# Two New Sesquiterpenes from *Ligularia lankongensis*

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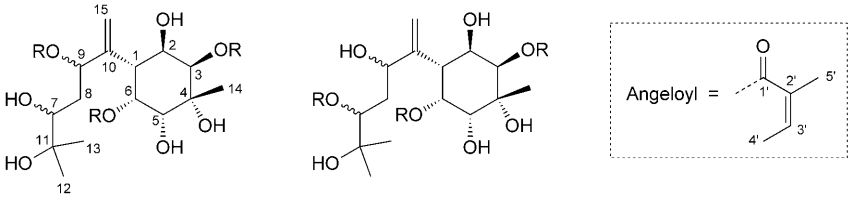
In continuation of a systematic investigation into the chemotaxonomic and bioactive components of pyrrolizidine alkaloids and sesquiterpenoids of *Ligularia* plants, two new, highly oxygenated, rare bisabolene sesquiterpenes, compounds **1** and **2**, were isolated from the roots of *Ligularia lankongensis*. Their structures and relative configurations were elucidated by means of 1D- and 2D-NMR as well as MS analyses.

**Introduction.** – The genus *Ligularia* has been taxonomically placed in the Compositae (tribe Senecioneae), and comprises *ca*. 100 species in China [1]. Some 27 species in this genus have long been used as folk remedies for their antibiotic, antiphlogistic, and antitumor activities [2]. *Ligularia* plants are chemotaxonomically characteristic due to the presence of typical pyrrolizidine alkaloids and sesquiterpenoids. In previous studies [3][4], we reported on several new pyrrolizidine alkaloids.

*Ligularia lankongensis* (FRANCH.) HAND.-MAZZ. mainly grows in the northern parts of Yunnan and in the southwest of Sichuan, China. Its underground parts have been used as expectorant and antibechic remedy in the local areas. Up to date, the bioactive components of this herb have not been disclosed. In continuation of our phytochemical investigations on *Ligularia* species, we herein report two new, highly oxygenated bisabolane sesquiterpenes, compounds **1** and **2**, from *L. lankongensis*. Bisabolane sesquiterpenes are most distributed in Compositae, especially in the genera *Cremanthodium* [5], *Ligularia* [6], *Artemisia* [7], and *Senecio* [8].




**Results and Discussion.** – Compound **1** was obtained as a colorless gum. The $^1$H-and $^{13}$C-NMR (DEPT) spectra indicated 15 skeletal C-atoms: three Me, two $CH_2$, and seven

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CH groups, as well as three quaternary C-atoms. FAB-MS exhibited the $[M+1]^+$ signal at *m/z* 583, with fragment peaks at *m/z* 483 ($[M+1-C_5H_8O_2]^+$) and 383 ($[M+1-(C_5H_8O_2)_2]^+$) due to the successive loss of angelic acid (Ang-OH)[1]) moieties, with *m/z* 83 ($Ang^+$) being the base peak. The molecular formula of **1** was determined as $C_{30}H_{46}O_{11}$, which suggested at least five OH groups.

In the IR spectrum, a strong absorption at 3424 $cm^{-1}$ confirmed the presence of OH groups. Further, the signals of six oxygenated methines at $\delta$(H) 4.74 (*dd*, *J* = 11.7, 2.9), 5.55 (*d*, *J* = 2.9), 3.89 (*d*, *J* = 3.9), 5.68 (*dd*, *J* = 3.9, 2.6), 5.79 (*dd*, *J* = 9.8, 3.7), and 3.55 (*dd*, *J* = 10.5, 1.4 Hz) were observed in the $^1$H-NMR spectrum (*Table*), the corresponding $^{13}$C-NMR resonances appearing at $\delta$(C) 66.9, 78.8, 72.4, 74.2, 77.5, and 76.0, respectively. Since there were two oxygenated quaternary C-atoms ($\delta$(C) 75.5, 73.8), we concluded that there were five OH groups present in **1**, as well as three Ang ester functions. Thus, to accommodate nine degrees of unsaturation, compound **1** was proposed to have a monocyclic sesquiterpene skeleton, with a terminal C=C bond [$\delta$(H) 5.53, 5.29 (2 br. *s*, 1 H each); $\delta$(C) 144.9 (*s*), 117.0 (*t*)], three angeloyloxy moieties, and five OH groups.

A series of $^1$H,$^1$H-COSY and HMQC experiments showed two main structural sequences for **1**: CH(OH)–$CH_2$–CH(OR) and CH(OR)–CH(OH)–CH(CR)–CH(OR)–CH(OH), which were connected based on HMBC experiments and by considering $^2J$ and $^3J$ couplings constants. The following HMBC correlations were observed: H–C(2)/C(1,3), H–C(3)/C(2,4,5,14); H–C(5)/C(6,14), H–C(6)/C(2,4,5), H–C(1)/C(2,9,10,15), H–C(9)/C(1,7,8,10,15), H–C(8)/C(9,10), H–C(7)/C(9,11,12,13), H–C(12)/C(7,11,13), H–C(13)/C(7,11,12), H–C(14)/C(3,4,5), and H–C(15)/C(1,9,10). From the above data, compound **1** was identified as a bisabolene sesquiterpene [5][6]. HMBC Experiments further exhibited the following correlations between bisabolane H-atoms and Ang C=O groups (primed numbers): H–C(3)/C(1′), H–C(6)/C(1″), and H–C(9)/C(1‴).Thus, the three angeloyloxy groups were at C(3), C(6), and C(9). As a consequence, the OH groups had to be at C(2), C(4), C(5), C(7), and C(11) (*Table*).

The relative configuration of **1** was derived from the $^1$H-NMR coupling constants *J*(2,3) = 2.9, *J*(5,6) = 3.9, *J*(1,6) = 2.6, and *J*(1,2) = 11.7 Hz, in combination with NOESY data (*Fig.*). The $\beta$-orientation of the 2-OH and 3-O-Ang groups was deduced from the NOE of H–C(2)/H–C(3), and those of Me(14)/H–C(5), H–C(5)/H–C(6), and H–C(6)/H–C(1) established the $\alpha$-orientation of the 4-OH, 5-OH, 6-O-Ang groups, as well as of the substituent in position 1, respectively. Based on the above experiments, the structure of compound **1** was, thus, assigned as (1$\alpha$,2$\beta$,3$\beta$,4*R**,5$\alpha$,6$\alpha$)-3,6,9-tris[(angeloyl)oxy]bisabol-10(15)-ene-2,4,5,7,11-pentol[2]).

The spectroscopic data of **2** were very similar to those of **1**. FAB-MS exhibited the $[M+1]^+$ peak at *m/z* 583, identical to that of **1**. The $^1$H-NMR spectrum of **2** showed an upfield-shifted signal for H–C(9) and a downfield-shifted one for H–C(7) relative to those of **1** (*Table*). The $^{13}$C-NMR spectrum of **2** also showed an upfield-shifted carbon signal for C(9) and a downfield-shifted one for C(7). The chemical shifts of all the other H- and C-atoms of **2** were basically identical to those of **1**. This clearly indicated that **2** was a positional isomer of **1**. Thus, the three angeloyloxy groups in **2** were placed at

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[1]) Angelic acid = (*Z*)-2-methylbut-2-enoic acid; Ang = angeloyl.

[2]) For fully systematic names, see the *Exper. Part*.

Table. *$^1$H- and $^{13}$C-NMR Data of* **1** *and* **2**. At 500/125 MHz, resp., in $CD_3OD$; $\delta$ in ppm, $J$ in Hz. Asterisks (*) mark overlapping angeloyl (Ang) signals.

| Atom | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| H–C(1) | 3.19 (*dd*, *J* = 11.7, 2.6) | 43.6 (*d*) | 3.19 (*dd*, *J* = 11.8, 2.7) | 42.0 (*d*) |
| H–C(2) | 4.74 (*dd*, *J* = 11.7, 2.9) | 66.9 (*d*) | 4.75 (*dd*, *J* = 11.8, 3.0) | 66.5 (*d*) |
| H–C(3) | 5.55 (*d*, *J* = 2.9) | 78.8 (*d*) | 5.55 (*d*, *J* = 3.0) | 79.1 (*d*) |
| C(4) | | 75.5 (*s*) | | 75.5 (*s*) |
| H–C(5) | 3.89 (*d*, *J* = 3.9) | 72.4 (*d*) | 3.95 (*d*, *J* = 3.9) | 72.2 (*d*) |
| H–C(6) | 5.68 (*dd*, *J* = 3.9, 2.6) | 74.2 (*d*) | 5.72 (*dd*, *J* = 3.9, 2.7) | 76.1 (*d*) |
| H–C(7) | 3.55 (*dd*, *J* = 10.5, 1.4) | 76.0 (*d*) | 5.16 (*dd*, *J* = 9.7, 2.0) | 78.1 (*d*) |
| $CH_2$(8) | 2.21, 1.87 (2*m*) | 37.4 (*t*) | 2.20, 2.13 (2*m*) | 36.3 (*t*) |
| H–C(9) | 5.79 (*dd*, *J* = 9.8, 3.7) | 77.5 (*d*) | 4.36 (*dd*, *J* = 8.6, 5.4) | 76.5 (*d*) |
| C(10) | | 144.9 (*s*) | | 146.9 (*s*) |
| C(11) | | 73.8 (*s*) | | 73.0 (*s*) |
| Me(12) | 1.34 (*s*) | 25.7 (*q*) | 1.32 (*s*) | 25.6 (*q*) |
| Me(13) | 1.31 (*s*) | 25.4 (*q*) | 1.32 (*s*) | 27.2 (*q*) |
| Me(14) | 1.39 (*s*) | 24.3 (*q*) | 1.41 (*s*) | 24.3 (*q*) |
| $CH_2$(15) | 5.53, 5.29 (2*s*) | 117.0 (*t*) | 5.32, 5.21 (2*s*) | 116.5 (*t*) |
| C(1′) | | 168.6 (*s*) | | 168.7 (*s*) |
| C(2′) | | 139.8 (*s*) | | 140.1 (*s*) |
| H–C(3′) | 6.36* | 129.5 (*d*) | 6.30* | 129.8 (*d*) |
| H–C(4′) | 2.21* | 16.5 (*q*) | 2.19* | 16.5 (*q*) |
| H–C(5′) | 2.13* | 21.3 (*q*) | 2.16* | 21.4 (*q*) |
| C(1″) | | 168.5 (*s*) | | 169.7 (*s*) |
| C(2″) | | 139.7 (*s*) | | 138.9 (*s*) |
| H–C(3″) | 6.36* | 129.4 (*d*) | 6.30* | 129.5 (*d*) |
| H–C(4″) | 2.21* | 16.2 (*q*) | 2.19* | 16.3 (*q*) |
| H–C(5″) | 2.13* | 21.2 (*q*) | 2.16* | 21.1 (*q*) |
| C(1‴) | | 168.9 (*s*) | | 169.5 (*s*) |
| C(2‴) | | 139.9 (*s*) | | 140.4 (*s*) |
| H–C(3‴) | 6.28* | 129.5 (*d*) | 6.30* | 129.4 (*d*) |
| H–C(4‴) | 2.21* | 16.5 (*q*) | 2.19* | 16.3 (*q*) |
| H–C(5‴) | 2.13* | 21.4 (*q*) | 2.16* | 21.2 (*q*) |

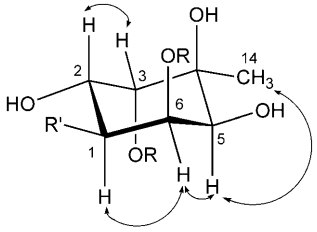


Figure. *Key NOEs for bisabolene* **1** (R = Ang)

C(3), C(6), and C(7), and the structure of **2** was deduced as (1$\alpha$,2$\beta$,3$\beta$,4*R**,5$\alpha$,6$\alpha$)-3,6,7-tris[(angeloyl)oxy]bisabol-10(15)-ene-2,4,5,9,11-pentol.

## Experimental Part

*General.* Column and thin-layer chromatography (CC and TLC, resp.) were performed on silica gel (200–300 mesh) and silica gel $GF_{254}$, resp., supplied by *Qingdao Marine Chemical Factory.* Optical rotation: *Perkin-Elmer-241* polarimeter. IR Spectra: *Nicolet-Impact-410* spectrometer; in $cm^{-1}$. $^1$H-, $^{13}$C-, and 2D-NMR Spectra: *Bruker-AM-400* or *-500* FT-NMR spectrometer; $\delta$ in ppm rel. to $Me_4Si$, $J$ in Hz. MS: *VG-AUTOSPCC-3000* apparatus; in $m/z$.

*Plant Material*. The roots of *Ligularia lankongensis* were collected in Lijiang, Yunnan Province, China, in 2001, and were identified by Dr. *M. Zhang*, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen was deposited at the Herbarium of the China Pharmaceutical University, Nanjing, P. R. China.

*Extraction and Isolation*. The air-dried, pulverized roots of *L. lankongensis* (3.0 kg) were extracted with 95% EtOH at reflux. After removal of the solvent by evaporation, the residue (200 g) was separated by CC (2 kg $SiO_2$; petroleum ether/acetone) to afford four crude fractions: *Fr. 1–4*. *Fr. 3*, eluted with petroleum ether/acetone 10:1, was further separated by CC ($SiO_2$; $CHCl_3$/MeOH 40:1.5) to afford **1** (20 mg) and **2** (42 mg).

*(1α,2β,3β,4*R*,5α,6α)-3,6,9-Tris[(angeloyl)oxy]bisabol-10(15)-ene-2,4,5,7,11-pentol* (= *(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-5-(4,5-Dihydroxy-5-methyl-2-{[(2Z)-2-methylbut-2-enoyl]oxy}-1-methylidenehexyl)-2,3,6-trihydroxy-2-methylcyclohexane-1,4-diyl (2Z,2′Z)-Bis(2-methylbut-2-enoate)*; **1**). Colorless gum. $[\alpha]_D^{20} = -18.37$ ($c = 0.78$, MeOH). IR (KBr): 3424, 2934, 1718, 1647, 1233, 1153, 1085, 931, 760. $^1$H- and $^{13}$C-NMR: see *Table*. FAB-MS (pos.): 583 (2, $[M+1]^+$). FAB-MS (neg.): 581 ($[M-1]^-$). HR-ESI-MS: 605.2924 ($[M+Na]^+$; $C_{30}H_{46}NaO_{11}^+$; calc. 605.2938).

*(1α,2β,3β,4*R*,5α,6α)-3,6,7-Tris[(angeloyl)oxy]bisabol-10(15)-ene-2,4,5,9,11-pentol* (= *(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-5-(2,5-Dihydroxy-5-methyl-4-{[(2Z)-2-methylbut-2-enoyl]oxy}-1-methylidenehexyl)-2,3,6-trihydroxy-2-methylcyclohexane-1,4-diyl (2Z,2′Z)-Bis(2-methylbut-2-enoate)*; **2**). Colorless crystals. M.p. 139.0–140.9°. $[\alpha]_D^{20} = -16.00$ ($c = 0.16$, MeOH). IR (KBr): 3470, 2980, 2956, 2930, 1719, 1696, 1647, 1239, 1162, 1046, 917, 851, 766. FAB-MS (pos.): 583 (53, $[M+1]^+$). HR-ESI-MS: 605.2950 ($[M+Na]^+$; $C_{30}H_{46}NaO_{11}^+$; calc. 605.2938).

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