(H) 2.07–2.08 and 2.12–2.16) was connected to the CH$_2$(1'') group (δ(C) 36.9, δ(H) 1.64–1.65), and the CH$_2$(5'') group (δ(C) 36.6, δ(H) 1.86–1.87 and 2.15–2.16) was connected to CH$_2$(6'') (δ(C) 29.4, δ(H) 1.17–1.18 and 1.58–1.60). The HMBC spectra demonstrated that the CH$_2$(3') group (δ(C) 24.3, δ(H) 2.35 and 2.64) was connected to the CO group (δ(C) 161.3 (C(2))).



1 **2**

Fig. 2. *Key HMBCs of compounds* **1** *and* **2**

The relative configuration of **1** was determined by examination of coupling constants in the ^{1}H-NMR and by the correlations in the NOESY plot (*Fig. 3*). The correlation between the signals δ(H) 3.78 (H–C(4')) and δ(H) 2.64 (H$_a$–C(3')) indicated that H–C(4') and H$_a$–C(3') are positioned on the same side of the ring. The signal δ(H) 3.78 (H–C(4')) was correlated with the signal δ(H) 1.64–1.65 (CH$_2$(1'')), suggesting that H–C(4') and CH$_2$(1'') were on the same side of the ring system. The CD spectrum (*Fig. 4*) showed a clearly positive *Cotton* effect at 249 nm, which is consistent with the axiom of an α,β-unsaturated cycloketone [7]. The structure and absolute



1 **2**

Fig. 3. *Key NOESY correlations of compounds* **1** *and* **2**

configuration of **1** were, therefore, elucidated as (3*S*,4′*S*,5′*R*)-5′-[(3*Z*)-7,8-dihydroxy-4,8-dimethylnon-3-en-1-yl]-4′,5′-dihydro-4′,8-dihydroxy-5′-methyl-3′*H*,5*H*-spiro[1,4-benzodioxepine-3,2′-furan]-2,5-dione, named ferulactone A.

Compound **2**, was isolated as a yellow amorphous solid, and its molecular formula, $C_{24}H_{30}O_7$ was deduced from the HR-ESI-MS (*m*/*z* 429.1935, $[M-H]^+$), indicating the presence of ten degrees of unsaturation. Analysis of the ^{1}H- and ^{13}C-NMR spectra established that **2** was a sesquiterpene derivate too. Compared with compound **1**, one more C=C bond (δ(C) 124.9 (C(7″)) and 131.4 (C(8″))) was found in compound **2**. The resonances of H−C(7″) (δ(H) 3.01–3.05, δ(C) 77.1) and C(8″) (δ(C) 71.6) for compound **1** were shifted downfield to H−C(7″) (δ(H) 5.16–5.18, δ(C) 124.9) and C(8″) (δ(C) 131.4), respectively, for compound **2**. The resonances of Me(9″) (δ(C) 26.3, δ(H) 1.03 (*s*)) and Me(11″) (δ(C) 24.6, δ(H) 0.97 (*s*)) for compound **1**. connected to the O-bearing quaternary C-atom C(8″) (δ(C) 71.6), had changed to the signals for Me(9″) (δ(C) 25.8, δ(H) 1.65 (*s*)) and Me(11″) (δ(C) 17.8, δ(H) 1.59 (*s*)) in compound **2**, connected to quaternary C-atom C(8″) (δ(C) 131.4). The linkage and O-bearing patterns of the C-atoms were determined by analysis of the ^{1}H- and ^{13}C-NMR spectra and HMBCs (*Fig. 2*).

The relative configuration of compound **2** was confirmed by a NOESY spectrum (see *Fig. 3*). The correlation between the signals δ(H) 5.16–5.18 (H−C(7″)) and δ(H) 1.65 (Me(9″)) indicated that H−C(7″) and Me(9″) (δ(C) 25.8, δ(H) 1.65 (*s*)) were situated in *cis*-orientation. Compared with compound **1**, the relative configuration of the remaining part of compound **2** was identical to the one of compound **1**. In the CD spectrum, it showed a clearly positive *Cotton* effect at 249 nm (see *Fig. 4*), being consistent with the proposed absolute configuration [7]. The structure and absolute configuration of **2** was therefore elucidated as (3*S*,4′*S*,5′*R*)-5′-[(3*Z*)-4,8-dimethylnona-3,7-dien-1-yl]-4′,5′-dihydro-4′,8-dihydroxy-5′-methyl-3′*H*,5*H*-spiro[1,4-benzodioxepine-3,2′-furan]-2,5-dione, named ferulactone B.

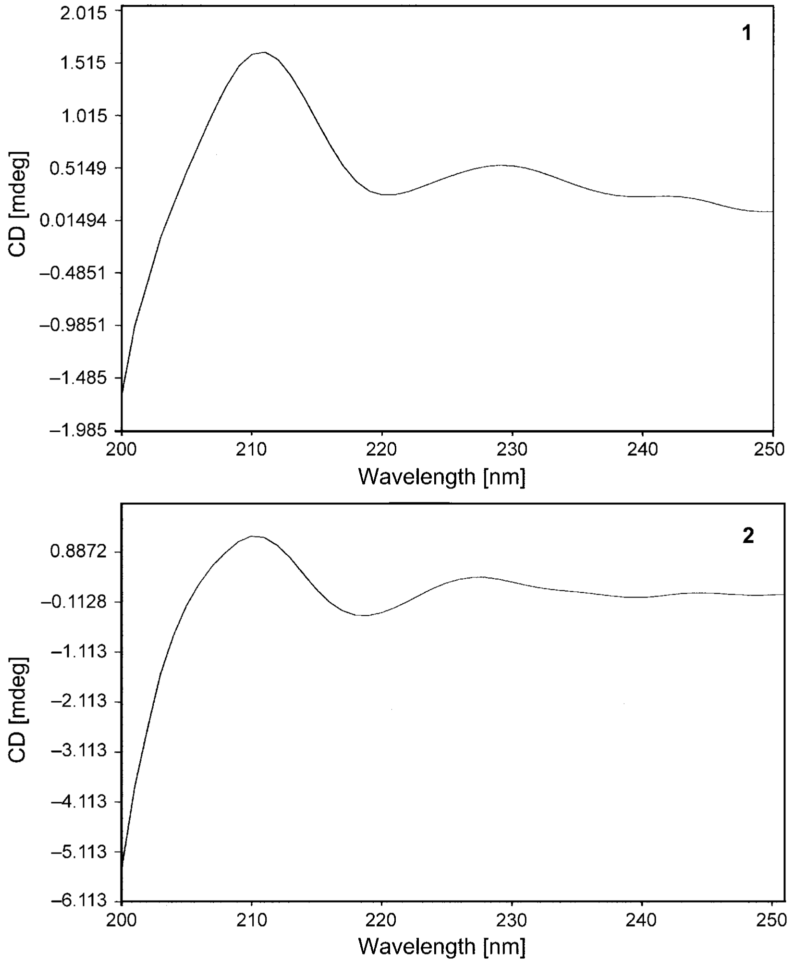


Fig. 4. *CD Curves of compounds* **1** *and* **2**

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Group, Co.*); *Sephadex LH-20* (*Pharmacia, Co.*). Prep. HPLC: *Hitachi-L-7110* pump, *Hitachi L-7420* UV spectrophotometric detector at 210 nm, *YMC C_{18}* reversed-phase column (5 μm, 10 × 250 mm; flow rate 2.5 ml/min). CD Spectrum: *MOS-450* spectrometer; MeOH soln.; in nm (mdeg). NMR Spectra: *Bruker AV-600* and *ARX-300* spectrometer; δ in ppm rel. to TMS as internal standard, J in Hz. HR-ESI-MS: *BIC micro TOF-Q* mass spectrometer; in *m/z*.

Plant Material. The roots of *Ferula ferulaeoides* (Steud.) Korov. were collected in Shawan (Xinjiang Province, P. R. China) and identified by Prof. *Ping Yan*, School of Pharmacy, Shihezi University. A voucher specimen (No. 20050803) was deposited with the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation. The dried roots of *Ferula ferulaeoides* (Steud.) Korov. (1.3 kg) were extracted with 95% EtOH (5 × 1.3 l) under reflux conditions to give a crude extract, which was suspended in H_2O and extracted with petroleum ether (PE), AcOEt, and BuOH, successively, to yield a PE-soluble fraction (70.8 g), an AcOEt-soluble fraction (147.3 g) and a BuOH-soluble fraction (50.1 g).

A part of the AcOEt-soluble fraction (120.0 g) was subjected to CC (SiO_2; gradient of PE/acetone) to furnish a mixture (20.6 g). This fraction was resubjected to CC (SiO_2; gradient $CHCl_3$/acetone): *Frs. A* and *B*. *Fr. A* was separated by CC (*ODS*; H_2O containing an increasing amount of MeOH) to yield *Fr. A.2* which was further subjected prep. reversed-phase HPLC (33% MeCN): **1** (12 mg). *Fr. B* was separated by CC (*ODS*; H_2O containing an increasing amount of MeCN) to yield **2** (800 mg).

Ferulactone A (=(3S,4′S,5′R)-5′-[(3Z)-7,8-Dihydroxy-4,8-dimethylnon-3-en-1-yl]-4′,5′-dihydro-4′,8-dihydroxy-5′-methyl-3′H,5H-spiro[1,4-benzodioxepine-3,2′-furan]-2,5-dione*; **1**). Cinnamon amorphous solid. CD: 210 (+1.52), 230 (+0.52), 249 (+0.28). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 463.1994 ($[M-H]^+$, $C_{24}H_{31}O_9^+$; calc. 463.1968).

Ferulactone B (=(3S,4′S,5′R)-5′-[(3Z)-4,8-Dimethylnona-3,7-dien-1-yl]-4′,5′-dihydro-4′,8-dihydroxy-5′-methyl-3′H,5H-spiro[1,4-benzodioxepine-3,2′-furan]-2,5-dione*; **2**). Yellow amorphous solid. CD: 210 (+0.92), 230 (+0.23), 249 (+0.15). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 429.1935 ($[M-H]^+$, $C_{24}H_{29}O_7^+$; calc. 429.1913).

REFERENCES

[1] J. R. Yang, G. J. Li, Z. H. Li, *Nat. Prod. Res. Dev.* **2006**, *18*, 246.
[2] M. Fatehi, F. Farifteh, Z. Fatehi-Hassanabad, *J. Ethnopharmacol.* **2004**, *91*, 321.
[3] M. Sayyah, A. Mandgary, M. Kamalinejad, *J. Ethnopharmacol.* **2002**, *82*, 105.
[4] F. Eftekhar, M. Yousefzadi, K. Borhani, *Fitoterapia* **2004**, *75*, 758.
[5] M. H. Homady, K. M. Khleifat, K. Tarawneh, I. A. AlRaheil, *Theriogenology* **2002**, *57*, 2247.
[6] D. W. Song, W. J. Zhao, X. P. Wu, Z. J. Cao, L. Gao, *Chin. Tradit. Pat. Med.* **2005**, *3*, 329.
[7] L. J. Wu, 'Shiyong Tianran Youji Chanwu Huaxue', People's Medical Publishing House, 2007, p. 330.

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