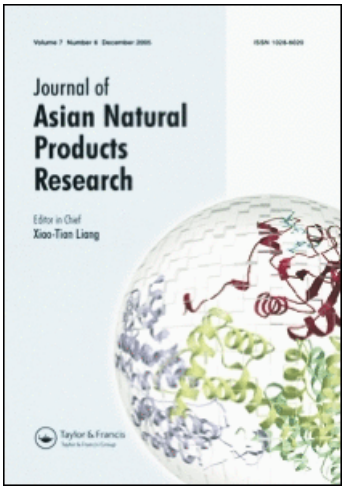


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# Four new terpenoids from the roots of *Ligularia narynensis*

Xue Gao[a]; Wei-Dong Xie[a]; Zhong-Jian Jia[a]
[a] State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, China

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# Four new terpenoids from the roots of *Ligularia narynensis*

XUE GAO, WEI-DONG XIE and ZHONG-JIAN JIA*

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China

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Four new oplopane and guaiane type sesquiterpenoids (**1**–**3**), and a monoterpenoid (**4**) together with three known monoterpenoids (**5**–**7**), have been isolated from the roots of *Ligularia narynensis*. The structures of **1**–**4** were elucidated as 3β,4-diacetoxy-8α-(2-methylbutyryloxy)-9α-(4-methylsenecioyloxy)-11α,12-epoxyoplop-10 (14)-ene (**1**), 3β,4-diacetoxy-9α-(4-acetoxy-4-methylsenecioyloxy)-2β,8α-di (2-methylbutyryloxy)-11α,12-epoxyoplop-10 (14)-ene (**2**), 2α-hydroxy-1βH,7αH,10αH-guai-4,11 (12)-dien-3-one (**3**) and 1α,2β,3α,6α-tetrahydroxy-*p*-menthane (**4**) by spectroscopic methods. **1** and **2** were evaluated for their *in vitro* cytotoxic activity against cultured SMMC-7721 (human hepatoma), L02 (human hepatocyte), and HL-60 (human promyelocytic leukaemia) cell lines.

*Keywords*: *Ligularia narynensis*; Compositae; Sesquiterpenoids; Monoterpenoids

## 1. Introduction

The genus *Ligularia* has been placed taxonomically in the sub-group Senecioneae of the family Compositae, with about 110 species distributed within mainland China [1]. More than 27 *Ligularia* species have been used as traditional Chinese medicinal herbs for the treatment of fever, pain, inflammation, and intoxication, and to invigorate blood circulation [2]. In our previous chemical and biological investigation of the genus *Ligularia*, we found that eremophilanolides and benzofuranolides are the most widespread secondary metabolites, however, germacranolides, bisabolanolides and guaianolides were also found in this genus [3–10]. However, so far oplopane sesquiterpenes have not been found in the genus. In this paper, we report the isolation and structural elucidation of three new sesquiterpenoids (**1**–**3**) and one new monoterpenoid (**4**), along with three known monoterpenoids: 5α-hydroxy-*p*-menth-1-en-2-one (**5**) [11], 5β-hydroxy-*p*-menth-1-en-2-one (**6**) [11], 3β,6β-dihydroxy-*p*-menth-1-ene (**7**) [12] from the roots of *Ligularia narynensis*. The cytotoxicity of compounds **1** and **2** against SMMC-7721 (human hepatoma), L02 (human hepatocyte), and HL-60 (human promyelocytic leukaemia) cell lines was tested.

*Corresponding author. Email: jiazj@lzu.edu.cn

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## 2. Results and discussion

The air-dried and pulverised roots of *L. narynensis* were extracted with petroleum ether/$Et_2O$/$CH_3OH$ (1:1:1). After repeated column chromatographic separation with different eluates, four new terpenoids (**1**–**4**) were isolated together with three known compounds **5**–**7** (figure 1). The structures of the known compounds **5**–**7** were identified by comparison of their spectral data with those reported in the literature.

Compound **1** was obtained as colourless needles. Its molecular formula was determined as $C_{30}H_{44}O_9$ by HRESI-MS, giving a quasi-molecular ion $[M + Na]^+$ at *m*/*z* 571.2876. Its IR spectrum showed the presence of ester group (1735 $cm^{-1}$) and double bond (1646 $cm^{-1}$) absorptions. The $^1H$ NMR and $^{13}C$ NMR (DEPT) spectra (table 1) of **1** displayed the signals of one 2-methylbutyryloxy [$\delta_H$ 2.37 (1H, m), 1.76, 1.40 (each 1H, m), 1.14 (3H, d, $J = 7.0$ Hz), 0.88 (3H, t, $J = 7.0$ Hz)], one 4-methylsenecioyloxy [$\delta_H$ 5.61 (1H, brs), 2.14 (2H, brq, $J = 7.2$ Hz), 2.13 (3H, brs), 1.08 (3H, t, $J = 7.0$ Hz) [13], and two acetoxy groups

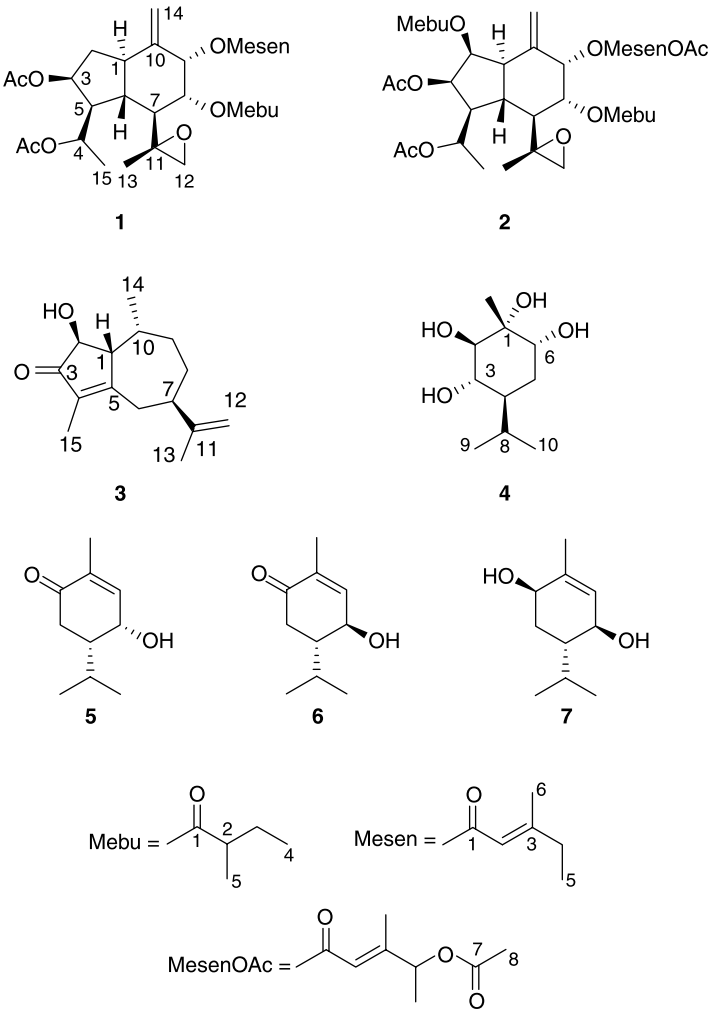


Figure 1. Structures of compounds **1**–**7**.

Table 1. $^{1}$H NMR and $^{13}$C NMR spectral data of **1** and **2** ($CDCl_3$, TMS, $\delta_{ppm}$, $J_{Hz}$).

| | *$^{1}$H NMR* | | *$^{1}$C NMR* | |
|---|---|---|---|---|
| *No.* | ***1*** | ***2*** | ***1*** | ***2*** |
| 1 | 2.34 (ddd, 11.4, 11.0, 3.0) | 2.51 (dd, 11.6, 3.2) | 41.4 (d) | 44.6 (d) |
| 2a | 1.71 (ddd, 11.0, 11.0, 11.0) | 5.67 (dd, 4.4, 3.2) | 33.6 (t) | 71.0 (d) |
| 2b | 2.12 (ddd, 11.0, 6.8, 3.0) | | | |
| 3 | 5.52 (ddd, 11.0, 11.0, 6.8) | 5.55 (dd, 10.8, 4.4) | 72.9 (d) | 72.2 (d) |
| 4 | 5.12 (dq, 6.6, 4.0) | 5.17 (dq, 6.8, 4.0) | 70.1 (d) | 69.7 (d) |
| 5 | 2.64 (ddd, 11.0, 11.0, 4.0) | 2.73 (ddd, 11.0, 10.8, 4.0) | 45.3 (d) | 44.9 (d) |
| 6 | 1.42 (ddd, 11.4, 11.0, 11.0) | 2.00 (ddd, 11.6, 11.0, 11.0) | 47.4 (d) | 43.7 (d) |
| 7 | 1.82 (dd, 11.0, 10.6) | 1.78 (dd, 11.0, 10.6) | 49.0 (d) | 49.2 (d) |
| 8 | 5.03 (dd, 10.6, 3.2) | 5.07 (dd, 10.6, 3.2) | 73.2 (d) | 72.3 (d) |
| 9 | 5.72 (br d, 3.2) | 5.67 (br d, 3.2) | 72.2 (d) | 73.2 (d) |
| 10 | | | 142.9 (s) | 137.8 (s) |
| 11 | | | 55.1 (s) | 55.1 (s) |
| 12a | 2.74 (d, 4.0) | 2.76 (d, 4.0) | 53.0 (t) | 53.0 (t) |
| 12b | 2.81 (d, 4.0) | 2.84 (d, 4.0) | | |
| 13 | 1.19 (s) | 1.21 (s) | 16.1 (q) | 16.3 (q) |
| 14a | 4.93 (br s) | 4.84 (br s) | 112.0 (t) | 115.2 (t) |
| 14b | 5.21 (br s) | 5.24 (br s) | | |
| 15 | 1.46 (d, 6.6) | 1.44 (d, 6.8) | 16.1 (q) | 16.2 (q) |
| OAc: 1 | | | 170.6 (s) | 170.2 (s) |
| 2 | 2.10 (s) | 2.11 (s) | 21.3 (q) | 21.2 (q) |
| OAc: 1 | | | 170.3 (s) | 169.8 (s) |
| 2 | 1.98 (s) | 2.04 (s) | 21.0 (q) | 21.0 (q) |
| OMebu: 1 | | | | 176.0 (s) |
| 2 | | 2.40 (m) | | 41.1 (d) |
| 3 | | 1.48 (m), 1.75 (m) | | 26.3 (t) |
| 4 | | 0.89 (t, 7.0) | | 11.5 (q) |
| 5 | | 1.14 (d, 7.1) | | 16.2 (q) |
| OMebu: 1 | | | 176.1 (s) | 176.1 (s) |
| 2 | 2.37 (m) | 2.40 (m) | 41.1 (d) | 41.1 (d) |
| 3 | 1.40 (m), 1.76 (m) | 1.48 (m), 1.75 (m) | 26.2 (t) | 26.5 (t) |
| 4 | 0.88 (t, 7.0) | 0.97 (t, 7.1) | 11.5 (q) | 11.7 (q) |
| 5 | 1.14 (d, 7.0) | 1.19 (d, 7.0) | 16.1 (q) | 17.5 (q) |
| OMesen: 1 | | OMesenOAc: | 165.4 (s) | 164.9 (s) |
| 2 | 5.61 (br s) | 5.82 (br s) | 114.0 (d) | 114.9 (d) |
| 3 | | | 162.7 (s) | 158.0 (s) |
| 4 | 2.14 (br q, 7.0) | 5.25 (q, 7.0) | 33.7 (t) | 73.5 (d) |
| 5 | 1.08 (t, 7.0) | 1.33 (d, 7.0) | 11.7 (q) | 19.3 (q) |
| 6 | 2.13 (br s) | 2.08 (br s) | 18.8 (q) | 15.4 (q) |
| 7 | | | | 169.9 (s) |
| 8 | | 1.96 (s) | | 19.6 (q) |

[$\delta_H$ 2.10, 1.98 (each 3H, s); $\delta_C$ 170.6 (s), 170.3 (s), 21.3 (q), 21.0 (q)]. The FAB-MS of **1** exhibited fragment peaks at *m/z* 489.4 $[M + H\text{-}HOAc]^+$, 447.1 $[M + H\text{-}HOMebu]^+$, 435.3 $[M + H\text{-}HOMesen]^+$, and 213.3 $[M + H\text{-}HOMesen\text{-}HOMebu\text{-}2HOAc]^+$. Besides these data, the $^{1}$H NMR and $^{13}$C NMR (DEPT) spectra of **1** showed resonances for an exomethylene [$\delta_H$ 4.93, 5.21 (each 1H, brs), $\delta_C$ 112.0 ($CH_2$), 142.9 (C)] and an epoxide [$\delta_H$ 2.74, 2.81 (each 1H, d, $J = 4.0$ Hz); $\delta_C$ 53.0 ($CH_2$), 55.1 (C)]. The remaining NMR signals indicated one methylene, eight methines including four oxygenated ones, and two methyls in which one was tertiary and the other was secondary. To accommodate 9 degrees of unsaturation, compound **1** was proposed to have a bicyclic sesquiterpene skeleton with a terminal double bond, an epoxy group, two acetoxy groups, one 2-methylbutyryloxy group and one 4-methylsenecioyloxy group. $^{1}$H–$^{1}$H COSY spectrum showed two partial structure sequences for the skeleton of compound **1**: CH (1)$CH_2$ (2)CH (3)CH (5)CH (6)CH (7)CH (8)CH (9) and CH (1)CH (6)CH (5)CH (4)$CH_3$ (15). The C—C interconnectivity of both the

fragments was established by analysis of the HMBC correlations of H-14 with C-9, C-1 and C-10, H-13 with C-7, C-11 and C-12, H-12 with C-7, C-11 and C-13, and H-15 with C-5 and C-4. Above information proved **1** to be an oplopanol derivative [14]. The positions of the four ester groups were also confirmed by the correlations in the HMBC spectrum: H-3 ($\delta_H$ 5.52), H-4 ($\delta_H$ 5.12), H-8 ($\delta_H$ 5.03), and H-9 ($\delta_H$ 5.72), respectively, with ester carbonyl carbons ($\delta_C$ 170.6, 170.3, 176.1, and 165.4) attributed to two acetoxy groups, one 2-methylbutyryloxy, and one 4-methylsenecioyloxy. The relative stereochemistry of **1** was deduced from NOESY spectrum, along with inspection of the molecular model. Clear NOESY correlations of H-3 with H-1, H-1 with H-7, and H-7 with H-5 and H-12b indicated that these protons were all oriented on the bottom face of the molecule and were assigned as the α-protons. On the other hand, NOESY correlations of H-8 with H-6 and H-13 demonstrated they were β-oriented on the top face of the molecule. Because the coupling constant $J_{7,8}$ was 10.6 Hz, therefore H-8 was axial form, then H-9 was deduced to be equatorial form from the small coupling constant $J_{8,9} = 3.2$ Hz, so H-8 and H-9 had the same orientation. Hence, compound **1** was structurally determined as 3β,4-diacetoxy-8α-(2-methyl-butyryloxy)-9α-(4-methylsenecioyloxy)-11α,12-epoxyoplop-10 (14)-ene.

Compound **2** was isolated as colourless gum, and the molecular formula $C_{37}H_{54}O_{13}$, was revealed by HRESI-MS (*m/z* 729.3458 $[M + Na]^+$) and the NMR spectra. Its IR spectrum showed the presence of ester group (1735 $cm^{-1}$) and double bond (1656 $cm^{-1}$) absorptions. The $^1H$ and $^{13}C$ NMR (DEPT) spectroscopic features (table 1) of **2** closely resembled those of **1**, but the signals of the 4-methylsenecioyloxy disappeared, and signals for a 4-acetoxy-4-methylsenecioyloxy and an extra 2-methylbutyryloxy arose, correspondingly, the signals for the methylene of H-2 [$\delta_H$ 1.71 (1H, ddd, $J = 11.0, 11.0, 11.0$ Hz), 2.12 (1H, ddd, $J = 11.0, 6.8, 3.0$ Hz), $\delta_C$ 33.6 (C-2)] in **1** were replaced by those for an oxymethine [$\delta_H$ 5.67 (1H, dd, $J = 4.4, 3.2$ Hz), $\delta_C$ 71.0 (C-2)] in **2**. In the HMBC experiment, the correlations between H-2 and the carbonyl signal of 2-methylbutyryloxy at $\delta_C$ 176.0 and between H-9 and the carbonyl signal of 4-acetoxy-4-methylsenecioyloxy at $\delta_C$ 164.9 established the substituted position. The relative stereochemistry of **2** was deduced by the same correlations in NOESY spectrum and coupling constants as those of **1**. We can indicate H-2 ($\delta_H$ 5.67) was in α-orientation from inspection of the molecular model, because H-1 ($\delta_H$ 2.51) was axial and the coupling constant $J_{1,2}$ was 3.2 Hz. So the relative configuration of **2** was assigned as 3β,4-diacetoxy-9α-(4-acetoxy-4-methyl-senecioyloxy)-2β,8α-di(2-methylbutyryloxy)-11α,12-epoxy-oplop-10 (14)-ene.

Compound **3** was obtained as colourless gum. IR spectrum (KBr) showed characteristic absorptions for hydroxyl (3413 $cm^{-1}$), ketone carbonyl (1707 $cm^{-1}$), and double bonds (1637 $cm^{-1}$). The molecular formula was assigned as $C_{15}H_{22}O_2$ on the basis of the $[M + Na]^+$ peak at *m/z* 257.1517 in its HRESI-MS, which was also supported by the NMR spectral data. The $^1H$ NMR spectrum displayed signals for one methyl at $\delta_H$ 1.16 (d, $J = 6.8$ Hz), two olefinic methyls at 1.75 (br s), 1.73 (d, $J = 2.3$ Hz), one characteristic terminal double bond at 4.73 (2H, br s) and an oxygen bearing methine at 3.89 (d, $J = 3.2$ Hz). The $^{13}C$ NMR and DEPT spectra ($CH_3 \times 3$, $CH_2 \times 4$, CH × 4, C × 4) gave corresponding resonances of one terminal double bond and an oxygen methine at $\delta_C$ 149.5 (C), 109.6 ($CH_2$) and 74.7 (CH), respectively. Furthermore, one α,β-unsaturated ketone was given at $\delta_C$ 208.4 (C), 133.8 (C), and 174.0 (C). Above information suggested that compound **3** was very similar to the known compound, 6-hydroxyguaia-4,11 (12)-diene-3-one [15], but the position of the hydroxyl was varied. The long-range correlations in the

HMBC experiment of **3** were observed between H-14 ($\delta_H$ 1.16) with C-1 ($\delta_C$ 54.8), C-9 ($\delta_C$ 31.2), and C-10 ($\delta_C$ 34.1), H-13 ($\delta_H$ 1.75) with C-7 ($\delta_C$ 48.7), C-12 ($\delta_C$ 109.6), and C-11 ($\delta_C$ 149.5), H-15 ($\delta_H$ 1.73) with C-3 ($\delta_C$ 208.4), C-5 ($\delta_C$ 174.0), and C-4 ($\delta_C$ 133.8), and H-2 ($\delta_H$ 3.89) with C-10 ($\delta_C$ 34.1), C-1 ($\delta_C$ 54.8), and C-3 ($\delta_C$ 208.4), which further supported the structure of 2-hydroxyguaia-4,11 (12)-diene-3-one. The relative stereochemistry of **3** was determined by the coupling constants and NOE difference spectra. The coupling constants of H-7 (br dd, $J_{7,6a} = 12.0$ Hz and $J_{7,8a} = 14.0$ Hz) showed that H-7 was axial form, hence the isoprenyl was equatorial form. If the isoprenyl at C-7 was in β-orientation, H-6a was also in β-orientation. Irradiation at H-6a produced positive NOE effect on H-1 and H-10, hence H-1 and H-10 were axial form in β-orientations; however, irradiation at H-2 produced positive NOE effect on H-14, so H-2 the same as H-14 was in α-orientation. Thus, the structure of compound **3** was confirmed as 2β-hydroxy-1βH,7αH, 10βH-guai-4,11 (12)-dien-3-one (**3**).

Compound **4** was obtained as colourless needles. The molecular formula was assigned as $C_{10}H_{20}O_4$ by the $[M + H]^+$ peak at *m/z* 205.1424 in its HRESI-MS and the NMR spectral data. The IR spectrum (KBr) showed the presence of hydroxyl groups (3416 $cm^{-1}$). Both $^1H$ NMR and $^{13}C$ NMR spectra (10 carbons including 3 × $CH_3$, 1 × $CH_2$, 5 × CH, 1 × C), showed the presence of an isopropyl at $\delta_H$ 0.95, 0.81 (each 3H, d, $J = 6.8$ Hz), 2.33 (1H, dqq, $J = 2.8$, 6.8, 6.8 Hz) and $\delta_C$ 15.1 ($CH_3$), 20.8 ($CH_3$), 26.7 (CH), a singlet methyl at $\delta_H$ 1.49 (3H, s) and $\delta_C$ 25.5 ($CH_3$), three oxymethine at $\delta_H$ 3.85 (1H, d, $J = 9.2$ Hz), 3.97 (1H, dd, $J = 9.2$, 11.2 Hz), 4.13 (1H, dd, $J = 2.4$, 3.0 Hz) and $\delta_C$ 76.0 (CH), 68.4 (CH), 63.4 (CH), and a quaternary carbon at $\delta_C$ 75.2 (C). Analysis of the overall NMR spectral data, suggested that compound **4** could be an monoterpenoid of *p*-methane [16] with four hydroxyl. The $^1H$–$^1H$ COSY spectrum established the correlation of the cyclohexane ring: CH (2)CH (3)CH (4)$CH_2$ (5)CH (6). In the HMBC spectrum, correlations of H-7 with C-6 ($\delta_C$ 63.4), C-2 ($\delta_C$ 76.0) and C-1 ($\delta_C$ 75.2), H-3 with C-8 ($\delta_C$ 26.7) and C-4 ($\delta_C$ 41.9), and H-9 and H-10 with C-4 ($\delta_C$ 41.9) and C-8 ($\delta_C$ 26.7) were shown. So the structure of **4** was elucidated as 1,2,3,6-tetrahydroxy-menthane. The relative stereochemistry of **4** was determined by the coupling constants and NOE difference spectra. The $J_{2,3}$ (9.2 Hz), $J_{3,4}$ (11.2 Hz) and $J_{4,5a}$ (12.0 Hz) indicated that these protons were all *trans*-diaxial configuration, if the isopropanyl at C-4 was in β-orientation, the hydroxyl in C-2 was the same, whereas the hydroxyl in C-3 was in *α*-orientation. Meanwhile, the $J_{5a,6}$ and $J_{5e,6}$ (3.0 and 2.4 Hz) indicated that H-6 was β-orientated. Existence of an NOE effect between H-3 and H-7, suggested that H-7 was also in β-orientation. Thus, the structure of compound **4** was confirmed as 1α,2β,3α,6α-tetrahydroxy-*p*-menthane.

The cytotoxicities of compounds **1** and **2** against SMMC-7721 (human hepatoma), L02 (human hepatocyte), and HL-60 (human promyelocytic leukaemia) cells were screened, however they were inactive ($IC_{50} > 100$ μg/ml).

## 3. Experimental

### *3.1 General experimental procedures*

Melting points were determined on an X-4 digital display micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 Polarimeter. IR spectra were determined on a Nicolet 170SX FT-IR instrument in KBr pellets. HRESI-MS were measured on a Bruker APEX II instrument and FAB-MS on a VG-ZAB-HS mass

spectrometer (at 70 eV). NMR spectra were taken with TMS as internal standard on a Varian mercury-400 BB-NMR spectrometer. Column chromatography was performed on silica gel (200–300 mesh and 300–400 mesh, Marine Chemical factory, Qingdao, China), and RP-18 gel (10 μ, E. Merck, Darmstadt, Germany), and TLC was conducted on silica GF254 (Marine Chemical factory, Qingdao, China).

### *3.2 Plant material*

The roots of *Ligularia narynensis* were collected from Tianshan Mountains, Xinjiang province, China, in August 2003. Taxonomic identification was performed by Professor Guo-Liang Zhang, School of Life Science, Lanzhou University. A voucher specimen (No. 20030823) has been deposited at the College of Chemistry and Chemical Engineering, Lanzhou University.



### *3.3 Extraction and isolation*

The air-dried roots of *Ligularia narynensis* (3.5 kg) were pulverised and extracted with petroleum ether (60–90°C)/$Et_2O$/$CH_3OH$ (1:1:1) three times (7 days each time) at room temperature. The extract was concentrated under reduced pressure to afford a residue (70 g). This residue was subjected to silica gel column chromatography (200–300 mesh, 700 g) with a gradient of petroleum ether/acetone (30:1, 15:1, 8:1, and 2:1) as eluent. Four fractions were collected according to TLC analysis. Fraction II (petroleum ether/acetone 15:1, 9.6 g) was separated by silica gel column chromatography (300–400 mesh, 96 g) with petroleum ether/EtOAc (10:1 and 4:1) as eluent to give two fractions (Fr-1 and Fr-2). Fr-1 (petroleum ether/EtOAc 10:1, 3.8 g) was purified by repeated chromatography over a RP-18 column with acetone-$H_2O$ (5:4) to afford **1** (65 mg), **2** (20 mg) and Fr-1-2. Fr-1-2 was further purified by Prep. TLC (petroleum ether/AcOEt, 10:1, two times) to afford pure **5** (2 mg, $R_f = 0.5$), **6** (3 mg, $R_f = 0.4$). Fraction III (petroleum ether/acetone 8:1, 9.2 g) after further repeated silica gel column chromatography (petroleum ether/EtOAc, 6:1 and 2:1) gave **3** (4 mg), **4** (3 mg), and **7** (15 mg).

**3.3.1 3β,4-Diacetoxy-8α-(2-methylbutyryloxy)-9α-(4-methylsenecioyloxy)-11α,12-epoxyoplop-10-(14)-ene (1)**. Colourless needles; mp 169–170°C; $[\alpha]_D^{20}$ + 167.0 (*c* 0.30, $CHCl_3$); IR (KBr) $\nu_{max}$ 2973, 1735, 1646, 1255 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR (DEPT) spectral data, see table 1; FAB-MS *m/z* 489.4 $[M + H\text{-}HOAc]^+$, 447.1 $[M + H\text{-}HOMebu]^+$, 435.3 $[M + H\text{-}HOMesen]^+$, and 213.3 $[M + H\text{-}HOMesen\text{-}HOMebu\text{-}2HOAc]^+$; HRESI-MS *m/z* 571.2876 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_9Na$, 571.2878).

**3.3.2 3β,4-Diacetoxy-9α-(4-acetoxy-4-methylsenecioyloxy)-2β,8α-di(2-methylbutyryloxy)-11α,12-epoxyoplop-10-(14)-ene (2)**. Colourless gum; $[\alpha]_D^{20}$ + 100.0 (*c* 0.20, $CHCl_3$); IR (KBr) $\nu_{max}$ 2969, 1735, 1656, 1240 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR (DEPT) spectral data, see table 1; FAB-MS *m/z* 646.8 $[M–HOAc]^+$, 604.7 $[M–HOMebu]^+$, 534.6 $[M–HOMesen\text{-}HOAC]^+$, 330.4 $[M–HOMesenOAc\text{-}HOMebu\text{-}2HOAc]^+$, 312.4 $[M–HOMesenOAc\text{-}2HOMebu\text{-}HOAc]^+$; HRESI-MS *m/z* 729.3458 $[M + Na]^+$ (calcd for $C_{37}H_{54}O_{13}Na$, 729.3457).

**3.3.3 2α-Hydroxy-1βH,7αH,10αH-guai-4,11-(12)-dien-3-one (3)**. Colourless gum; $[\alpha]_D^{20}$ −26 (*c* 0.5, $CHCl_3$). UV $\lambda_{max}$ $CH_3OH$ (log ε): 301 (4.51) nm; IR (KBr) $\nu_{max}$ 3413 (OH), 1707 (ketone carbonyl), 1637 (double bond) $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR (DEPT) spectral data, see table 2; HRESI-MS *m/z* 257.1517 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1512).

**3.3.4 α,2β,3α,6α-tetrahydroxy-*p*-menthane (4)**. Colourless gum; $[\alpha]_D^{20}$-43.3 (*c* 0.3, $CHCl_3$); IR (KBr) $\nu_{max}$ 3416 (OH) $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR (DEPT) spectral data, see table 2; HRESI-MS *m/z* 205.1424 $[M + H]^+$ (calcd for $C_{10}H_{21}O_4$, 205.1434).

### *3.4 Cytotoxicity assays*

The cytotoxic activities *in vitro* against SMMC-7721 (human hepatoma), L02 (human hepatocytes) and HL-60 (human promyelocytic leukaemia) cell lines of compounds **1** and **2** were carried out according to the sulphorhodamine B (SRB) method [17]. Briefly, exponentially growing cells were harvested and seeded in 96-well plates with the final volume 100 μl containing $5 \times 10^3$ cells per well. After 24 h incubation, cells were treated with various concentrations of those compounds and 10-hydroxycamptothecin (used as a positive control) for 48 h. The absorbency of extracted sulphorhodamine B at 515 nm was measured on a microplate reader (Bio-Rad). The experiments were carried out in triplicate. Each run entailed 5–6 concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%. Because only very small quantities of **3** and **4** were obtained, it was not possible to screen their bioactivity against tumour cell lines.

Table 2. $^1H$ NMR and $^{13}C$ NMR spectral data of **3** and **4** ($CDCl_3$, TMS, $\delta_{ppm}$, $J_{Hz}$)[a].

| | ***3*** | | ***4*** | |
|---|---|---|---|---|
| *No.* | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 2.80 (m) | 54.8 (d) | | 75.2 (s) |
| 2 | 3.89 (d, 3.2) | 74.7 (d) | 3.85 (d, 9.2) | 76.0 (d) |
| 3 | | 208.4 (s) | 3.97 (dd, 9.2, 11.2) | 68.4 (d) |
| 4 | | 133.8 (s) | 2.15 (dddd, 2.8, 2.8, 11.2, 12.0) | 41.9 (d) |
| 5 | | 174.0 (s) | 2.09 (ddd, 2.4, 12.0, 12.0) | 27.9 (t) |
| | | | 1.80 (ddd, 2.8, 3.0, 12.0) | |
| 6 | 2.28 (dd, 12.0, 12.0) | 34.3 (t) | 4.13 (dd, 3.0, 2.4) | 63.4 (d) |
| | 2.75 (br d, 12.0) | | | |
| 7 | 1.87 (br dd, 14.0, 12.0) | 48.7 (d) | 1.49 (s) | 25.5 (q) |
| 8 | 1.83 (m), 1.43 (m) | 35.7 (t) | 2.33 (dqq, 2.8, 6.8, 6.8) | 26.7 (d) |
| 9 | 1.10 (m) | 31.2 (t) | 0.95 (d, 6.8) | 20.8 (q) |
| 10 | 2.17 (m) | 34.1 (d) | 0.81 (d, 6.8) | 15.1 (q) |
| 11 | | 149.5 (s) | | |
| 12 | 4.73 (br s) | 109.6 (t) | | |
| 13 | 1.75 (br s) | 20.8 (q) | | |
| 14 | 1.16 (d, 6.8) | 20.4 (q) | | |
| 15 | 1.73 (d, 2.3) | 7.7 (q) | | |

[a]The $^1H$ NMR spectra of **3** and **4** were obtained at 400 MHz, and the $^{13}C$ NMR spectra of **3** and **4** were obtained at 100 MHz.

## References

[1] A.M. Lu. *The Geography of Spermatophytic Families and Genera*, p. 530, Science Press, Beijing (1999).
[2] L.S. Chen. *Chin. Tradit. Herb. Drugs*, **18**, 1431 (1987).
[3] Z.J. Jia, H.M. Chen. *Phytochemistry*, **30**, 3132 (1991).
[4] Y. Zhao, Z.J. Jia, R.X. Tan, L. Yang. *Phytochemistry*, **31**, 2785 (1992).
[5] Y. Zhao, S. Parsons, B.A. Smart, R.X. Tan, Z.J. Jia, H. Sun, W.H. Rankin David. *Tetrahedron*, **53**, 6195 (1997).
[6] W.S. Wang, K. Gao, L. Yang, Z.J. Jia. *Planta Med.*, **66**, 189 (2000).
[7] X.Q. Li, K. Gao, Z.J. Jia. *Planta Med.*, **69**, 350 (2003).
[8] K. Gao, L. Yang, Z.J. Jia. *Chem. J. Chin. Univ.*, **18**, 748 (1997).
[9] K. Gao, L. Yang, Z.J. Jia. *Planta Med.*, **63**, 46 (1997).
[10] H. Chen, B.G. Wang, Z.J. Jia. *Indian. J. Chem. Sect. B: Org. Chem. Incl. Med. Chem.*, **35B**, 1304 (1996).
[11] T.J. De Pascual, I.S. Bellido, C. Torres, B.A. Sastre, M. Grande. *Phytochemistry*, **20**, 163 (1981).
[12] D. Guillermo, Y.R. Maria. *Phytochemistry*, **30**, 3129 (1991).
[13] K. Masao, S. Noriko. *Chem. Pharm. Bull.*, **40**, 2753 (1992).
[14] A. Arciniegas, A.L. Perez-Castorena, S. Reyes, J.L. Contreras, A.R. de Vivar. *J. Nat. Prod.*, **66**, 225 (2003).
[15] M. Silva, A. Wiesenfeld, P.G. Sammes, T.W. Tyler. *Phytochemistry*, **16**, 379 (1977).
[16] T.J. de Pascual, I.S. Bellido, C. Torres, B.A. Sastre, M. Grande. *Phytochemistry*, **20**, 163 (1981).
[17] P. Skehan, R. Storeng, D. Sncdiero. *J. Natl. Cancer Inst.*, **82**, 1107 (1990).