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EREMOPHILANE SESQUITERPENE LACTONES FROM *Ligularia intermedia* NAKAI OF SHANXI

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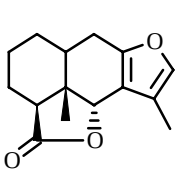
In China, about 30 *Ligularia* species have long been used as folk remedies due to their antibiotic, antiphlogistic, and antitumor activities [1]. The most widespread constituents of this genus are pyrrolizidine alkaloids and sesquiterpenes [2, 3]. Some research groups have carried out phytochemical studies on this plant [4–8]. However, it is well known that the climate and ecological environment can affect the chemical constituents of plants. Namely, plants of different species have different chemical constituents, and plants of the same species may have different chemical constituents because of environmental influence. In order to research the influence of different ecological environments on chemical constituents of plants, we carried out systematically phytochemical investigation on *Ligularia intermedia* Nakai collected from Guan Di mountain, Shanxi province in China.

General Methods. Melting points were determined on a X-4 micro-melting point apparatus and without correction. IR spectra were recorded on an IFS-120HR (German) spectrometer; ^{1}H NMR and ^{13}C NMR spectra and 2D NMR spectra (^{1}H-^{1}HCOSY, HMQC, HMBC) were taken with a Varian INOVA-400 using TMS as the internal standard. EIMS was measured on a VG-ZAB-HS mass spectrometer.

Plant Material. The whole plants of *Ligularia intermedia* were collected from Guan Di mountain, Shanxi in July 2004, and were identified by Professor Wenju Guo of the Institute of Drug Control, Shanxi province.

Extraction and Isolation. The fresh air-dried material of *Ligularia intermedia* Nakai (3.8 kg) were extracted with petroleum ether (60–90°)–Et_2O–MeOH (1 : 1 : 1) (v/v/v) at room temperature three times (five days each). This yielded 176 g of crude extract, 100 g of which was subjected to CC with petroleum ether–EtOAc (100 : 1>0 : 1) and MeOH. We obtained Fr. 1 to Fr. 11. Fr. 5 afforded compound **1** (795 mg); Fr. 6 afforded compound **3** (423 mg) and compound **4** (7.1 mg); Fr. 8 afforded compound **2** (536 mg), compound **5** (369 mg), and compound **6** (4.8 mg).



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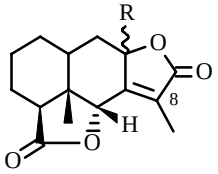

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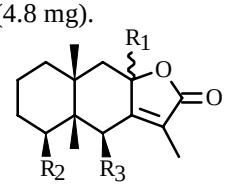

5, 6

2: R = β-OH; **3:** R = β-OCH_3; **4:** R = α-OH

5: R_1 = β-OH; **6:** R_1 = α-OCH_3; **5, 6:** R_2 = COOH, R_3 = OAng

Furanoeremophilan-4β,6α-olide (1). Colorless needles (petrol-EtOAc), mp 133–134°C; EI-MS *m/z*: 246$[M]^+$; IR (KBr, ν, cm^{-1}): 3143, 3000, 2939, 2884, 1772, 1639, 1566, 1452, 1425. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, TMS): 7.05 (1H, s, H-12), 5.07 (1H, s, H-6β), 2.31 (2H, brd, H-9), 2.26 (1H, dd, H-4α), 1.95 (1H, m, H-10), 1.90 (1H, m, H-3β), 1.86 (3H, s, CH_3-13), 1.83 (1H, m, H-1α), 1.76 (1H-2α), 1.62 (1H, m, H-3α), 1.52 (1H, m, H-2β), 1.44 (1H, m, H-1β), 1.23 (3H,

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s, CH_3-15). ^{13}C NMR (DEPT, 100 MHz, $CDCl_3$, δ, ppm): 8.24 (q, C-13), 18.74 (t, C-2), 20.30 (q, C-14), 20.48 (t, C-1), 23.14 (t, C-3), 25.24 (t, C-9), 36.95 (d, C-10), 41.27 (d, C-4), 41.42 (s, C-5), 81.63 (d, C-6), 114.68 (s, C-11), 120.08 (s, C-7), 138.56 (s, C-12), 150.78 (s, C-8), 176.65 (s, C-15). All the above data were consistent with the furanoeremophilan-4β,6α-olide reported in the literature [7, 9].

8β-Hydroxyeremophil-7(11)-ene-12,8α(4β,6α)-diolide (2). Colorless crystal (petrol–EtOAc), mp 188–189°C; EI-MS *m/z*: 278 $[M]^+$, 260, 191, 135, 109, 95, 79, 43; IR (KBr, ν_{max}, cm^{-1}): 3407, 2942, 2866, 1782, 1755, 1462, 1445; ^{1}H NMR (400 MHz, C_5D_5N, δ, ppm, TMS): 5.24 (1H, s, H-6), 3.12 (1H, brs, OH-8) C_5D_5N, 1.28-2.41 (10H, m, H-1, 2, 3, 4, 9,10), 1.93 (3H, s, H-13), 1.21 (3H, s, H-14). ^{13}C NMR (100 MHz, C_5D_5N, δ, ppm, TMS): 8.94 (q, C-13), 19.69 (t, C-1), 19.69 (q, C-14), 20.98 (t, C-2), 24.44 (t, C-3), 35.67 (d, C-10), 36.95 (t, C-9), 40.48 (d, C-4), 44.92 (s, C-5), 83.01 (d, C-6), 104.28 (s, C-8), 126.4 (s, C-11), 154.37 (s, C-7), 171.59 (s, C-12), 175.68 (s, C-15). All the above data were consistent with 8β-hydroxyeremophil-7(11)-ene-12, 8α(4β,6α)-diolide reported in the literature [9, 10]. The structure of this compound was further confirmed by the spectra data in ^{1}H-^{1}H COSY, HMQC, and HMBC.

8β-Methoxyeremophil-7(11)- ene-12,8α(4β,6α)-diolide (3). Colorless crystals (petrol–EtOAc), mp 180–181°C; EI-MS *m/z*: 292 $[M]^+$, 277, 261, 232, 220, 205, 195, 183, 159, 140, 109, 99, 77; IR (KBr, ν_{max}, cm^{-1}): 1785, 1759, 1449, 1303, 1197, 1007, 991; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, TMS): 4.87 (1H, s, H-6β), 3.19 (3H, s, H-OCH_3), 2.28 (2H, brd, H-9), 2.24 (1H, dd, H-4α). ^{13}C NMR (DEPT) (100 MHz, $CDCl_3$, δ, ppm): 9.06 (q, C-13), 19.19 (t,C-1), 20.05 (q, C-14), 20.88 (t, C-2), 24.18 (t, C-3), 34.56 (d, C-10), 35.26 (t, C-9), 40.83 (d, C-4), 44.64 (s, C-5), 50.51(-OCH_3), 82.49 (d, C-6), 105.59 (s, C-8), 129.28 (s, C-11), 151.10 (s, C-7), 170.37 (s, C-12), 174.87 (s, C-15). All the above data were consistent with 8α-methoxyeremophil-7(11)-ene-12,8α(4β,6α)-diolide reported in the literature [9, 10].

8β-Hydroxyeremophil-7(11)-ene-12,8β(4β,6α)-diolide (4). Colorless needles (Me_2CO), mp 207–208°C; EI-MS *m/z*: 278 $[M]^+$, 260, 191, 135, 109, 95, 79, 43; IR (KBr, ν_{max}, cm^{-1}): 3407, 2942, 2866, 1782, 1755, 1462, 1445, 1354, 1207, 1194, 1160, 1155, 1141, 1128, 1105, 1034, 918, 893; ^{1}H NMR (C_5D_5N, δ, ppm): 5.10 (1H, s, H-6), 2.98 (1H, brs, HO-8), 1.24–2.61 (10H, m, H-1, 2, 3, 4, 9, 10), 2.04 (3H, s, H-13), 1.43 (3H, s, H-14); ^{13}C NMR (DEPT) (100 MHz, C_5D_5N, δ, ppm, TMS): 8.47 (C-13), 19.58 (C-14), 19.60 (C-1), 20.95 (C-2), 24.52 (C-3), 35.62 (C-10), 36.38 (C-9), 40.25 (C-4), 44.84 (C-5), 82.74 (C-6), 103.53 (C-8), 126.58 (C-11), 153.53 (C-7), 170.89 (C-12), 175.34 (C-15). The structure of compound **4** was identified as 8α-hydroxyeremophil-7(11)-ene-12,8β(4β,6α)-diolide reported in the literature [8].

8β-Hydroxyl-6β-angloxyeremophil-7(11)-ene-12,8α-olide-15-oic Acid (5). Colorless crystals (Me_2CO), mp 208–210°C; EM-MS *m/z*: 378 $[M]^+$ 360, 232, 109, 83, 55. IR (KBr, ν_{max}, cm^{-1}): 3156, 2962, 1725, 1702, 1446, 1385, 1228, 1160, 1137, 958. ^{1}H NMR (400 MHz, C_5D_5N, δ, ppm, TMS): 6.41 (1H, m, H-3′), 5.91 (1H, s, H-6β), 2.61(1H, brs, OH), 2.58(1H, dd, H-4α), 2.00 (3H, dq, J = 7.5, 1.4, CH_3-4′), 1.93 (3H, s, CH_3-13), 1.91 (3H, brs, CH_3-5′), 1.26 (3H, brs, CH_3-14). ^{13}C NMR (DEPT) (100 MHz, C_5D_5N, δ, ppm): 9.50 (q, C-13), 15.88(q, C-5′), 18.85 (q, C-14), 19.06 (t, C-1), 20.74(q, C-4′), 25.29 (t, C-2), 25.51 (t, C-3), 36.18 (d, C-10), 39.40 (t, C-9), 41.73 (d, C-4), 41.76 (s, C-5), 72.26 (d, C-6), 105.31 (s, C-8), 127.70 (s, C-11), 129.08 (s, C-2′), 140.00 (d, C-3′), 153.41 (s, C-7), 166.75 (s, C-1′), 172.15 (s, C-12), 176.28 (C-15). All the above data were consistent with 8β-hydroxyl-6β-angloxyeremophil-7(11)-ene-12, 8α-olide-15-oic acid reported in the literature [10]. The structure of this compound was further confirmed by the spectra data in ^{1}H-^{1}H COSY, HMQC, and HMBC.

8α-methoxyl-6β-angloxyeremophil-7(11)-ene-12,8β-olide-15-oic Acid (6). Milky crystals (Me_2CO), mp 178–179°C. EM-MS *m/z*: 392 $[M]^+$, 360, 278, 260 , 149, 105, 83, 55. IR (KBr, ν_{max}, cm^{-1}) 3430, 2933, 1769, 1702, 1451, 1380, 1228, 1151, 960. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, TMS): 6.29 (1H, qq, H-3′), 5.10 (1H, s, H-6β), 2.80 (1H, dd, H-4α), 2.06 (3H, dq, J = 7.5, 1.4, CH_3-4′), 1.98 (3H, dq, CH_3-5′), 1.93 (3H, s, CH_3-13), 1.24 (3H, s, CH_3-14); ^{13}C NMR (100 MHz, C_5D_5N, δ, ppm) (DEPT): 9.66 (q, C-13), 18.89 (q, C-5′), 19.01 (t, C-1), 19.12 (q, C-14), 20.76 (q, C-4′), 24.76 (t, C-2), 25.11 (t, C-3), 35.08 (d, C-10), 38.16 (t, C-9), 41.62 (s, C-5), 41.85 (d, C-4), 50.77 (-OCH_3), 72.01 (d, C-6), 103.73 (s, C-8), 128.32 (s, C-2′), 129.43 (s, C-11), 141.07 (d, C-3′), 151.23 (s, C-7), 166.58 (s, C-1′), 171.53 (s, C-12), 175.73 (s, C-15). All the above data were consistent with 8α-methoxyl-6β-angloxyeremophil-7(11)-ene-12,8β-olide-15-oic acid reported in the literature [11].

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