Sesquiterpenoid Derivatives from *Ferula ferulioides*

Keisuke KOJIMA,*[,a] Kimio ISAKA,[a] Ondognii PUREV,[b] Gonbovanjilin JARGALSAIKHAN,[b] Dagdangin SURAN,[b] Hajime MIZUKAMI,[a] and Yukio OGIHARA*[,a]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] 3–1 Tanabe-Dori, Mizuho-ku, Nagoya 467–8603, Japan and Branch of Mongolian State University,[b] City Hovd, Mongolia. Received July 24, 1998; accepted August 21, 1998

Four new sesquiterpenoid derivatives, 1-(2,4-dihydroxyphenyl)-2-hydroxy-5,9,13-trimethyl-4(*E*),8(*E*),12-tetradecatrien-1-one, 1-(2,4-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadiene-1-one, 1-(2,4-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadiene-1,9-dione and 1-(2,4-dihydroxyphenyl)-3,7-dimethyl-3-vinyl-8-(4-methyl-2-furyl)-6(*E*)-octen-1-one, were isolated from the roots of *Ferula ferulioides*. The structures of these new compounds were established by comprehensive spectral analysis. The biosynthetic pathway leading to these compounds is proposed based on their structures.

Key words *Ferula ferulioides*; Umbelliferae; sesquiterpenoid; myristicin; nerolidol; dshamirone

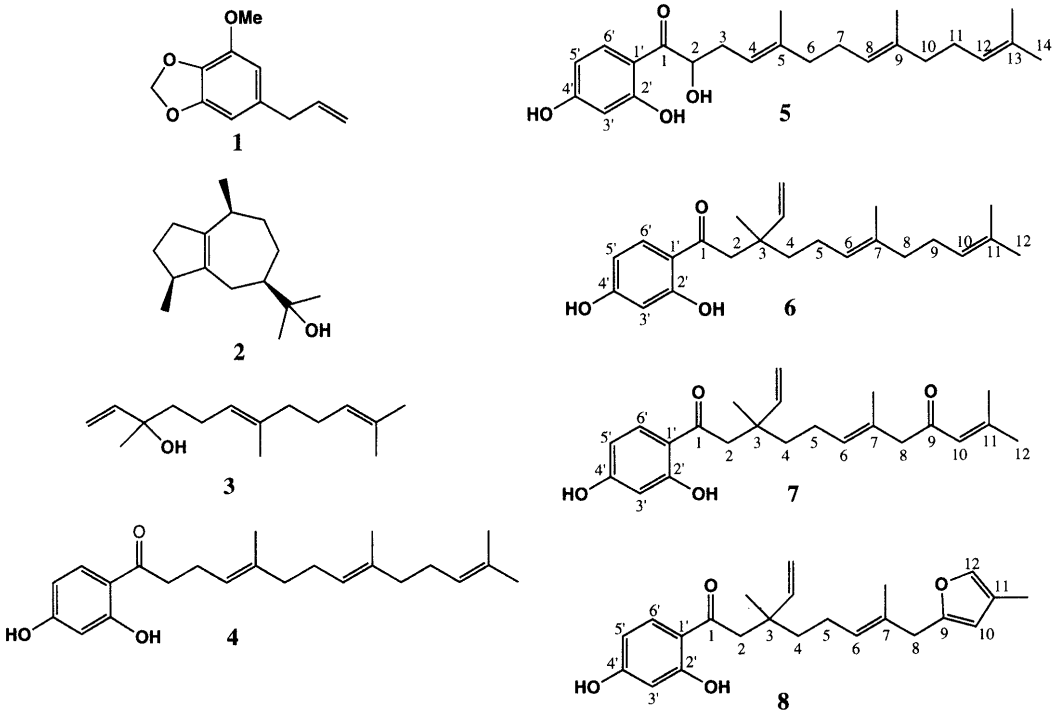
Ferula ferulioides (STEUD.) KOROVIN (Umbelliferae) grows in Bulgan Somon of Hovd City, Mongolia, and has been used as a traditional medicine for the treatment of spasm. The present paper deals with the isolation and the structural elucidation of four novel sesquiterpenoid derivatives (**5**—**8**), together with four known compounds (**1**—**4**) from *F. ferulioides*.

After drying and pulverization, the roots were extracted with methanol, and removal of the solvent gave a waxy solid which was successively extracted with ethyl acetate and water. From the ethyl acetate extract, four novel compounds (**5**—**8**) were isolated and identified with four known compounds, myristicin[1] (**1**), guaiol[2—5] (**2**), nerolidol[2] (**3**) and dshamirone[6] (**4**). Myristicin is a phenylpropanoid derivative; nerolidol and guaiol are sesquiterpenes.

The molecular formula of compound **5** was determined to be $C_{23}H_{32}O_4$ ($[M]^+$ at *m/z* 372.2298) by high-resolution mass spectrometry (HR–MS). The ^{1}H-NMR and ^{13}C-NMR spectra of **5** showed the existence of the aromatic protons of an ABC system (δ_H 6.43, 6.44, 7.54), four olefinic-methyl groups (δ_H 1.51, 1.59, 1.60, 1.68; δ_C 16.0, 16.3, 17.7, 25.7), three trisubstituted olefinic protons (δ_H 5.09, 5.09, 5.18), a carbonyl carbon (δ_C 203.4), a methine (δ_H 5.04; δ_C 71.9) bearing an oxygen atom and three methylene groups. The information concerning the location of these units was obtained from a heteronuclear multiple bond correlation (HMBC) spectrum. The structure of the side chain was deduced from a nuclear Overhauser effect spectroscopy (NOESY) experiment, cross-peaks were observed from the following pairs: H-4/H-6, H-8/H-10 and H-12/H-14. These results indicated an *E* configuration for the C-4–C-5 and C-8–C-9 double bonds.[7,8] Compound **5** is thus 1-(2,4-dihydroxyphenyl)-2-hydroxy-5,9,13-trimethyl-4(*E*),8(*E*),12-tetradecatrien-1-one.

As the optical rotation of **5** showed $[\alpha]_D^{22}=0°$, **5** is presumably a racemate at the chiral centre C-2. An HPLC experiment using a chiral column showed two peaks, indicating that **5** is a racemic mixture as shown in Fig. 1.

Compound **6** analysed for $C_{23}H_{32}O_3$ (*m/z* 356.2355 $[M]^+$). Compared to the ^{1}H-NMR and ^{13}C-NMR spectra of **5**, those of **6** showed the presence of a vinyl system (δ_H 4.95, 5.01; δ_C



* To whom correspondence should be addressed.

112.2, 145.8) in place of the trisubstituted olefin system at C-4–C-5, and the substitution of a methyl bound to a quaternary centre C-3 (δ_C 40.3). The stereochemistry at the C-6–C-7 double bond was established as *E* from the cross-peak of H-6 and H-8 by NOESY experiment. The structure of **6** was confirmed as 1-(2,4-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadien-1-one.

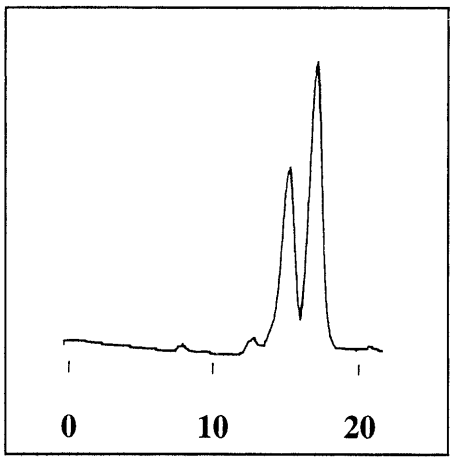


Fig. 1. HPLC Chart Using a Chiral Column
Column: CHIRALCEL OD, solvent: hexane/2-propanol (9 : 1), wave length: 221 nm.

Compound **7** showed an $[M]^+$ peak at *m/z* 370.2144 ($C_{23}H_{30}O_4$), and gave rise to ^{1}H- and ^{13}C-NMR spectra that were similar to those of **6**, except for the appearance of one more carbonyl signal (δ_C 200.3) assigned to C-9, **7** is thus 1-(2,4-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadiene-1,9-dione.

Compound **8** had an $[M]^+$ peak at *m/z* 368.2002 ($C_{23}H_{28}O_4$). The ^{1}H- and ^{13}C-NMR spectra of **8** were similar to those of **7** except for the appearance of a 4-methyl-2-furyl system (δ_H 1.98, 5.86, 7.06; δ_C 9.8, 108.8, 120.5, 137.7, 154.4) at C-8, instead of ketone at C-9 and trisubstituted olefin at C-10–C-11. Based on these spectral data, **8** was determined to be 1-(2,4-dihydroxyphenyl)-3,7-dimethyl-3-vinyl-8-(4-methyl-2-furyl)-6(*E*)-octen-1-one. Compounds **6**—**8** were all racemic mixtures at C-3; none of them showed any optical activity.

As shown in Chart 1, we now propose that dshamirone (**4**) and compounds **5**—**8** may be biosynthesized through condensation of a C6–C3 derivative (A) and farnesyl pyrophosphate (B). Condensation of A at C-1 position of B leads to **5**, whereas condensation of A at the C-3 position of B leads to **6**—**8**. Compound A has been reported as an intermediate during β-oxidation of cinnamic acids derivatives.[9]

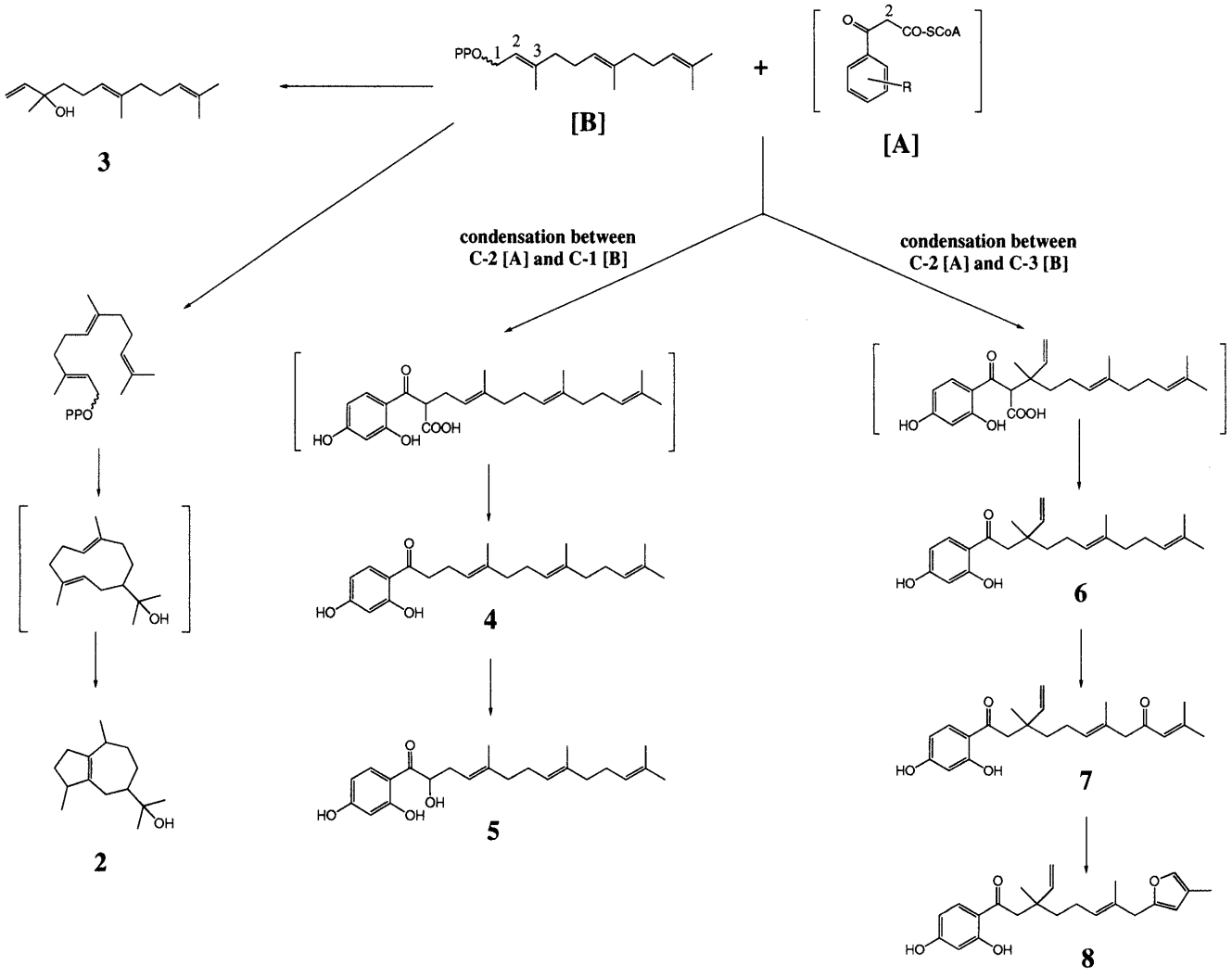


Chart 1. Proposed Biosynthesis Pathway
Compounds **2**—**8** were isolated in this investigation. The compounds in the brackets are hypothetical precursors.

Table 1. ^{1}H-NMR Spectral Data for Compounds **4**—**5** in $CDCl_3$

	4	5
2	2.92 (2H, t, J=8 Hz)	5.04 (1H, t, J=5.5 Hz)
3	2.42 (2H, q, J=7 Hz)	2.40 (1H, m)
		2.61 (1H, m)
4	5.17 (1H, t, J=7 Hz)	5.18 (1H, t, J=7 Hz)
6	1.98[a)]	1.98 (2H, m)
7	2.06[a)]	2.04 (2H, m)
8	5.09[a)]	5.09[a)]
10	1.98[a)]	1.98 (2H, m)
11	2.06[a)]	2.04 (2H, m)
12	5.09[a)]	5.09[a)]
14	1.68 (3H, s)	1.68 (3H, br s)
5Me	1.63 (3H, s)	1.51 (3H, s)
9Me	1.59 (3H, s)	1.59 (3H, s)
13Me	1.60 (3H, s)	1.60 (3H, s)
3′	6.39[a)]	6.43[a)]
5′	6.38[a)]	6.44[a)]
6′	7.65 (1H, d, J=9 Hz)	7.54 (1H, d, J=9 Hz)

a) Overlapped with other signals.

Table 3. ^{13}C-NMR Spectral Data for Compounds **4**—**8** in $CDCl_3$

	4	5		6	7	8
1	204.7	203.4	1	204.0	203.9	203.9
2	38.1	71.9	2	47.1	46.9	47.0
3	23.3	35.5	3	40.3	40.2	40.3
4	122.4	117.5	4	41.0	40.5	40.8
5	136.9	139.6	5	22.9	23.3	23.0
6	39.7	39.8	6	124.3	129.6	126.7
7	26.6	26.7	7	135.1	129.5	131.9
8	124.1	124.4	8	39.7	55.8	38.4
9	135.1	135.2	9	26.7	200.3	154.4
10	39.7	39.7	10	124.3	122.9	108.8
11	26.8	26.5	11	131.4	156.7	120.5
12	124.4	123.9	12	25.7	27.8	137.7
13	131.3	131.3	3Me	23.3	23.3	23.3
14	25.7	25.7	7Me	16.0	16.4	16.4
5Me	16.1	16.3	11Me	17.7	20.9	9.8
9Me	16.0	16.0	Vinyl-CH	145.8	145.7	145.7
13Me	17.7	17.7	Vinyl-CH_2	112.2	112.3	112.3
1′	113.9	110.8	1′	115.1	114.9	114.9
2′	165.2	165.6	2′	165.6	165.6	165.6
3′	103.6	103.8	3′	103.5	103.6	103.6
4′	162.7	163.8	4′	162.8	163.2	163.2
5′	107.7	108.4	5′	107.5	107.7	107.7
6′	132.4	131.8	6′	133.2	133.1	133.1

Table 2. ^{1}H-NMR Spectral Data for Compounds **6**—**8** in $CDCl_3$

	6	7	8
2	2.89 (2H, d, J=3 Hz)	2.87 (2H, d, J=3 Hz)	2.88 (2H, d, J=3 Hz)
4	1.53 (2H, m)	1.53 (2H, m)	1.57 (2H, m)
5	1.95[a)]	1.98 (2H, m)	1.98 (2H, m)
6	5.09[a)]	5.22 (1H, t, J=6 Hz)	5.19 (1H, br t, J=6 Hz)
8	1.95[a)]	3.04 (2H, s)	3.21 (2H, s)
9	2.04 (2H, m)		
10	5.08[a)]	6.12 (1H, s)	5.86 (1H, s)
12	1.68 (3H, s)	1.89 (3H, s)	7.06 (1H, s)
3Me	1.17 (3H, s)	1.15 (3H, s)	1.16 (3H, s)
7Me	1.58 (3H, s)	1.59 (3H, s)	1.58 (3H, s)
11Me	1.60 (3H, s)	2.15 (3H, s)	1.98 (3H, s)
Vinyl-CH	5.87 (1H, dd, J=11, 17.5 Hz)	5.85 (1H, dd, J=11, 17.5 Hz)	5.86 (1H, dd, J=11, 17.5 Hz)
Vinyl CH_2	4.95 (1H, br d, J=17.5 Hz)	4.95 (1H, brd, J=17.5 Hz)	4.95 (1H, br d, J=17.5 Hz)
	5.01 (1H, br d, J=11 Hz)	5.01 (1H, brd, J=11 Hz)	5.01 (1H, br d, J=11 Hz)
3′	6.36[a)]	6.37[a)]	6.36[a)]
5′	6.37[a)]	6.38[a)]	6.37[a)]
6′	7.63 (1H, d, J=9 Hz)	7.61 (1H, d, J=9 Hz)	7.61 (1H, d, J=9 Hz)

a) Overlapped with other signals.

Experimental

General Procedures NMR spectra were recorded on a JEOL JNM-A500 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Electon impact mass spectrum (EI-MS) were recorded on a JEOL JMS-DX300 spectrometer. Optical rotation were measured with a JASCO DIP-4 digital polarimeter.

Plant Material The roots of *Ferula ferulioides* (STEUD.) KOROVIN were collected in July 1996 from Bulgan Somon of Hovd City. Voucher specimens have been deposited in the Botanical Department of Mongolian State University.

Extraction and Isolation The dried and pulverized roots of *Ferula ferulioides* (400 g) were extracted successively with methanol under reflux. After evaporation of this extract, a part of the methanol extract (20 g) was partitioned between ethyl acetate and water. The ethyl acetate layer was dried and evaporated under reduced pressure. The residue was chromatographed on silica gel with hexane–ethyl acetate (10 : 1—1 : 1) to afford six fractions. Fraction 1 was subjected to RP-18 Lober chromatography (70% CH_3CN) to give **1** (14 mg). Fraction 2 was subjected to RP-18 Lober chromatography (80% CH_3CN) to give **2** (61 mg). Fraction 3 was subjected to RP-18 Lober chromatography (70% CH_3CN) to give **3** (66 mg). Fraction 4 was subjected to RP-18 Lober chromatography (80% CH_3CN) to give **4** (16 mg) and **6** (9 mg). Fraction 5 was subjected to RP-18 Lober chromatography (75% CH3CN), **8** (70 mg) was isolated. Fraction 6 was subjected to RP-18 Lober chromatography (45—65% CH_3CN) to give **5** (10 mg) and **7** (7 mg), respectively.

1-(2,4-Dihydroxyphenyl)-2-hydroxy-5,9,13-trimethyl-4(*E*),8(*E*),12-tetradecatrien-1-one (**5**): $[\alpha]_D^{22}$ ±0° (*c*=0.8, $CHCl_3$), EI-MS *m/z*: 372 $[M]^+$, 137 $[C_7H_5O_3]^+$, HR-MS *m/z*: 372.2298 $[M]^+$ (Calcd for $C_{23}H_{32}O_4$: 372.2300). ^{1}H- and ^{13}C-NMR: Table 1, 3.

1-(2,4-Dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadiene-1-one (**6**): $[\alpha]_D^{22}$ ±0° (*c*=1.7, $CHCl_3$), EI-MS *m/z*: 356 $[M]^+$, 137 $[C_7H_5O_3]^+$, HR-MS *m/z*: 356.2355 $[M]^+$ (Calcd for $C_{23}H_{32}O_3$: 356.2351). ^{1}H- and ^{13}C-NMR: Table 2, 3.

1-(2,4-Dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadiene-1,9-dione (**7**): $[\alpha]_D^{22}$ ±0° (*c*=1.0, $CHCl_3$), EI-MS *m/z*: 370 $[M]^+$, 137 $[C_7H_5O_3]^+$, HR-MS *m/z*: 370.2144 $[M]^+$ (Calcd for $C_{23}H_{30}O_4$: 370.2144). ^{1}H- and ^{13}C-NMR: Table 2, 3.

1-(2,4-Dihydroxyphenyl)-3,7-dimethyl-3-vinyl-8-(4-methyl-2-furyl)-6(*E*)-octen-1-one (**8**): $[\alpha]_D^{22}$ ±0° (*c*=2.3, $CHCl_3$), EI-MS *m/z*: 368 $[M]^+$, 137 $[C_7H_5O_3]^+$, HR-MS *m/z*: 368.2002 $[M]^+$ (Calcd for $C_{23}H_{28}O_4$: 368.1987).

^{1}H- and ^{13}C-NMR: Table 2, 3.

References

1) Kumamoto J., Scora R. W., *J. Agr. Food. Chem.*, **18**, 544—545 (1970).
2) Cornwell P. A., Barry B. W., *J. Pharm. Pharmacol.*, **46**, 261—269 (1994).
3) Takeda K., Kubota T., Nagata W., *Chem. Pharm. Bull.*, **1**. 241—243 (1953).
4) Ninato H., *Tetrahedron*, **18**, 365—371 (1962).
5) Takeda K., Minato H., Terasawa T., Yanaihara C., *Chem. Pharm. Bull.*, **13**, 942—947 (1965).
6) Kamilov Kh. M., Nikonov G. K., *Khim. Prir. Soedin.*, **6**, 817—818 (1976).
7) Lamnaouer D., Bodo B., Martin M. T., Molho D., *Phytochemistry*, **26**, 1613—1615 (1987).
8) Miski M., Jakupovic J., *Phytochemistry*, **29**, 1995—1998 (1990).
9) Gross G. G., "The Biochemistry of Plants," vol. 7, ed. by Conn E. E., Academic Press, 1981, pp. 301—316.