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Eremophilane Sesquiterpenoids from *Farfugium japonicum*

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Farfugium japonicum (L.) Kitam, a perennial herb of the Compositae family, is mainly distributed in south and southeast China, Korea, and Japan. It is called "Lian-Peng-Cao" in China and is used as a Chinese folk medicine for the treatment of sore throat, cold and cough.[1] Its young leaves and stalks are used as vegetable food in Japan.[2] Phytochemical investigations on this plant collected in Japan have resulted in the isolation of sesquiterpenoids,[3-10] triterpenoids,[4] sterols,[8] fatty acids,[8] and pyrrolizidine alkaloids.[11] In the course of searching for antibacterial sesquiterpenes from Compositae plants, a new eremophilane sesquiterpenoid, 3β-angeloyloxy-6α-hydroxy-8β-methoxy-9α-senecioyloxyeremophila-7(11)-en-12,8α-lactone (**1**), and three known ones, 3β-angeloyloxy-8β,10β-dihydroxyeremophila-7(11)-en-12,8α-lactone (**2**),[5] 3β-angeloyloxy-10β-hydroxy-8β-methoxyeremophila-7(11)-en-12,8α-lactone (**3**)[10] and 3β-angeloyloxy-10β-hydroxy-8α-methoxyeremophila-7(11)-en-12,8β-lactone (**4**),[12] were isolated from the roots of *F. japonicum*. We herein report the isolation and structure characterization of **1**. This is also the first report on the ^{13}C-NMR data for the compound **2**. In addition, the antibacterial activity of compounds **1**-**4** against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* was evaluated.

Compound **1** was isolated as colorless crystal with the molecular formula of $C_{26}H_{36}O_8$ determined by the quasi molecular ion peak at *m*/*z* 494.2755 ($[M + NH_4]^+$, Calcd for $C_{26}H_{40}NO_8^+$: 494.2748) in HR-ESI-MS spectrometry. Its IR spectrum showed the absorption bands of hydroxyl (3550 cm^{-1}), double bond (1647 cm^{-1}), ester carbonyl (1710 cm^{-1}) and γ-lactone moiety (1772 cm^{-1}). The ^{1}H-, ^{13}C-NMR and DEPT spectra (Table 1) displayed the signals of an angeloyl group [δ_H 6.10 (1H, qq, J = 7.2, 1.5 Hz, H-3'), 1.99 (3H, dq, J = 7.2, 1.5 Hz, H-4'), 1.89 (3H, d, J = 1.5 Hz, H-5'); δ_C 167.4 (C), 139.0 (CH), 127.8 (C), 20.5 (CH_3), 15.8 (CH_3)], a senecioyl group [δ_H 5.51 (1H, m, H-2"), 2.15 (3H, d, J = 1.2 Hz, H-4"), 1.88 (3H, d, J = 1.2 Hz, H-5"); δ_C 164.7 (C), 159.0 (C), 115.2 (CH), 27.4 (CH_3), 20.3 (CH_3)], and a methoxyl group [δ_H 3.24 (3H, s); δ_C 50.2 (CH_3)]. Furthermore, there were fifteen carbon signals left in the ^{13}C-NMR spectrum, indicating the presence of sesquiterpene skeleton. The characteristic signals of three methyls at δ_H 2.13 (3H, d, J = 1.6 Hz, H-13), 0.96 (3H, s, H-14), 0.89 (3H, d, J = 7.1 Hz, H-15) in the ^{1}H-NMR spectrum, along with a ^{13}C-NMR signal for a quaternary carbon at δ_C 105.7, suggested that **1** has an eremophila-7(11)-en-12,8-lactone skeleton with an oxygened group at C-8.[13] Comparing its NMR data (Table 1) to those of an analogous compound 3β-angeloyloxy-8β-methoxy-9β-senecioyloxyeremophila-7(11)-en-12,8α-lactone,[8] the ^{1}H-NMR signals at δ_H 5.12 (1H, m), 5.13 (1H, d, J = 1.5 Hz) and 5.02 (1H, d, J = 1.6 Hz) could be assigned to H-3, H-9 and H-6, respectively.

The HMBC correlations (Figure 2) further confirmed the eremophilane skeleton and the locations of functional groups in **1**. The HMBC correlations H_3-15 (δ_H 0.89)/C-3 (δ_C 71.0), and H-3 (δ_H 0.89)/C-1' (δ_C 167.9) suggested that C-3 is oxygenated and the angeloyloxyl group was at C-3. The coupling pattern of H-9 (δ_H 5.13, d, J = 1.5 Hz) and the HMBC correlations of H-9 (δ_H 5.13) with C-5 (δ_C 47.3), C-10 (δ_C 105.5) and C-1" (δ_C

Table 1. ^{1}H-, ^{13}C-NMR and DEPT data of **1** ($CDCl_3$, J in Hz, δ ppm, TMS)[a]

No.	δ_H	δ_C
1	2.01 (1H, m), 1.76 (1H, m)	25.0 t
2	1.74 (2H, m)	24.6 t
3	5.12 (1H, m)	71.0 d
4	2.36 (1H, m)	35.8 d
5	-	47.3 s
6	5.02 (1H, d, J = 1.6 Hz)	69.9 d
7	-	155.0 s
8	-	105.5 s
9	5.13 (1H, d, J = 1.5 Hz)	74.1 d
10	1.98 (1H, m)	40.3 d
11	-	128.6 s
12	-	171.2 s
13	2.13 (3H, d, J = 1.6 Hz)	9.0 q
14	0.96 (1H, s)	20.1 q
15	0.89 (3H, d, J = 7.1 Hz)	8.8 q
Ang		
1'	-	167.9 s
2'	-	127.9 s
3'	6.10 (1H, qq, J = 7.2, 1.5 Hz)	138.6 d
4'	1.99 (3H, dq, J = 7.2, 1.5 Hz)	15.8 q
5'	1.89 (3H, d, J = 1.5 Hz)	20.5 q
Sen		
1"	-	164.7 s
2"	1.99 (1H, m)	115.2 d
3"	-	159.0 s
4"	1.88 (3H, d, J = 1.2 Hz)	27.4 q
5"	2.15 (3H, d, J = 1.2 Hz)	20.3 q
OCH_3	3.24 (3H, s)	50.2 q
OH	3.74 (1H, s)	-

[a]Measured at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR.

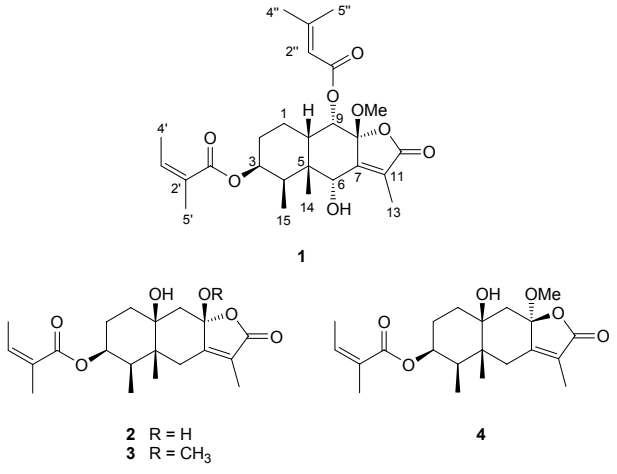

Figure 1. The Structures of **1**-**4**.

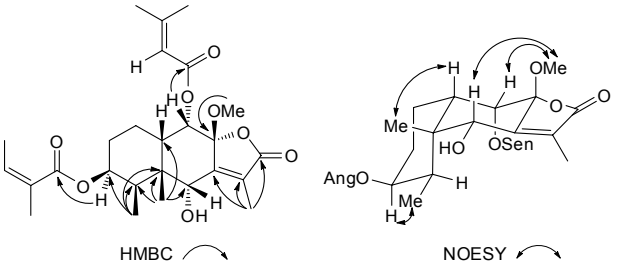

Figure 2. The Key HMBC and NOESY correlations for **1**.

164.6) suggested the senecioxylxyl group location at C-9. The presence of hydroxy at C-6 could be deduced by the HMBC correlation H_3-14 (δ_H 0.96)/C-6 (δ_C 69.9) and H-6 (δ_H 5.02)/C-14 (δ_C 20.1). The HMBC correlation of the methoxyl protons with C-8 (δ_C 105.5) indicated the position of the methoxyl group to be at C-8. As for stereochemistry in **1**, the H_3-14 and H_3-15 were assigned to be β configuration in biogenetic consideration.[14] The NOESY cross peaks observed between H_3-14 and H-10 (Figure 2) implied a *cis*-junction for the two rings.[9] The methoxy group at C-8 was deduced to be β orientation based on the downfield shifts of H_3-14 compared to H_3-15.[15] The α configuration of the hydroxyl group at C-6 was deduced by the homoallylic coupling ($J_{6\beta,13}$ = 1.6 Hz) between olefinic methyl (CH_3-13) protons and the allylic proton (H-6β).[16] The small coupling constants ($J_{9,10}$ = 1.5 Hz) between H-9 and H-10 indicated the senceioyloxyl group was α orientation. The NOESY correlations (Figure 2) of methoxy protons with H-6 and H-9 also supported the relative configurations of C-6, C-8 and C-9. The absence of NOESY correlation between H_3-14 and H-3 suggested the angeloyoxyl group be β orientation.[17] Therefore, the structure of **1** was determined as 3β-angeloyloxy-6α-hydroxy-8β-methoxy-9α-senecioyloxyeremophila-7(11)-en-12,8α-lactone (Figure 1).

The antibacterial activity of **1**-**4** was determined against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. At the concentration of 100 μg/mL, all tested compounds possess minor antibacterial activity (diameters of growth inhibition < 10 mm).

Experimental Section

General Procedures. Melting points were determined on Kofler melting point apparatus and uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were obtained from Nicolet NEXUS 670 FT-IR. ^{1}H-, ^{13}C-NMR (DEPT) and 2D NMR were recorded on Bruker AVANCE 500 spectrometer with TMS as internal reference. HR-ESI-MS spectra were obtained on Bruker APEX II spectrometer. Silica gel (200 - 300 mesh and 300 - 400 mesh) used for column chromatography (CC) and silica GF_{254} for thin layer chromatography (TLC) were purchased from Qingdao Marine Chemical Factory in China. Silica gel C-18 used for low pressure CC were purchased from Merck. Spots were detected on TLC under UV light at 254 and 365 nm or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

Plant Material. The rhizome of *Farfugium japonicum* were collected in Hangzhou, Zhejiang Province, People Republic of China in 2009, and identified by Associate Prof. Hong Zhao, Marine College, Shandong University at Weihai. A voucher specimen (NO. HZ09002) is deposited in the Laboratory of Botany, Marine College, Shandong University at Weihai.

Extraction and Isolation. The air-dried rhizome of *Farfugium japonicum* (2.8 kg) were extracted three times with petroleum ether (60 - 90 ^{o}C)-Et_2O-CH_3OH (1:1:1) (seven days each time) at room temperature. The extract was concentrated under reduced pressure to afford a residue (136 g) which was subjected to silica gel CC (200 - 300 mesh, 1500 g) eluting with *n*-hexane-acetone (10:1, 5:1, 3:1, 1:1, 0:1) to give five fractions (Fr.1 - Fr.5). Fr.1 (10:1, 35.6 g) was subjected to silica gel CC eluted with *n*-hexane-EtOAc (30:1 to 0:1) give five subfractions (Fr.1A - Fr.5E). Fr.1B (1.2 g) was isolated by silica gel CC with a *n*-hexane-acetone (25:1 to 0:1) elution, and further purified by low pressure C-18 CC with H_2O-CH_3OH (1:3) elution to give **1** (15 mg). Fr.1C (4.2 g) was purified by silica gel CC with an *n*-hexane-acetone (20:1 to 0:1) gradient to give five subfractions (Fr.1Ca - Fr.1Ce). Fr.1Cb (1.3 g) was purified by silica gel CC with a *n*-hexane-EtOAc (15:1) eluent to give **3** (160 mg) and **4** (90 mg). Fr. 2 (8.6 g) was purified by silica gel CC with a *n*-hexane-acetone (10:1 to 0:1) as elution to give three subfractions (Fr.2A - Fr.2C). Fr.2B (360 mg) was purified by repeated silica gel CC eluted with *n*-hexane-acetone (6:1) as elution to give **2** (90 mg).

3β-Angeloyloxy-6α-hydroxy-8β-methoxy-9α-senecioyloxyeremophila-7(11)-en-12,8α-lactone (1). Colorless crystal; mp 175 - 176 ^{o}C; $[\alpha]_D^{20}$ = −208 (*c* 1.25, $CHCl_3$); IR (KBr): ν_{max} 3438, 2974, 2940, 1772, 1710, 1644; HR-ESI-MS *m*/*z* 494.2755 ($[M + NH_4]^+$, Calcd for $C_{26}H_{40}NO_8^+$: 494.2748). ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (DEPT) (125 MHz, $CDCl_3$): see Table 1.

3β-Angeloyloxy-8β,10β-dihydroxyeremophila-7(11)-en-12,8α-lactone (2). White power; $[\alpha]_D^{20}$ = +50.9 (*c* 0.55, $CHCl_3$); IR (KBr): ν_{max} 3351, 3368, 3012, 2958, 1757, 1708, 1691; HR-ESI-MS *m*/*z* 382.2230 ($[M + NH_4]^+$, Calcd for $C_{20}H_{32}NO_6^+$: 382.2224); ^{1}H-NMR (400 MHz, $CDCl_3$): 6.08 (1H, m, H-3'), 5.01 (1H, brs, H-3), 2.65 (1H, brd, *J* = 14.2 Hz, H-6), 2.44 (1H, brd, *J* = 14.2 Hz, H-6), 2.00 (3H, brs, H-4'), 1.91 (3H, brs, H-5'), 1.84 (3H, brs, H-13), 1.27 (3H, s, H-14), 0.96 (3H, d, *J* = 7.5 Hz,

H-15); ^{13}C-NMR (100 MHz, $CDCl_3$): 30.4 (C-1), 27.7 (C-2), 72.3 (C-3), 36.6 (C-4), 46.1 (C-5), 31.3 (C-6), 158.5 (C-7), 103.0 (C-8), 42.6 (C-9), 74.4 (C-10), 123.0 (C-11), 172.5 (C-12), 8.6 (C-13), 18.3 (C-14), 12.5 (C-15), 167.5 (C-1'), 127.8 (C-2'), 139.3 (C-3'), 15.9 (C-4'), 21.1 (C-5').

3*β*-Angeloyloxy-10*β*-hydroxy-8*β*-methoxyeremophila-7(11)-en-12,8*α*-lactone (3). Colorless crystal; $[\alpha]_D^{20}$ =+210 (*c* 0.50, $CHCl_3$); IR (KBr): ν_{max} 3517, 2985, 2949, 1759, 1712, 1644; HR-ESI-MS *m/z* 396.2376 ($[M + NH_4]^+$, Calcd for $C_{21}H_{34}NO_6^+$: 396.2381); ^{1}H-NMR (500 MHz, $CDCl_3$): 6.01 (1H, qq, *J* = 7.2, 1.5 Hz, H-3'), 4.94 (1H, m, H-3), 3.74 (1H, s, -OH), 3.11 (3H, s, $-OCH_3$), 2.57 (1H, brd, *J* = 14.2 Hz, H-6), 2.14 (1H, brd, *J* = 14.2 Hz, H-6), 1.94 (3H, dq, *J* = 7.2, 1.5 Hz, H-4'), 1.84 (3H, q, *J* = 1.5 Hz, H-5'), 1.83 (3H, brs, H-13), 1.20 (3H, s, H-14), 0.89 (3H, d, *J* = 7.2 Hz, H-15); ^{13}C-NMR (125 MHz, $CDCl_3$): 30.0 (C-1), 27.7 (C-2), 72.2 (C-3), 36.7 (C-4), 46.5 (C-5), 31.6 (C-6), 155.8 (C-7), 105.7 (C-8), 42.5 (C-9), 73.5 (C-10), 126.1 (C-11), 171.3 (C-12), 8.8 (C-13), 18.3 (C-14), 12.6 (C-15), 167.4 (C-1'), 127.8 (C-2'), 139.1 (C-3'), 15.9 (C-4'), 21.1 (C-5'), 50.5 ($-OCH_3$).

3*β*-Angeloyloxy-10*β*-hydroxy-8*α*-methoxyeremophila-7(11)-en-12,8*β*-lactone (4). Colorless crystal; ^{1}H-NMR (400 MHz, $CDCl_3$): 6.10 (1H, qq, *J* = 7.2, 1.2 Hz, H-3'), 5.30 (1H, m, H-3), 3.12 (3H, s, $-OCH_3$). 2.75 (1H, brd, *J* = 13.6 Hz, H-6), 2.62 (1H, brd, *J* = 13.6 Hz, H-6), 2.00 (3H, brd, *J* = 7.2 Hz, H-4'), 1.91 (3H, brs, H-5'), 1.86 (3H, brs, H-13), 1.19 (3H, d, *J* = 7.2 Hz, H-15), 0.93 (3H, s, H-14); ^{13}C-NMR (100 MHz, $CDCl_3$): 34.2 (C-1), 22.1 (C-2), 71.8 (C-3), 42.6 (C-4), 45.1 (C-5), 34.9 (C-6), 156.5 (C-7), 105.3 (C-8), 47.5 (C-9), 73.5 (C-10), 127.3 (C-11), 171.7 (C-12), 8.5 (C-13), 19.4 (C-14), 10.4 (C-15), 167.8 (C-1'), 128.2 (C-2'), 138.5 (C-3'), 16.0 (C-4'), 20.8 (C-5'), 50.3 ($-OCH_3$).

Antibacterial Assay. The antibacterial activity of isolates against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* was evaluated with the cup-plate method.[17]

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