# Terpenoids from the Roots of *Ligularia muliensis*

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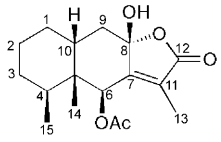
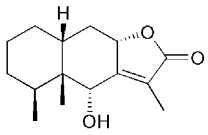
Three new eremophilane-type sesquiterpenes, (6$\beta$,8$\alpha$)-6-(acetyloxy)-8-hydroxyeremophil-7(11)-en-12,8-olide (**1**), (6$\alpha$,8$\alpha$)-6-hydroxyeremophil-7(11)-en-12,8-olide (**2**), and (6$\alpha$,8$\alpha$)-6-(acetyloxy)eremophil-7(11)-en-12,8-olide (**3**) ((8$\alpha$)-eremophil-7(11)-en-12,8-olide = (4a*R*,5*S*,8a*R*,9a*S*)-4a,5,6,7,8,8a,9,9a-octahydro-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one), besides the recently elucidated eremoligularin (**4**) and bieremoligularolide (**5**), as well as a new highly oxygenated monoterpene, *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*)-*p*-menthane-1,2,3,5-tetrol (**12**), together with six known constituents, *i.e.*, the sesquiterpenes **6** and **7**, the norsesquiterpenes **8**–**10**, and the monoterpene **13**, were isolated from the roots of *Ligularia muliensis*. In addition, an attempt to dimerize **1** to a bieremophilenolide (*Scheme*) resulted in the generation of the new derivative (6$\beta$,8$\beta$)-6-(acetyloxy)-8-chloroeremophil-7(11)-en-12,8-olide (**11**). The new structures were established by means of detailed spectroscopic analysis (IR, FAB-, EI-, or HR-ESI-MS as well as 1D- and 2D-NMR experiments). Compounds **4** and **5** were evaluated for their antitumor effects *in vitro* (*Table 3*).

**1. Introduction.** – The genus *Ligularia* has been taxonomically placed in the Compositae (tribe Senecioneae) with *ca.* 100 species distributed within China [1]. Many of them have long been used as traditional herbal medicines with antibiotic, antiphlogistic, and antitumour activities [2]. For example, *L. virgaurea spp. oligocephala* is used to treat stomachache and nausea [3], and *L. duciformis* and *L. fischeri* are used to treat apoplexy, inflammations, and coughs [4]. Phytochemical investigations of many species of *Ligularia* have been reported by our groups [5], which established that the genus *Ligularia* is an important source of sesquiterpenes, many of which show to a certain extent antibacterial and anticancer bioactivity in the bioactive screening. *Ligularia muliensis* grows in mountainous areas in Sichuan Province of China and its phytochemical investigation has not been reported up to now, except for the recent isolation of compounds **4** and **5** (Ang = angeloyl = (2*Z*)-2-methyl-1-oxobut-2-enyl) from the roots of this plant by our group [6]. Herein, we give a detailed description of isolation and purification of all components **1**–**10**, **12**, and **13** from this species, the structural elucidation of the new compounds **1**–**3** and **12**, and the biological activities of **4** and **5**. Moreover, the synthesis of the new chloroeremophilane derivative **11** is described.

**2. Results and Discussion.** – The known compounds $\beta$-sitosterol, **6** [7], **7** [7], **8** [8], **9** [9], **10** [9], and **13** [10] were identified by comparison of their physical and spectral data



**1** trivial numbering[1])



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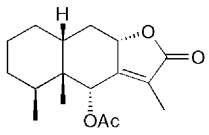

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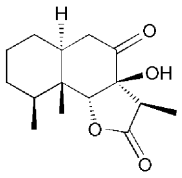

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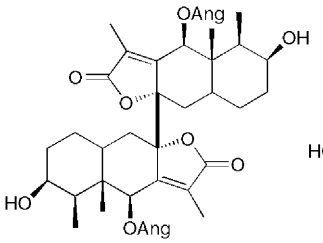
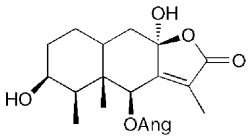


**5** Ang = angeloyl



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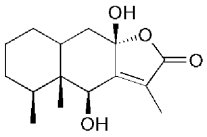

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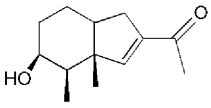

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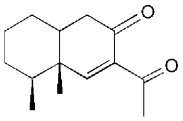

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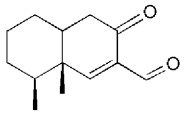

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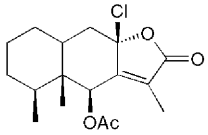
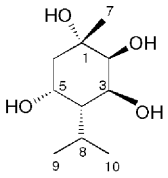


**11**



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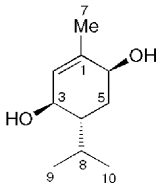

**13**

with those reported in the literature. The structures of compounds **4** and **5** have already been reported in a preliminary communication [6].[1])

Compound **1** was isolated as optically active colorless crystals. Its EI-MS gave a molecular-ion peak at $m/z$ 308, and HR-ESI-MS showed peaks for $[2M+NH_4]^+$ and

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

$[M+NH_4]^+$ at *m/z* 634.3585 and 326.1917, respectively, corresponding to a molecular formula $C_{17}H_{24}O_5$. The IR spectrum of **1** suggested the presence of an OH (3418 $cm^{-1}$), ester carbonyl (1734 $cm^{-1}$), and $\alpha,\beta$-unsaturated $\gamma$-lactone moiety (1714 $cm^{-1}$, 1649 $cm^{-1}$). A detailed analysis of the $^1$H- and $^{13}$C-NMR data (*Tables 1* and *2*) and their comparison with literature data established the structure of **1** as (6$\beta$,8$\alpha$)-6-(acetyloxy)-8-hydroxyeremophil-7(11)-en-12,8-olide[1]).

The $^1$H-NMR spectrum of **1** showed two Me *s* at $\delta$ 1.01 and 1.88 (Me(14) and Me(13)), and a Me *d* at $\delta$ 0.77 (Me(15)). These were characteristic signals for an eremophilene-type sesquiterpene lactone [11]. In addition, a Me *s* of an acetyl group at $\delta$ 2.03 and an oxygenated CH proton at $\delta$ 5.55 suggested the presence of an AcO group in **1**, which was confirmed by 17 C-signals appearing in the $^{13}$C-NMR spectrum. The signal of a quaternary C-atom at $\delta$ 104.6 (C(8)) implied the presence of a hemiacetal moiety. In the HMBC spectrum, the correlations of Me(13) with an ester carbonyl C-atom at $\delta$ 171.7 (C(12)) and olefinic C-atoms at $\delta$ 152.6 (C(7)) and 127.8 (C(11)) showed that **1** was an 8-hydroxyeremophil-7(11)-en-12,8-olide, and the correlations of the oxygenated CH proton at $\delta$ 5.55 with the carbonyl C-atom of the Ac at $\delta$ 171.1, C(7), and C(8) suggested that the AcO was at C(6). In a number of eremophilanolide derivatives, the angular Me–C(5) of the *cis*-fused system appears at $\delta$(H) 0.9–1.2, while in the *trans*-fused system, its $\delta$(H) is 0.5–0.9 ($CDCl_3$ solutions) [12][13]. Thus, **1** should be *cis*-fused since Me(14) resonated at $\delta$ 1.01. The Me(14) and Me(15) groups were $\beta$-orientated [14] as expected from biogenesis. Also OH–C(8) was $\beta$-orientated as suggested by the more downfield shifted $\delta$(H) of Me(14) compared to that of Me(15) [15]. The $\beta$-position of AcO–C(6) was deduced from the absence of a homoallylic $^1$H,$^1$H-coupling between H–C(6) and Me(13) [15]. This was further confirmed by the NOEs at the signals of Me(14) (4.46%) and Me(15) (6.14%) on irradiation of H–C(6).

Table 1. *$^1$H-NMR* (300 MHz, $CDCl_3$) *Data of Compounds* **1**–**3**, **11**, and **12**. $\delta$ in ppm, *J* in Hz. Trivial numbering[1]).

| No. | **1** | **2** | **3** | **11** | **12** |
|---|---|---|---|---|---|
| $CH_2$(1) | 1.74–1.78 (*m*), 1.31–1.35 (*m*) | 1.65–1.71 (*m*), 1.42–1.45 (*m*) | 1.77–1.81 (*m*), 1.31–1.34 (*m*) | 1.75–1.85 (*m*), 1.45–1.55 (*m*) | – |
| $CH_2$(2) or H–C(2) | 1.74–1.78 (*m*), 1.20–1.22 (*m*) | 1.62–1.66 (*m*), 1.35–1.37 (*m*) | 1.77–1.81 (*m*), 1.27–1.31 (*m*) | 1.75–1.85 (*m*), 1.45–1.55 (*m*) | 3.36 (*d*, *J*=2.4) |
| $CH_2$(3) or H–C(3) | 1.39–1.42 (*m*) | 1.42–1.45 (*m*) | 1.40–1.46 (*m*) | 1.45–1.55 (*m*) | 3.76 (*dd*, *J*=2.4, 10.5) |
| H–C(4) | 1.31–1.35 (*m*) | 1.35–1.37 (*m*) | 1.31–1.34 (*m*) | 1.33–1.40 (*m*) | 1.37 (*ddd*, *J*=10.5, 6.0, 2.4) |
| H–C(5) | – | – | – | – | 3.90 (*q*, *J*=2.4) |
| H–C(6) or $CH_2$(6) | 5.55 (*s*) | 4.68 (*d*, *J*=1.8) | 5.74 (br. *s*) | 5.54 (*s*) | 1.60 (*m*), 1.50 (*m*) |
| Me(7) | – | – | – | – | 1.45 (*s*) |
| H–C(8) | – | 5.09 (*ddd*, *J*=10.8, 6.6, 1.8) | 4.88 (*ddd*, *J*=9.0, 3.3, 1.8) | – | 2.07 (*m*) |
| $CH_2$(9) or Me(9) | 2.22 (*dd*, *J*=12.9, 3.6), 1.98 (br. *d*, *J*=12.9) | 2.05 (br. *d*, *J*=14.4, 3.0), 1.72 (br. *d*, *J*=14.4, 3.3) | 2.05 (br. *d*, *J*=12.1), 2.10–2.14 (*m*) | 1.94 (br. *d*, *J*=11.7), 1.87 (br. *d*, *J*=11.7) | 0.91 (*d*, *J*=6.9) |
| H–C(10) or Me(10) | 1.31–1.34 (*m*) | 1.35–1.37 (*m*) | 1.69–1.73 (*m*) | 1.33–1.36 (*m*) | 0.81 (*d*, *J*=6.9) |
| Me(13) | 1.88 (*s*) | 1.84 (br. *d*, *J*=1.8) | 1.93 (br. *d*, *J*=1.8) | 2.02 (*s*) | – |
| Me(14) | 1.01 (*s*) | 1.12 (*s*) | 1.26 (*s*) | 1.09 (*s*) | – |
| Me(15) | 0.77 (*d*, *J*=6.0) | 0.77 (*d*, *J*=6.0) | 0.83 (*d*, *J*=6.3) | 0.80 (*d*, *J*=5.4) | – |
| AcO | 2.03 (*s*) | – | 2.07 (*s*) | 2.09 (*s*) | – |
| OH–C(8) | 3.96 (br. *s*) | – | – | – | – |

Table 2. *$^{13}$C-NMR* (75 MHz, $CDCl_3$) *Data of Compounds* **1**–**3**, **11**, and **12**. $\delta$ in ppm. Trivial numbering[1]).

| No. | **1** | **2** | **3** | **11** | **12** |
|---|---|---|---|---|---|
| $CH_2$(1) or C(1) | 25.3 | 25.7 | 25.9 | 25.0 | 66.2 |
| $CH_2$(2) or $CH_2$) | 19.6 | 19.9 | 20.1 | 19.8 | 64.2 |
| $CH_2$(3) or CH(3) | 30.2 | 30.5 | 30.5 | 30.0 | 67.1 |
| CH(4) | 28.8 | 29.3 | 29.4 | 28.9 | 37.8 |
| C(5) or CH(5) | 41.9 | 42.8 | 42.1 | 42.1 | 69.5 |
| CH(6) or $CH_2$(6) | 71.7 | 69.6 | 72.0 | 70.7 | 30.4 |
| C(7) or Me(7) | 152.6 | 162.2 | 157.0 | 153.5 | 21.5 |
| C(8) or CH(8) | 104.6 | 78.6 | 78.2 | 98.3 | 25.4 |
| $CH_2$(9) or Me(9) | 38.6 | 34.9 | 34.9 | 34.6 | 20.9 |
| CH(10) or Me(10) | 34.8 | 33.7 | 35.2 | 34.4 | 16.5 |
| C(11) | 127.8 | 121.2 | 124.6 | 128.5 | |
| C(12) | 171.7 | 175.1 | 174.3 | 170.5 | |
| Me(13) | 8.5 | 8.6 | 8.7 | 8.8 | |
| Me(14) | 16.2 | 16.3 | 16.5 | 16.2 | |
| Me(15) | 16.1 | 16.1 | 16.3 | 16.2 | |
| *Me*COO | 20.8 | | 21.0 | 20.7 | |
| Me*C*OO | 171.1 | | 170.3 | 169.8 | |

Compound **2** was obtained as optically active colorless crystals. Its molecular formula $C_{15}H_{22}O_3$ was deduced by HR-ESI-MS due to the ion $[M+Na]^+$ at *m*/*z* 273.1464. The IR and NMR data of **2** (*Tables 1* and *2*) were very similar to those of **1**, so compound **2** was also an eremophilenolide. Its structure was established as (6$\alpha$,8$\alpha$)-6-hydroxyeremophil-7(11)-en-12,8-olide[1]).

The differences in the NMR spectra of **1** and **2** were that the Ac signals were absent in **2**, H–C(6) of **1** at $\delta$(H) 5.55 (*s*) was shifted to $\delta$(H) 4.68 (*d*, *J* = 1.8 Hz) in **2** and of **1** C(8) of **1** at $\delta$(C) 104.6 (C) was shifted to $\delta$(C) 78.2 (CH) in **2**. In addition, an additional proton at $\delta$ 5.09 (*ddd*, *J* = 10.8, 6.6, 1.8 Hz) appeared in **2**, instead of the hemiacetal OH–C(8) of **1**. The configurations of **2** were in accordance with those of **1**, except for the configuration at C(6) since **2** exhibited a homoallylic $^1$H,$^1$H-coupling between H–C(6) and Me(13) (*J* = 1.8 Hz). Thus OH–C(6) of **2** was $\alpha$-orientated according to the rule reported by *Naya et al.* [15].

Compound **3** was isolated as optically active colorless crystals. A quasi-molecular-ion peak $[M+H]^+$ at *m*/*z* 293.1743 in the HR-ESI-MS showed that the molecular formula was $C_{17}H_{24}O_4$. The NMR spectra (*Tables 1* and *2*) were extremely similar to those of **2**, excepting the appearance of an AcO group ($\delta$(H) 2.07 (*s*); $\delta$(C) 170.3 and 21.0) in **3** and the downfield shift of its H–C(6) signal ($\delta$(H) 4.68 (*d*, *J* = 1.8 Hz) in **2** *vs.* 5.74 (br. *s*) in **3**), which showed that **3** was an acetylated derivative of **2**. Therefore, compound **3** was assigned as (6$\alpha$,8$\alpha$)-6-(acetyloxy)-eremophil-7(11)-en-12,8-olide[1]).

Compound **12** was obtained as an optically active colorless oil. Its IR spectrum showed an important absorption band for OH groups at 3387 cm$^{-1}$. The EI-MS gave a molecular-ion peak at *m*/*z* 204 and fragment-ion peaks at *m*/*z* 186 ($[M-H_2O]^+$), 168 ($[M-2\,H_2O]^+$), 153 ($[M-2\,H_2O-Me]^+$), 125 ($[M-2\,H_2O-$isopropyl$]^+$) and 107 ($[M-3\,H_2O-$isopropyl$]^+$), and the HR-ESI-MS showed a $[M-H_2O+Na]^+$ ion at *m*/*z* 209.1152 corresponding to a molecular formula $C_{10}H_{20}O_4$. The structure of **12**

was established by the $^1$H- and $^{13}$C-NMR (*Tables 1* and *2*), $^1$H,$^1$H-COSY, HMBC (*Figure*), and NOESY data as *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*)-*p*-menthane-1,2,3,5-tetrol[1]).

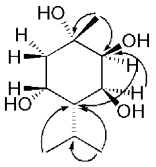


Figure. *Selected HMBC Correlations of Compound* **12**

The $^1$H-NMR, $^{13}$C-NMR, and DEPT spectra of **12** showed signals for 1 C, 5 CH, 1 $CH_2$, and 3 Me. The Me groups were at $\delta$ 1.45 (*s*), 0.91 (*d*, $J$=6.9 Hz), and 0.81 (*d*, $J$=6.9 Hz), and three out of five CH were O-bearing, with signals at $\delta$ 3.36 (*d*, $J$=2.4 Hz, H–C(2)), 3.76 (*dd*, $J$=10.5, 2.4 Hz, H–C(3)), and 3.90 (*t*, $J$=2.4 Hz, H–C(5)). Compound **12** was thus deduced as a menthane monoterpene derivative with four OH groups [16]. The cross-peaks H–C(5)/H–C(4) ($\delta$ 1.37) and $CH_2$(6) ($\delta$ 1.50, 1.60), H–C(4)/H–C(3), H–C(5), and H–C(8) ($\delta$ 2.07), and H–C(2)/H–C(3) in the $^1$H,$^1$H-COSY of **12** and the HMBC correlations Me(7) ($\delta$ 1.45)/C(1) ($\delta$ 66.2) and C(2) ($\delta$ 64.2) as well as other correlations (*Figure*) showed that the four OH groups were located at C(1), C(2), C(3), and C(5) and the $^i$Pr group at C(4). The relative configuration of **12** was deduced from the $^1$H,$^1$H-coupling pattern of the ring protons. Thus, the small $J$(5,6ax)=$J$(5,6eq)=2.4 Hz was characteristic of an equatorial H–C(5), $J$(3,4)=10.5 Hz of the axial positions of H–C(4) and H–C(3), and $J$(2,3)=2.4 Hz of an equatorial and axial orientation H–C(2) and H–C(3), respectively. The configuration at C(1) was determined from a NOESY plot, in which a cross-peak between Me(7) and $H_\beta$–C(6) was observed.

It had been reported that eremophilane derivatives were potent on cytotoxicity. The antitumor activities of compounds **4** and **5** *in vitro* against human leukaemia cell (HL-60), human hepatoma cell (SMMC-7721), and human cervical carcinoma cell (HeLa) were tested by the method of the cells stained with sulforhodamine (SRB). Compound **5** showed strong cytotoxicity, whereas **4** showed weak cytotoxicity against the above three cells (*Table 3*).

Table 3. *Cytotoxocity* ($IC_{50}$ in μg/ml) *of Compounds* **4** *and* **5**

| | HL-60 cell | SMMC-7721 cell | HeLa cell |
|---|---|---|---|
| **4** | 80.22±2.48 | 78.46±10.13 | 57.80±4.00 |
| **5** | 3.81±0.59 | 11.16±1.18 | 6.15±1.12 |
| 10-Hydroxycamptothecine[a]) | 0.02±0.01 | 0.35±0.10 | 0.14±0.02 |

[a]) Hydroxycamptothecine (HCPT) was purchased from Hainan *Weikang Pharmaceutical Co., Ltd*., Hainan, China.

Since compound **5** is a rare novel bieremophilenolide and showed strong antitumor effects, we attempted to synthesize its analog **14** from compound **1** (*Scheme*). However, we only obtained an intermediate **11** which is a new chloroeremophilane derivative.

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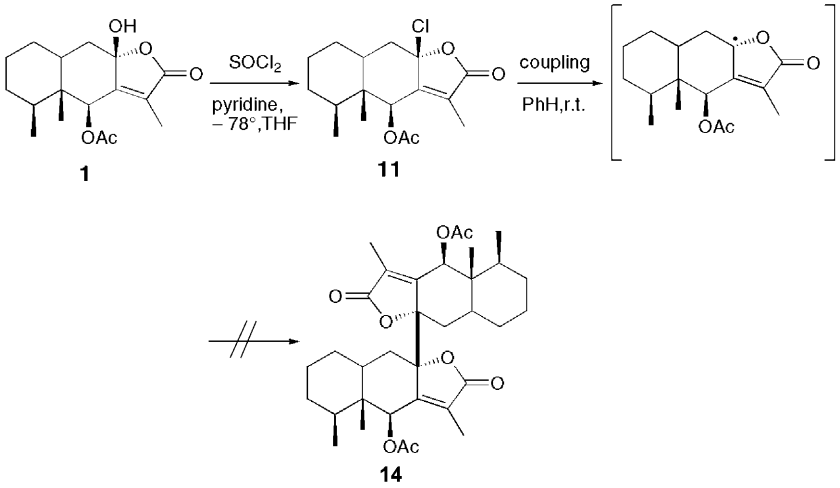
Scheme. *Attempted Dimerization of* **1**




## Experimental Part

*General*. Column chromatography (CC): silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. TLC: silica gel $GF_{254}$ (10–40 μ) from *Qingdao Marine Chemical Factory*; detection at 254 nm or by heating after spraying with 5% $H_2SO_4$ in EtOH (*v*/*v*). $[\alpha]_D$: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet-NEXUS-670-FT-IR* spectrometer; $\tilde{\nu}$ in $cm^{-1}$. NMR Spectra: *Varian-Mercury-300BB* instrument; $^1H$ at 300 and $^{13}C$ at 75 MHz; $SiMe_4$ as internal standard and $CDCl_3$ as solvent. MS: *VG-ZAB-HS* instrument at 70 eV (EI), *ZAB-HS* instrument (FAB), and *Bruker-APEX-II* instrument with glycerol as the matrix (HR-ESI); in *m*/*z*.

*Plant Material*. The dried roots of *Ligularia muliensis* were collected from the Muli autonomic county of Sichuan Province, P. R. China, in August 2003, and authenticated by Prof. *Guoliang Zhang* from the School of Life Science, Lanzhou University.

*Extraction and Isolation*. Dried and powdered roots of *Ligularia muliensis* (1.9 kg) were extracted four times (7 days each time) with petroleum ether/$Et_2O$/acetone 1:1:1 (6 l) and filtered. The combined filtrate was evaporated at 55°: 66.7 g of extract. The extract was subjected to CC (silica gel (700 g; 8.0 × 90 cm), petroleum ether/acetone step gradient 60:1, 10:1, 4:1, and 2:1, and finally MeOH; 450 ml each eluate): *Fr. 1* and *2* (with petroleum ether/acetone 60:1), *Fr. 3–6* (with petroleum ether/acetone 10:1), *Fr. 7* (with petroleum ether/acetone 4:1), *Fr. 8* (with petroleum ether/acetone 2:1), and *Fr. 9* (with MeOH). *Fr. 1* and *Fr. 2* contained paraffin wax and volatile oil and was not further separated. From *Fr. 3*, β-sitosterol (*ca.* 200 mg) was obtained. *Fr. 4* (13.2 g) was subjected to CC (silica gel (200–300 mesh, 200 g; 7.0 × 100 cm), petroleum ether/acetone 30:1 → 0:1): *Fr. 4.1–4.6*. *Fr. 4.1* (1.68 g) was further purified by CC (silica gel, petroleum ether/AcOEt 8:1 → 1:1 followed by crystallization: **4** (7 mg; $R_f$ 0.45 petroleum ether/acetone 3:1)). *Fr. 4.2* was subjected to CC (silica gel, $CHCl_3$/AcOEt 20:1 → 1:1) to give a mixture **5**/**6** which was separated by prep. TLC (silica gel $GF_{254}$ (10–40 μ; 25 × 25 cm), $CHCl_3$/AcOEt 5:1): **5** (20 mg; $R_f$ 0.34) and **6** (5 mg; $R_f$ 0.57). *Fr. 4.4* (1.74 g) was further purified by CC (silica gel, petroleum ether/AcOEt 8:1): **8** (8 mg). *Fr. 4.5* was further purified by prep. TLC (silica gel, petroleum ether/AcOEt 5:1): **3** (18 mg; $R_f$ 0.5). *Fr. 4.6* (1.73 g) was further purified by CC (silica gel, petroleum ether/AcOEt 10:1 → 1:1) followed by crystallization: **2** (210 mg; $R_f$ 0.45 (petroleum ether/acetone 3:1)). *Fr. 5* (5.95 g) was subjected to CC (silica gel (200–300 mesh; 120 g), petroleum ether/acetone 30:1, 20:1, 15:1, 10:1, 8:1, 5:1): *Fr. 5.1–5.3*. *Fr. 5.2* was further purified by repeated CC

(silica gel (200–300 mesh), petroleum ether/acetone 8:1 → 2:1) and then by prep. TLC (silica gel, petroleum ether/AcOEt 5:1): **10** (2 mg; $R_f$ 0.5). From *Fr. 6* (5.95 g), crude crystal line **7** was obtained and then recrystallized from acetone: **7** (197 mg). The rest of *Fr. 6* was further purified by CC (silica gel, petroleum ether/acetone 8:1 → 1:1): *Fr. 6.1–6.4*. *Fr. 6.1* was further purified by CC (silica gel, petroleum ether/AcOEt 15:1 → 2:1): **9** (2 mg; $R_f$ 0.34). From *Fr. 6.2*, the crude crystalline **1** was obtained and then recrystallized from acetone: **1** (97 mg; $R_f$ 0.45). *Fr. 6.3* was purified by CC (silica gel, $CHCl_3$/acetone) and then recrystallized: **13** (10 mg). *Fr. 6.4* was subjected to CC (silica gel (200–300 mesh; 12 mg), with $CHCl_3$/acetone 8:1, 5:1, and 3:1) to afford crude **12** which was further purified by prep. TLC (silica gel, $CHCl_3$/acetone 3:1): **12** (2 mg; $R_f$ 0.5).

*(4*S*,4a*R*,5*S*,8a*R*,9a*R*)-4-(Acetyloxy)-9a-chloro-4a,5,6,7,8,8a,9,9a-octahydro-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one* (**11**). Treatment of **1** (95 mg) with excess thionyl chloride afforded **11**. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*.

The critical dimerization step was then attempted by treating **11** (48 mg) under the dimerization conditions reported in [17][18]. A soln. of **11** in benzene was added to freshly prepared $[CoCl(PPh_3)_3]$ under Ar, and the resulting mixture was stirred at r.t. No dimer was formed, maybe due to the small amount of **11** used.

*Assays of Cytotoxicity. Cell Cultures.* Human leukaemia cells (HL-60), human hepatoma cells (SMMC-7721), and human cervical carcinoma cells (HeLa) were cultured with 10% bovine serum at 37° and with 5% $CO_2$. The survival rates were determined by the sulforhodamine B (SRB) method [19].

*Testing of Cytotoxicity.* Testing for *in vitro* antitumor activities of compounds **4** and **5** against HL-60, SMMC-7721, and HeLa was carried out by the method of the cells stained with SRB [19]: the exponentially growing cells were harvested and seeded in 96-well plates with the final volume 100 µl containing $4\times10^3$ cells per well. After 24 h incubation, cells were treated with various concentrations of **4** or **5** for 48 h. The cultures were fixed at 4° for 1 h by addition of ice-cold 50% aq. $CCl_3COOH$ soln. to give a final concentration of 10%. Fixed cells were rinsed 5 times with deionized $H_2O$ and stained for 10 min with 0.4% SRB dissolved in 0.1% aq. AcOH soln. The wells were washed 5 times with 0.1% aq. AcOH soln. and left to dry overnight. The absorbed SRB was dissolved in 150 µl of unbuffered 1% aq. *Tris* soln. (=tris(hydroxymethyl)aminomethane=2-amino-2-(hydroxymethyl)propane-1,3-diol) (pH 10.5). The absorbency of extracted SRB 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%.

*(6β,8α)-6-(Acetyloxy)-8-hydroxyeremophil-7(11)-en-12,8-olide* (=*(4*S*,4a*R*,5*S*,8a*R*,9a*S*)-4-(Acetyloxy)-4a,5,6,7,8,8a,9,9a-octahydro-9a-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **1**). Colorless crystals. M.p. 129–130°. $[\alpha]_D^{20}=+28$ ($c=1.0$, EtOH). IR: 3418, 1734, 1714, 1649. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-ESI-MS: 634.3585 ($[2M+NH_4]^+$, $C_{34}H_{52}NO_{10}^+$; calc. 634.3586), 326.1917 ($[M+NH_4]^+$, $C_{17}H_{28}NO_5^+$; calc. 326.1954).

*(6α,8α)-6-Hydroxyeremophil-7(11)-en-12,8-olide* (=*(4*R*,4a*R*,5*S*,8a*R*,9a*S*)-4a,5,6,7,8,8a,9,9a-Octahydro-4-hydroxy-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **2**). Colorless crystals. M.p. 187–190°. $[\alpha]_D^{20}=+8.4$ ($c=0.5$, EtOH). IR: 3446, 1935, 1742, 1689, 1714, 1020. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 250, 232, 217, 141, 126, 109, 69, 55, 43. HR-ESI-MS: 273.1464 ($[M+Na]^+$, $C_{15}H_{22}NaO_3^+$; calc. 273.1461).

*(6α,8α)-6-(Acetyloxy)eremophil-7(11)-en-12,8-olide* (=*(4*R*,4a*R*,5*S*,8a*R*,9a*S*)-4-(Acetyloxy)-4a,5,6,7,8,8a,9,9a-octahydro-3,4a,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **3**). Colorless crystals. M.p. 195–198°. $[\alpha]_D^{20}=+23$ ($c=0.5$, EtOH). IR: 3429, 2932, 1760, 1698, 1230. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-ESI-MS: 293.1743 ($[M+H]^+$, $C_{17}H_{25}O_4^+$; calc. 293.1747).

rel-*(1R,2*R*,3*R*,4*S*,5*S*)*-p-*Menthane-1,2,3,5-tetrol* (=*(rel-*1*R,2*R*,3*R*,4*S*,5*S*)-4-Isopropyl-1-methylcyclohexane-1,2,3,5-tetrol*; **12**). Colorless oil. $[\alpha]_D^{14}=-4.2$ ($c=0.5$, EtOH). IR: 3387, 1065, 1032. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 204 ($M^+$), 186 ($[M-H_2O]^+$), 168 ($[M-2\,H_2O]^+$), 153 ($[M-2\,H_2O-Me]^+$), 125 ($[M-2\,H_2O-\text{isopropyl}]^+$), 107 ($[M-3\,H_2O-\text{isopropyl}]^+$). HR-ESI-MS: 209.1152 ($[M-H_2O+Na]^+$, $C_{10}H_{18}NaO_3^+$; calc. 209.1148).

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