

## Study on the Terpene Compounds from *Ligularia kanaitzensis*\*

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Received April 30, 2002; Accepted June 2, 2002

### Introduction

The Genus *Ligularia* belongs to Tribe Senecioneae, Family Compositae and comprises more than 110 species occurring in China, of which about 40 species of *Ligularia* have been used as Chinese traditional or folk herbs. *Ligularia kanaitzensis* (Franch.) Hand.-Mazz is grown in Southwest China. Its roots and rhizomes are used as a remedy for cough and inflammation<sup>[1]</sup>. The presence of eremophilane sesquiterpenes and pyrrolizidine alkaloids in many other species of Genus *Ligularia* is well documented. In this paper, we report the isolation and structural elucidation of 11-hydroperoxy-6, 9-eremophiladien-8-one (**1**), 6 $\alpha$ -hydroxy-7(11)-eremophien-12, 8 $\beta$ -olide (**2**), oleanic acid (**3**),  $\beta$ -amyrin (**4**),  $\beta$ -amyrin (**5**) and lupol (**6**) from the EtOH extracts of the roots and rhizomes of *Ligularia kanaitzensis*.

### Plant material

The roots and rhizomes of *Ligularia kanaitzensis* were collected from Yulong Mountain (1999/8), Lijiang Prefecture of Yunnan Province and authenticated by Dr. Zhang Mian. Voucher specimens (No. 990008-Li) were deposited in the Herbarium of China Pharmaceutical University.

### General experimental

Optical rotations were determined by: JASCO DEP-370 polarimeter. Melting points were obtained on Koffler apparatus and uncorrected. IR spectra were obtained on Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on Bruker AM-400 instrument with TMS as internal standard and CDCl<sub>3</sub> or C<sub>5</sub>D<sub>5</sub>N as solvent. <sup>1</sup>H NMR spectra were measured at 400 MHz; <sup>13</sup>C NMR and DEPT spectra were recorded at 100 MHz. The EI-MS

experiments were carried out on VG Auto Spec-3000 spectrometer at 70 eV or 20 eV.

### Extraction and isolation

Dried and powdered roots and rhizomes of *Ligularia kanaitzensis* (5.5 kg) were extracted with EtOH (20L  $\times$  3) at room temperature. After removal of the solvent *in vacuo*, an extract of 330.0 g was afforded. The extract was suspended in H<sub>2</sub>O and partitioned with petroleum ether (60–90°C) (2L  $\times$  3) and EtOAc (2L  $\times$  3) successively. 140.0 g of the evaporated petroleum ether extract were subjected to repeated chromatography on a silica gel column (200–300 mesh, 1.0 kg), eluted with a gradient of petroleum ether and EtOAc. Four crude fractions were obtained. The first fraction was chromatographed on a silica gel column (silica gel H, 100 g) with petroleum ether/EtOAc (20:1) and afforded **5** (15 mg) through further purifying. The second fraction was isolated on CC (silica gel H, 200 g) with CHCl<sub>3</sub>/(Me)<sub>2</sub>CO (10:1) and further purified on a Sephadex LH-20 column with CHCl<sub>3</sub> or RP-18 (eluted with MeOH/H<sub>2</sub>O 1:9) to obtain **1** (50 mg), **2** (30 mg), **4** (20 mg), **6** (11 mg). The fourth fraction was further chromatographed on a silica gel column (silica gel H, 100 g) with CHCl<sub>3</sub>/(Me)<sub>2</sub>CO (10:1) and afforded **3** (100 mg).

### Structure identification

**Compound 1** C<sub>15</sub>H<sub>22</sub>O<sub>3</sub>, colourless needles (petroleum ether-EtOAc). Reaction with peroxide visualization reagent is positive. EI-MS m/z (%): 251 [M + 1]<sup>+</sup> (56), 233 (45), 217 (99), 203 (89), 191 (40), 177 (72), 164 (45), 159 (100), 147 (79), 135 (77), 121 (66), 105 (35), 91 (67), 83 (24), 77 (36), 69 (39), 55 (46). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

\* First published in Chinese in Pharmaceutical Journal 2002, 37: 12.

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$\delta$ : 2.40 (1H, m, H-1a), 2.32 (1H, m, H-1b), 1.48 (H, m, H-4), 7.26 (1H, s, H-6), 6.07 (1H, s, H-6), 6.07 (1H, br.s, H-9), 1.56 (3H, s, H-12), 1.53 (3H, s, H-13), 1.14 (3H, s, H-14), 1.10 (3H, d,  $J = 5.96$  Hz, H-15).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 32.8 (t, C-1), 30.6 (t, C-2), 28.4 (t, C-3), 42.3 (d, C-4), 43.9 (s, C-5), 125.5 (d, C-6), 168.2 (s, C-7), 186.5 (s, C-8), 152.3 (d, C-9), 139.5 (s, C-10), 83.8 (s, C-11), 25.2 (q, C-12), 24.9 (q, C-13), 17.5 (q, C-14), 16.7 (q, C-15). Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of 11-hydroperoxyeremophil-6, 9-dien-8-one<sup>[2,3]</sup>. Therefore, compound **1** was identified as 11-hydroperoxyeremophil-6,9-dien-8-one.

**Compound 2**  $\text{C}_{15}\text{H}_{22}\text{O}_3$ , colourless needles ( $\text{Me}_2\text{CO}$ ), mp 78 – 80°C. IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3440, 1790, 1710. EI-MS  $m/z$  (%): 250  $[\text{M}]^+$  (23), 232  $[\text{M}-\text{H}_2\text{O}]^+$  (29), 222 (61), 217 (6), 205 (13), 189 (9), 177 (6), 161 (11), 141 (5), 126 (34), 109 (100), 98 (13), 91 (29), 81 (30), 67 (31), 55 (54).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.65 (3H, d,  $\text{C}_4\text{-Me}$ ), 0.90 (3H, s,  $\text{C}_5\text{-Me}$ ), 1.64 (3H, s,  $\text{C}_{11}\text{-Me}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 20.4 (t, C-1), 26.1 (t, C-2), 30.5 (t, C-3), 29.5 (d, C-4), 40.2 (s, C-5), 34.9 (t, C-6), 160.0 (s, C-7), 104.3 (s, C-8), 38.8 (t, C-9), 39.5 (d, C-10), 121.7 (s, C-11), 173.2 (s, C-12), 7.9 (q, C-13), 21.2 (q, C-14), 15.8 (q, C-15). Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of 8 $\alpha$ -hydroxy-7(11)-eremophil-12, 8 $\beta$ -olide<sup>[4]</sup>. Thus, compound **2** was identified as 8 $\alpha$ -hydroxy-7(11)-eremophil-12, 8 $\beta$ -olide.

**Compound 3**  $\text{C}_{30}\text{H}_{48}\text{O}_3$ , colourless powder, mp 282 – 284°C. Liebermann-Burchard reaction is positive. EI-MS  $m/z$  (%): 456  $[\text{M}]^+$  (6), 248 (100), 207 (21), 189  $[\text{207}-\text{H}_2\text{O}]^+$ . Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of oleanic acid<sup>[5]</sup>.

Therefore, compound **3** was identified as oleanic acid.

**Compound 4**  $\text{C}_{30}\text{H}_{50}\text{O}$ , colourless needles ( $\text{EtOAc}$ ), mp 196 – 198°C. Liebermann-Burchard reaction is positive. EI-MS  $m/z$  (%): 426  $[\text{M}]^+$  (10), 411 (4), 218 (100), 203 (28), 189 (9), 175 (7), 135 (10), 109 (8), 93 (17), 81 (16), 69 (33), 57 (39).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.18 (1H, t,  $J = 6.8$  Hz), 3.22 (1H, dd,  $J = 5.6, 3.4$  Hz), 1.13 (3H, s), 0.99 (3H, s), 0.95 (3H, s), 0.93 (3H, s), 0.89 (3H, s), 0.87 (3H, s), 0.83 (3H, s), 0.79 (3H, s). Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of  $\beta$ -amyrin<sup>[6]</sup>. Thus, compound **4** was identified as  $\beta$ -amyrin.

**Compound 5**  $\text{C}_{32}\text{H}_{52}\text{O}_2$ , colourless needles (petroleum ether-EtOAc), mp 235 – 236°C. Liebermann-Burchard reaction is positive. EI-MS  $m/z$  (%): 469  $[\text{M}+1]^+$  (45), 289 (11), 218 (100), 189 (29), 121 (22), 109 (31), 95 (39), 69 (44), 57 (34).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.16 (t,  $J = 6.4$  Hz), 4.50 (dd,  $J = 11.6, 4.2$  Hz), 2.09 (3H, s), 1.17 (3H, s), 0.98 (3H, s). Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of  $\beta$ -amyrin acetate<sup>[6]</sup>. Thus, compound **5** was identified as  $\beta$ -amyrin acetate.

**Compound 6**  $\text{C}_{30}\text{H}_{50}\text{O}$ , colourless needles ( $\text{EtOAc}$ ), mp 213 – 214°C,  $[\alpha]_{\text{D}}^{26} = +27.6^\circ$  ( $\text{CHCl}_3$ ,  $c = 0.646$ ). Liebermann-Burchard reaction is positive. EI-MS  $m/z$  (%): 426  $[\text{M}]^+$  (45), 411 (15), 393 (14), 316 (4), 247 (27), 218 (31), 207 (25), 189 (34).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 4.70, 4.58 (2H, d,  $J = 1.83$  Hz), 3.20 (1H, dd,  $J = 11.1, 4.8$  Hz), 0.68 – 1.98 (25H, m), 1.66 (3H, s), 0.74 (3H, s), 0.80 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.04 (3H, s). Data of EI-MS,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were in accord with those of lupeol<sup>[5,6]</sup>. Therefore, compound **6** was identified as lupeol.

**Key words** Eremophilane-type sesquiterpenes; Triterpenes; Peroxide; *Ligularia kanaitzensis*

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## 干崖子橐吾的萜类成分研究

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**摘要** 目的 对橐吾属药用植物干崖子橐吾 *ligularia kanaitzensis* (Franch.) Hand.-Mazz. 药用部位的化学成分进行研究。方法 通过多次柱层析和重结晶分离纯化, 并运用 IR, EI-MS, <sup>13</sup>C NMR, <sup>1</sup>H NMR 等光谱方法进行结构鉴定。结果 分离鉴定了 2 个倍半萜成分: 11-hydroperoxy-6, 9-eremophiladien-8-one (1), 6 $\alpha$ -hydroxy-7(11)-eremopholen-12, 8 $\beta$ -olide (2) 和 4 个三萜成分: 齐敦果酸 (3),  $\beta$ -香树脂醇 (4),  $\beta$ -香树脂醇乙酸酯 (5), 羽伞豆醇 (6)。结论 上述 6 个化合物均为首次从干崖子橐吾中分离得到, 化合物 1 为一过氧化物, 其碳谱数据系首次报道。

**关键词** 干崖子橐吾; 倍半萜; 过氧化物; 三萜