# Novel Sesquiterpenes from *Ligularia virgaurea* spp. *oligocephala*

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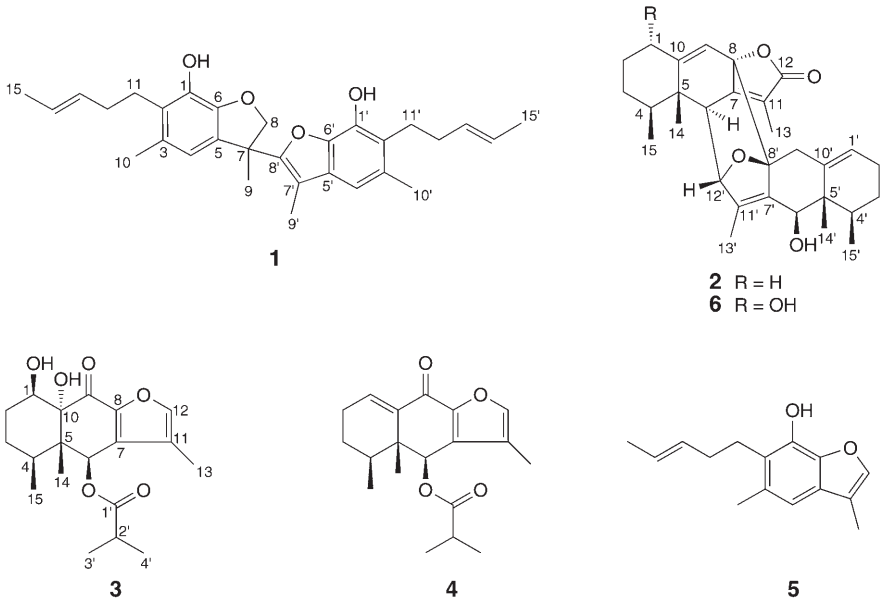
Two novel sesquiterpene dimers, ligularin A (**1**) and ligulolide D (**2**), and one new sesquiterpenoid, 1$\beta$,10$\alpha$-dihydroxy-6$\beta$-[(2-methylpropyl)oxy]furanoeremophil-9-one (**3**), as well as two known sesquiterpenoids, 6$\beta$-[(2-methylpropyl)oxy]-furanoeremophil-1(10)-en-9-one (**4**) and 1-hydroxy-3,7-dimethyl-2-(pent-3-enyl)benzofuran (**5**), were isolated from the petroleum-ether fraction from an alcoholic extract of the whole plant of *Ligularia virgaurea* spp. *oligocephala*. Their structures were elucidated by 1D and 2D-NMR spectroscopy together with HR-ESI-MS analysis, and comparison of the spectroscopic data with those reported for structurally related compounds. In addition, the cytotoxicities against human gastric cancer SGC-7910 cell were measured *in vitro*, the results demonstrated that these sesquiterpenes have no cytotoxicity against the selected tumor cell (all $IC_{50}$ values $>200$ μM).

**Introduction.** – The large family Compositae is a rich source of sesquiterpene natural products. Accordingly, the investigation of bioactive sesquiterpenoids from the family has been one of the subjects of our studies [1–6]. The genus *Ligularia* (Compositae) belongs to the tribe Senecioneae with *ca.* 100 species distributed within China [7], of which more than 27 species have been used as folk remedies due to their antibiotic, antiphlogistic, and antitumor activites [8]. We have been focused on the chemical constituents of *Ligularia* species and reported the isolation of some new sesquiterpenoids [9][10]. And recently, we reported several structurally novel constituents including ligulolides A, B, and C, from the AcOEt and BuOH fractions from alcoholic extracts of *L. virgaurea* spp. *oligocephala* [11–15], which has long been used as a traditional Chinese medicine for the treatment of stomach ache and nausea [16]. In our further studies of the petroleum-ether fraction of the plant, three novel sesquiterpenes, ligularin A (**1**), ligulolide D (**2**), and 1$\beta$,10$\alpha$-dihydroxy-6$\beta$-[(2-methylpropyl)oxy]furanoeremophil-9-one (**3**), and two known compounds, 6$\beta$-[(2-methylpropyl)oxy]furanoeremophil-1(10)-en-9-one (**4**) [17], 1-hydroxy-3,7-dimethyl-2-(pent-3-enyl)benzofuran (**5**), were isolated [18]; we herein report the isolation and structure elucidation of these compounds. Compounds **1**–**5** were also evaluated for their cytotoxicity against SGC-7910 cell.

**Results and Discussion.** – Compound **1** was obtained as yellow gum. $[\alpha]_D^{20} = -22$ ($c = 0.10$, $CHCl_3$). The molecular formula was determined as $C_{30}H_{36}O_4$ on the basis of




the $[M + Na]^+$ peak at $m/z$ 483.2496 (calc. 483.2506 for $C_{30}H_{36}NaO_4$) in its HR-ESI-MS, which was supported by evidence from the $^{13}$C-NMR analysis combined with the DEPT experiment (30 C-atoms as six Me, five $CH_2$, six CH groups, and 13 quaternary C-atoms). The $^1$H- and $^{13}$C-NMR spectra indicated the presence of two pent-3-enyl groups (see *Table 1*); two aromatic Me groups ($\delta$(H) 2.18 (*s*) and 2.35 (*s*), and $\delta$(C) 19.4 and 20.0), a tertiary Me group ($\delta$(H) 1.81 (*s*) and $\delta$(C) 24.9), a Me group ($\delta$(H) 1.84 (*s*) and $\delta$(C) 8.4 on the furan ring appearing as a *singlet* and having no long-range coupling with the $H_\alpha$-atom of the furan ring) and two aromatic H-atoms ($\delta$(H) 6.51 (*s*) and 6.77 (*s*)). All signals mentioned suggested that compound **1** was a benzofuranosesquiterpene dimer. In the NMR spectrum of **1**, the absence of $H_\alpha$-signals of furan ring and the existence of an oxygenated $CH_2$ group ($\delta$(H) 4.42 (*d*, $J = 4.8$ Hz, 1 H) and 4.77 (*d*, $J =$ 4.8 Hz, 1 H), and $\delta$(C) 84.1 (*t*)), and the chemical shifts of C(7) and C(9) changed from $\delta$(C) 116.0 and 7.8, to $\delta$(C) 48.4 and 24.9, respectively, led to the partial structure **I** (*Fig. 1*), which was similar to the structure of compound **5** (*cf. Table 3*). The IR spectrum revealed the presence of OH (3336 cm$^{-1}$), and *g*HMBC correlations between OH, and C(1), C(3), and C(6) indicated that the OH group was at C(1). Extensive analysis with $^1$H- and $^{13}$C-NMR spectra led to the partial structure **II** (*Fig. 1*). The two

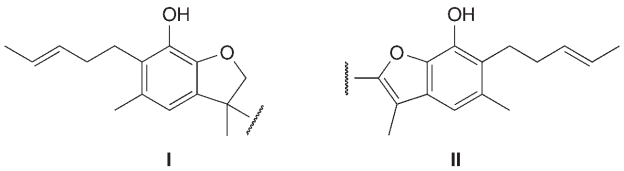



Fig. 1. *Partial structures from 2D-NMR for* **1**

Table 1. *NMR Data of Compound* **1** (in $CDCl_3$)

| Position | $\delta$(H)[a]) | $\delta$(C)[b]) | *g*HMBC[c]) |
|---|---|---|---|
| 1 | | 138.2 (*s*) | |
| 2 | | 129.6 (*s*) | |
| 3 | | 127.4 (*s*) | |
| 4 | 6.51 (*s*) | 116.6 (*s*) | 3, 5, 6, 7, 10 |
| 5 | | 130.2 (*s*) | |
| 6 | | 144.5 (*s*) | |
| 7 | | 48.4 (*s*) | |
| 8 | 4.42 (*d*, *J* = 4.8), 4.77 (*d*, *J* = 4.8) | 84.1 (*t*) | 8′, 5, 6, 7, 9 |
| 9 | 1.81 (*s*) | 24.9 (*q*) | 8′, 5, 7, 8 |
| 10 | 2.18 (*s*) | 19.4 (*q*) | 1, 2, 3, 4, 6 |
| 11 | 2.68 (*t*, *J* = 7.6) | 26.7 (*t*) | 1, 2, 3, 12 |
| 12 | 2.17–2.22 (*m*) | 32.1 (*t*) | 11, 13, 14 |
| 13 | 5.47–5.55 (*m*) | 131.1 (*d*) | 12 |
| 14 | 5.47–5.55 (*m*) | 125.2 (*d*) | 15 |
| 15 | 1.65 (*d* , *J* = 4.8) | 17.9 (*q*) | 13, 14 |
| 1′ | | 138.0 (*s*) | |
| 2′ | | 131.6 (*s*) | |
| 3′ | | 122.6 (*s*) | |
| 4′ | 6.77 (*s*) | 111.3 (*d*) | 3′, 5′, 6′, 7′, 10′ |
| 5′ | | 129.9 (*s*) | |
| 6′ | | 140.3 (*s*) | |
| 7′ | | 111.1 (*s*) | |
| 8′ | | 153.8 (*s*) | |
| 9′ | 1.84 (*s*) | 8.4 (*q*) | 5′, 7′, 8′ |
| 10′ | 2.35 (*s*) | 20.0 (*q*) | 1′, 2′, 3′, 4′, 6′ |
| 11′ | 2.76 (*t*, *J* = 7.6) | 26.6 (*t*) | 1′, 2′, 3′, 12′ |
| 12′ | 2.17–2.22 (*m*) | 32.5 (*t*) | 11′, 13′, 14′ |
| 13′ | 5.47–5.55 (*m*) | 131.1 (*d*) | 12′ |
| 14′ | 5.47–5.55 (*m*) | 125.3 (*d*) | 15′ |
| 15′ | 1.64 (*d*, *J* = 4.8) | 17.9 (*q*) | 13′, 14′ |
| 1-OH | 5.32 (*s*) | | |
| 1′-OH | 5.47 (*s*) | | |

[a]) Recorded at 400.16 MHz. [b]) Recorded at 100.63 MHz, multiplicity deduced by HMQC. [c]) H-Atoms showing long-range correlation with indicated C-atoms. $\delta$ in ppm and TMS as the intensive standard.

structures, **I** and **II** could be assembled into a structure by key correlations in the *g*HMBC spectrum of H−C(8) with C(8′), C(5), C(6), C(7), and C(9), and H−C(9) with C(8′), C(5), C(7), and C(8) (*Fig. 2*). The remaining signals were unambiguously assigned by *g*COSY, *g*HMQC, and *g*HMBC experiments. So the structure of compound **1** was established, and it was named *ligularin A*.

A plausible biosynthetic pathway for the dimer **1** is shown in the *Scheme*. The naturally occurring sesquiterpene **5**, which was also isolated from this plant, is presumably the parent compound for this dimer. First, compound **5** was oxygenated to provide **5A**. Second, **5** could react with **5A** to establish the C−C bond between C(7) and C(8′) by the nucleophilicity at C(7) in **5**, and to give a key intermediate molecule

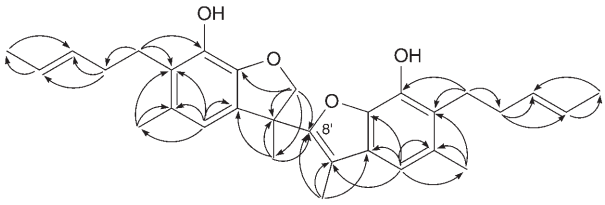
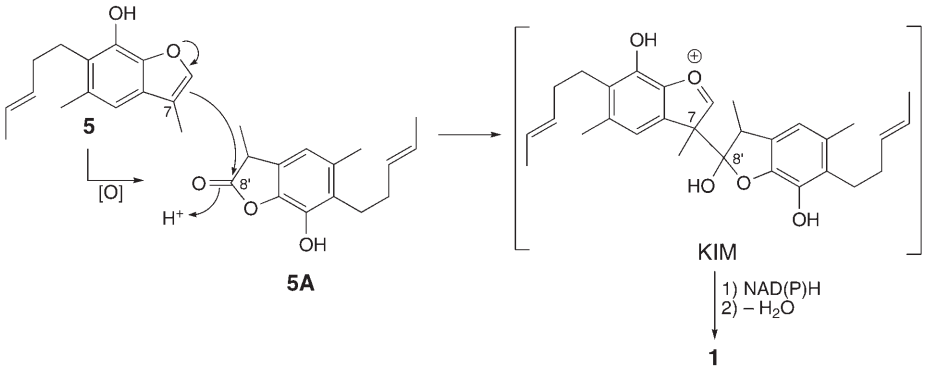



Fig. 2. *Significant* g*HMBC correlations* (H → C) *of* **1**

Scheme. *Possible Biosynthetic Pathway to* **1**




(KIM). Lastly, the incipient oxonium ion in the KIM could be reduced by NAD(P)H. Subsequent elimination of $H_2O$ would produce the dimer **1**.

Compound **2** was obtained as colorless gum. $[\alpha]_D^{20} = -49$ ($c = 0.09$, $CHCl_3$). The molecular formula was established as $C_{30}H_{38}O_4$ on the basis of the $[M + Na]^+$ peak at 485.2657 (calc. 485.2662 for $C_{30}H_{38}NaO_4$) in its HR-ESI-MS, which could be supported by evidence from $^{13}$C-NMR analysis combined with a DEPT experiment (30 C-atoms as six Me, six $CH_2$, and seven CH groups, and eleven quaternary C-atoms). In the NMR spectra (*Table 2*), there were six Me signals ($\delta$(H) 1.67(*s*) and 1.78 (*d*, $J = 2.0$ Hz), and $\delta$(C) 7.6 and 11.1 (olefinic Me groups); $\delta$(H) 0.97 (*s*) and 0.88 (*s*), and $\delta$(C) 19.2 and 14.9; and $\delta$(H) 0.92 (*d*, $J = 6.8$ Hz) and 0.95 (*d*, $J = 6.8$ Hz), and $\delta$(C) 15.2, 16.6, which were two typical eremophilane Me groups) [19][20]. Based on the above data and the molecular formula, compound **2** was assumed to be a dimeric eremophilane sesquiterpene. The IR spectrum showed absorption bands for OH (3465 $cm^{-1}$), and for an $\alpha,\beta$-unsaturated $\gamma$-lactone (1740 $cm^{-1}$), as well as C=C function (1699 $cm^{-1}$). The $^{13}$C resonances at $\delta$(C) 175.0, 164.5, and 116.2 together with the UV absorption at $\lambda_{max}$ (MeOH) 208 nm further evidenced that **2** possesses an $\alpha,\beta$-unsaturated $\gamma$-lactone substructure. Both the $^1$H- and $^{13}$C-NMR spectra also showed the presence of several other typical functions, such as three C=C functions at $\delta$(C) 151.9, 116.5, 126.2, 135.2, 137.6, and 135.9, and $\delta$(H) 5.55 (*d*, $J = 1.2$ Hz, 1 H) and 5.67 (*t*, $J = 3.0$ Hz, 1 H); two oxygenated quaternary C-atoms at $\delta$(C) 85.3 and 88.5, as well as two oxygenated CH groups ($\delta$(C) 77.0 and $\delta$(H) 4.11(*m*, 1 H), and $\delta$(C) 83.7 and $\delta$(H) 4.58 (*d*, $J = 2.0$ Hz, 1 H)). Comparing the data and features of 1D-NMR spectrum with those of a

Table 2. *NMR Data of Compound* **2** (in $CDCl_3$)

| Position | $\delta$(H)[a]) | $\delta$(C)[b]) | *g*COSY | *g*HMBC[c]) | NOESY |
|---|---|---|---|---|---|
| 1 | 2.04–2.07 (*m*) | 32.3 (*t*) | 2, 3 | 2, 3, 9, 10 | |
| 2 | 1.72–1.74 (*m*) | 27.0 (*t*) | 1, 3 | | |
| 3 | 1.34–1.37 (*m*), 1.55–1.59 (*m*) | 30.6 (*t*) | 1, 2 | 1, 4, 5 | |
| 4 | 2.07–2.08 (*m*) | 35.8 (*d*) | 15 | 5, 14, 15 | |
| 5 | | 47.1 (*s*) | | | |
| 6 | 2.79 (*d*, *J* = 2.0) | 45.7 (*d*) | 12′ | 5, 7, 8, 10, 11, 14 | 13, 14, 15, 12′, 13′ |
| 7 | | 164.5 (*s*) | | | |
| 8 | | 85.3 (*s*) | | | |
| 9 | 5.55 (*d*, *J* = 2.0) | 116.5 (*d*) | | 1, 5, 7 | |
| 10 | | 151.9 (*s*) | | | |
| 11 | | 116.2 (*s*) | | | |
| 12 | | 175.0 (*s*) | | | |
| 13 | 1.67 (*s*) | 7.6 (*q*) | | 7, 11, 12 | |
| 14 | 0.97 (*s*) | 19.2 (*q*) | | 4, 5, 6, 10 | 6, 15 |
| 15 | 0.92 (*d*, *J* = 6.8) | 15.2 (*q*) | 4 | 3, 4, 5 | 6, 14, 12′ |
| 1′ | 5.67 (br. *s*) | 126.9 (*d*) | 2′ | 2′, 3′, 5′, 9′ | 2′, 9′ |
| 2′ | 2.01–2.03 (*m*) | 22.7 (*t*) | 1′ | 1′, 3′, 10′ | |
| 3′ | 1.34–1.37 (*m*), 1.55–1.59 (*m*) | 26.6 (*t*) | 2′ | 1′, 2′, 4′, 5′ | |
| 4′ | 1.87–1.92 (*m*) | 33.2 (*d*) | 15′ | 2′, 3′, 5′, 15′ | 6′ |
| 5′ | | 44.2 (*s*) | | | |
| 6′ | 4.11 (*d*, *J* = 2.0) | 77.0 (*d*) | | | 4′ |
| 7′ | | 137.6 (*s*) | | | |
| 8′ | | 88.5 (*s*) | | | |
| 9′ | 2.50 (*d*, *J* = 13.6), 2.37 (*d*, *J* = 13.6) | 37.1 (*t*) | | 8, 1′, 5′, 7′, 8′, 10′, 11′ | |
| 10′ | | 135.2 (*s*) | | | |
| 11′ | | 135.9 (*s*) | | | |
| 12′ | 4.58 (*d*, *J* = 2.0) | 83.7 (*d*) | 6, 13′ | 7, 7′, 8′, 11′ | 6, 15 |
| 13′ | 1.78 (*d*, *J* = 2.0) | 11.1 (*q*) | 12′ | 7′, 11′, 12′ | |
| 14′ | 0.88 (*s*) | 14.9 (*q*) | | 4′, 5′, 6′, 10′ | 15′ |
| 15′ | 0.95 (*d*, *J* = 6.8) | 16.6 (*q*) | 4′ | 3′, 4′, 5′ | 14′ |

[a]) Recorded at 400.16 MHz. [b]) Recorded at 100.63 MHz, multiplicity deduced by HMQC. [c]) H-Atoms showing long-range correlation with indicated C-atoms. $\delta$ in ppm and TMS as the intensive standard.

sesquiterpene dimer (ligulolide B) isolated from the AcOEt fraction of the plant species, revealed that they were very similar, except for an oxygenated CH group ($\delta$(C) 69.3 and $\delta$(H) 4.15 (*m*)) in ligulolide B (**6**) instead of a $CH_2$ in **2** [13]. Therefore, a structure based on the known dimer **6** was inferred. In the *g*HMBC (*Fig. 3*) and *g*COSY experiments, the cross-peaks of H–C(9) at $\delta$(H) 5.55 (*d*, *J* = 2.0 Hz) with C(1) at $\delta$(C) 32.3 (*t*) established the absence of the OH group at C(1). The correlations between H–C(9′) with C(8), and H–C(12′) with C(7), as well as H–C(6) with H–C(12′) are consistent with the two substructures joined at C(6) with C(12′), and at C(8) with C(8′). The remaining signals were unambiguously assigned by *g*COSY, *g*HMQC, and *g*HMBC experiments. The relative configuration of the ring system in **2** could be determined on the basis of key NOESY correlations. The configuration of the OH group at C(6′) was deduced to be $\beta$ on the basis of the cross-peaks between H–C(6′) and H–C(4′). The H–C(6) was $\alpha$-oriented with the equatorial bond in the stereostructure, despite the

cross-peaks between H–C(6) with H–C(14) and H–C(15), while Me(14) and Me(15) were $\beta$-oriented [21]. The smaller coupling constants of $J(6,12') = 2.0$ Hz in the $^1$H-NMR revealed that the dihedral angle between H–C(6) and H–C(12′) was almost 90°, and that RO at C(8) was $\alpha$-oriented and RO at C(8′) was $\beta$-oriented [13][22][23]. Accordingly, the structure of **2** was elucidated as shown in *Fig. 3*, and it was named ligulolide D.

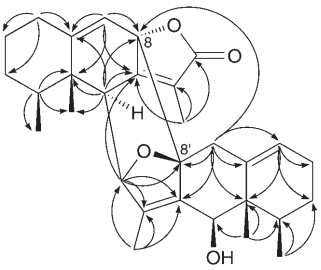



Fig. 3. *Significant* g*HMBC correlations* (H → C) *of* **2**

Compound **3** was obtained as colorless gum. $[\alpha]_D^{20} = +52$ ($c = 0.11$, $CHCl_3$). The molecular formula was deduced as $C_{19}H_{26}O_6$ on the basis of the $[M - H_2O + Na]^+$ peak at $m/z$ 355.1520 (calc. 355.1516 for $C_{19}H_{24}NaO_5$) in its HR-ESI-MS and was supported by evidence from $^{13}$C-NMR analysis combined with the DEPT experiment (19 C-atoms as five Me, two $CH_2$, and five CH groups, and seven quaternary C-atoms). The presence, in the $^1$H-NMR (*Table 3*), of a Me signal ($\delta$(H) 1.92 (*d*, $J = 1.2$ Hz)) and a C=C–H signal ($\delta$(H) 7.47), as well as two Me signals ($\delta$(H) 1.03 (*s*) and 1.13 (*d*, $J =$ 7.2 Hz)) showed that compound **3** was a furanoeremophilane sesquiterpene [24]. Both the $^1$H- and $^{13}$C-NMR spectra also showed the presence of several typical functions, such as a isobutyroyl group ($\delta$(H) 2.70 (*m*, 1 H), 1.23 (*d*, $J = 7.2$ Hz, 3 H), 1.26 (*d*, $J =$ 7.2 Hz, 3 H), and $\delta$(C) 176.5 (*s*), 34.2 (*d*), 18.6 (*q*), 19.5 (*q*)); two oxygenated CH groups ($\delta$(H) 3.92 (*d*, $J = 2.0$ Hz, 1 H) and 7.04 (*s*, 1 H), and $\delta$(C) 62.1 and 68.3); and an oxygenated quaternary C-atom ($\delta$(C) 80.5 (*s*)). The $^{13}$C resonance $\delta$(C) 186.4 coupled with IR bands (1736 and 1676 cm$^{-1}$) and the UV absorption (284 nm) evidenced that **3** possesses a 9-oxo-furanoeremophilane partial structure. The location of the isobutyroyl moiety at C(6) and the other oxygenated C-atoms C(1) and C(10) were deduced by *g*HMBC (*Fig. 4*) with correlations of H–C(6) at $\delta$(H) 7.04 with C(1′) at $\delta$(C) 176.5, C(8) at $\delta$(C) 146.0, C(7) at $\delta$(C) 139.6, C(5) at $\delta$(C) 50.2, and C(14) at $\delta$(C) 16.0, and H–C(1) at $\delta$(H) 3.92 with C(2) at $\delta$(C) 24.4, C(9) at $\delta$(C) 186.4, C(10) at $\delta$(C) 80.5, and C(5) at $\delta$(C) 50.2. On the basis of the absorption bands for OH (3452 cm$^{-1}$) in the IR spectrum and comparison with the known compounds 6$\beta$-(angeloyloxy)-1$\alpha$,10$\beta$-dihydroxy-9-oxofuranoeremophilane [18] and 1$\beta$,10$\beta$-epoxy-6$\beta$-(isobutyryloxy)-9-oxo-furanoeremophilane [25], **3** should possess two OH group at C(1) and C(10), instead of a 1,10-epoxy group. The pattern and small $J$ values between H–C(1) and H–C(2) ($J$(1e, 2e) = $J$(1e, 2a) = 3.0 Hz) indicated that the OH group at C(1) was $\beta$-oriented. The OH group at C(10) was $\alpha$-oriented, because rings A and B were *trans*-fused (*A*/*B-trans*) as deduced from the chemical shift of Me(14) at $\delta$(H) 1.03, (*s*) downfield to the Me(15) at $\delta$(H) 1.13 (*d*, $J = 7.2$ Hz) [23][26]. The configuration of the isobutyroyl moiety at C(6) was deduced to be $\beta$ from the NOESY spectrum, in which significant

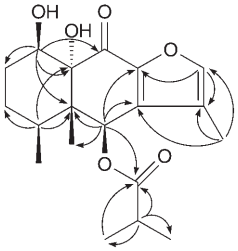



Fig. 4. *Significant* g*HMBC correlations* (H → C) *of* **3**

Table 3. *NMR Data of Compound* **3**, **4**, *and* **5** (in $CDCl_3$)[a])

| Position | **3** | | | **4** | | **5** | |
|---|---|---|---|---|---|---|---|
| | δ(H) | δ(C) | *g*HMBC | δ(H) | δ(C) | δ(H) | δ(C) |
| 1 | 3.92 (*t*, *J* = 3.0) | 62.1 (*d*) | 5, 9, 10 | 6.94 (*t*, *J* = 3.6) | 142.5 (*s*) | | 142.5 (*s*) |
| 2 | 1.72–1.76 (*m*, $H_\alpha$), 2.57–2.65 (*m*, $H_\beta$) | 24.4 (*t*) | | 2.18–2.22 (*m*) | 131.6 (*s*) | | 131.6 (*s*) |
| 3 | 2.42–2.45 (*m*, $H_\alpha$), 1.40–1.43 (*m*, $H_\beta$) | 23.4 (*t*) | | 1.51–1.54 (*m*) | 127.5 (*s*) | | 127.5 (*s*) |
| 4 | 1.61–1.65 (*m*) | 32.0 (*d*) | | 1.89–1.93 (*m*) | 111.5 (*d*) | 6.91 (*s*) | 111.5 (*d*) |
| 5 | | 50.2 (*s*) | | | 122.8 (*s*) | | 122.8 (*s*) |
| 6 | 7.04 (*s*) | 68.3 (*d*) | 5, 7, 8, 14, 1′ | 6.33 (*s*) | 138.5 (*s*) | | 138.5 (*s*) |
| 7 | | 139.6 (*s*) | | | 116.0 (*s*) | | 116.0 (*s*) |
| 8 | | 146.1 (*s*) | | | 140.5 (*d*) | 7.31 (*d*, *J* = 1.2) | 140.5 (*d*) |
| 9 | | 186.4 (*s*) | | | 7.8 (*q*) | 2.20 (*d*, *J* = 1.2) | 7.8 (*q*) |
| 10 | | 80.5 (*s*) | | | 19.9 (*q*) | 2.43 (*s*) | 19.9 (*q*) |
| 11 | | 121.8 (*s*) | | | 26.5 (*t*) | 2.85 (*t*, *J* = 9.2) | 26.5 (*t*) |
| 12 | 7.47 (*s*) | 147.4 (*d*) | 7, 8, 11 | 7.37 (*d*, *J* = 1.2) | 32.4 (*t*) | | 32.4 (*t*) |
| 13 | 1.92 (*s*) | 8.5 (*q*) | 7, 11, 12 | 1.88 (*d*, *J* = 1.2) | 131.1 (*d*) | 5.51–5.64 (*m*) | 131.1 (*d*) |
| 14 | 1.03 (*s*) | 16.0 (*q*) | 4, 5, 6, 10 | 1.10 (*s*) | 125.1 (*d*) | 5.51–5.64 (*m*) | 125.1 (*d*) |
| 15 | 1.13 (*d*, *J* = 7.2) | 16.0 (*q*) | 3, 4, 5 | 0.94 (*d*, *J* = 6.8) | 17.8 (*q*) | 1.70 (*dd*, *J* = 1.2, 4.8) | 17.8 (*q*) |
| 1′ | | 176.5 (*s*) | | | | | |
| 2′ | 2.66–2.71 (*m*) | 34.2 (*d*) | 1′, 3′, 4′ | 2.63–2.68 (*m*) | | | |
| 3′ | 1.23 (*d*, *J* = 7.2) | 18.6 (*q*) | 1′, 2′ | 1.24 (*d*, *J* = 7.2) | | | |
| 4′ | 1.26 (*d*, *J* = 7.2) | 19.5 (*q*) | 1′, 2′ | 1.25 (*d*, *J* = 7.2) | | | |

[a]) TMS was used as internal standard; δ in ppm. Recorded at 400.16 MHz for $^1H$ or at 100.63 MHz for $^{13}C$.

cross-peaks between H–C(6) and H–C(4) could be observed. Hence, **3** was elucidated as 1β,10α-dihydroxy-6β-[(2-methylpropyl)oxy]furanoeremophil-9-one[1]).

The structures of the known compounds **4** and **5** were elucidated by comparison of their NMR data with those reported in the literature.

[1]) For systematic names, see *Exper. Part.*

Sesquiterpenes **1**–**5** were tested for their ability to inhibit human gastric cancer SGC-7910 cell using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [27][28]: the results demonstrated that they have no cytotoxicity against the selected tumor cell (all $IC_{50}$ values $>200$ μM) compared to etoposide (VP-16).

## Experimental Part

*General.* Silica gel (200–300 mesh) used for column chromatography (CC) and silica gel ($GF_{254}$) for TLC were supplied by the *Qingdao Marine Chemical Factory* in China. Spots were detected on the TLC by visualization under UV light, or by heating at 110° and spraying with 98% $H_2SO_4$/EtOH 5:95 (*v*/*v*). Optical rotations: *Perkin-Elmer 241* polarimeter; in MeOH. UV Spectra: *Spect 50-UV/Vis* instrument (*Analytic Jena AG*); $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *FTS165-IR* instrument (*Bio-Rad*, USA); $\tilde{\nu}$ in $cm^{-1}$. $^1$H- (400.13 Hz) and $^{13}$C-NMR (100.62 Hz) spectra: *Varian INOVA-400* FT-NMR spectrometer (USA); in $CDCl_3$ with TMS as internal standard; $\delta$ in ppm, *J* in Hz. HR-ESI-MS: *Bruker APEX II*; in *m*/*z*.

*Plant Material.* The plant material, *L. virgaurea* spp. *oligocephala* (4.0 kg), was collected from Huzhu County, Qinghai Province, P. R. China in August 2002 and was identified by adjunct Prof. *Ji Ma*, Faculty of Pharmacy, First Military Medical University of PLA, Guangzhou, P. R. China. A voucher specimen (NO. 2002001) has been deposited at the Key Laboratory for Natural Medicine of Gansu Province.

*Extraction and Isolation.* The air-dried powder of the whole plants of *L. virgaurea* spp. *oligocephala* (4.0 kg) were extracted three times with 95% EtOH (each for 7 d) at r.t. The crude extract (340.0 g) was suspended in $H_2O$ and extracted successively with petroleum ether, AcOEt, and BuOH. The fraction of petroleum ether (100 g) was subjected to CC (silica gel; petroleum ether/AcOEt 40:1, 20:1, 10:1, 5:1, 1:1, 1:5 (*v*/*v*)) to give six fractions. Fractions were examined by TLC and combined to afford six subfractions (*Fr. 1A–1F*). *Fr. B* was further separated into three subfractions (*Fr. B1–B3*) by CC (petroleum ether/$Me_2CO$ 20:1 (*v*/*v*). *Fr. B1* was subjected to CC (silica gel; petroleum ether/AcOEt 15:1 (*v*/*v*) to produce **5** (70 mg). *Fr. B2* was further fractionated on silica-gel column with petroleum ether/$Me_2CO$ 15:1 (*v*/*v*) to produce **4** (18 mg). *Fr. C* was further subjected to CC (silica gel; petroleum ether/$Me_2CO$ 15:1 (*v*/*v*) to obtain **1** (12 mg). *Fr. D* was subjected to CC (silica gel, petroleum ether/$Me_2CO$ 10:1 (*v*/*v*) to yield the crude compound **2** and **3**. Crude **2** and **3** were further purified by CC (silica gel; petroleum ether/$Me_2CO$ 10:1 (*v*/*v*) for **2** and petroleum ether/$Me_2CO$ 8:1 (*v*/*v*) for **3** to afford pure **2** (40 mg) and pure **3** (9 mg).

*Ligularin A* (**1**). Yellow gum. $[\alpha]_D^{20} = -22$ ($c = 0.10$, $CHCl_3$). UV (MeOH): 210.0 (4.50), 265.0 (3.84). IR (Film): 3336, 2953, 1628, 1601, 1455, 1327, 962. $^1$H- and $^{13}$C-NMR: see *Table 1*. HR-ESI-MS: 483.2496 ($C_{30}H_{36}NaO_4^+$; calc. 483.2506).

*Ligulolide D* (**2**). Colorless gum. $[\alpha]_D^{20} = -49$ ($c = 0.09$, $CHCl_3$). UV (MeOH): 208.0 (4.24). IR (Film): 3465, 2927, 1740, 1699, 1440, 1376, 1233, 1046, 976. $^1$H- and $^{13}$C-NMR: see *Table 2*. HR-ESI-MS: 485.2657 ($C_{30}H_{38}NaO_4^+$; calc. 485.2662).

*1β,10α-Dihydroxy-6β-[(2-methylpropyl)oxy]furanoeremophil-9-one* (=*(4*S*,4a*S*,5*S*,8*R*,8a*R*)-4,4a,5,6,7,8,8a,9-Octahydro-8,8a-dihydroxy-3,4a,5-trimethyl-9-oxonaphtho[2,3-b]furan-4-yl 2-Methylpropanoate*; **3**). Colorless gum. $[\alpha]_D^{20} = +52$ ($c = 0.11$, $CHCl_3$). UV (MeOH): 284.0 (3.78). IR (Film): 3452, 2975, 1736, 1676, 1464, 1388, 1151, 969. $^1$H- and $^{13}$C-NMR: see *Table 3*. HR-ESI-MS: 355.1520 ([*M* − $H_2O$ + Na]$^+$, $C_{19}H_{24}NaO_5^+$; calc. 355.0516).

*Tests of Cytotoxicity against Human Gastric Cancer SGC-7910 Cell.* A MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in 96-well plates. The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to yield a blue formazan product that can be measured spectrophotometrically. In the experiment, the negative controls were isochoric normal saline, 1% DMSO or 0.1% DMSO; positive control was VP-16 at concentrations of 2.5, 5, 10, and 20 μM. SGC-7910 Cells at a log phase of their grown cycle ($1 \times 10^5$ cell/ml) were added to each well (90 μl/well), then treated in four replicates at various concentrations of the drugs with six vacant reference wells set in one plate (100 μl of cultured media in each well) and incubated for 44 h at 37° in a humidified atmosphere of 5% $CO_2$. After 44 h, 10 μl of MTT soln. (5 mg/ml) were added to each

well, which was incubated for another 4 h, then a soln. of 10% SDS was added to each well (100 µl/well). Twelve hours later at r.t., the OD of each well was recorded on an *ELISA* reader (*Bioteck EL-340*) at the wavelength of 570 nm.

This work was supported by the *National Natural Science Foundation of China* (NSFC No. 20475057 and 20372029) and the *Natural Science Foundation of Gansu Province* (No. 3ZS051-A25-077).

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*Received June 4, 2007*