# Six New Sesquiterpenes from *Eupatorium lindleyanum*

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The six new germacrane-type sesquiterpenoid lactones **1**–**6** were isolated from the $CHCl_3$ extract of *Eupatorium lindleyanum* DC. The structures of these compounds were determined by spectroscopic analyses, including interpretation of 1D- and 2D-NMR spectra. Compounds **1**–**6** were tested for cytotoxicity against NB4 and K562 leukemia cell lines, showing that **2** and **5** possess potent cytotoxicity (*Table 3*).

**Introduction.** – The plant *Eupatorium lindleyanum* DC., which shows antihistamine and antibacterial activities, is indigenous to China. The whole plant called 'Ye-Ma-Zhui' by local residents is used for the treatment of cough and tracheitis [1]. In previous studies, triterpenoids [2], flavonoids [3], and sesquiterpenes [4–8] were isolated from this plant. These isolates showed a variety of biological activities, especially the sesquiterpenes exhibited potent cytotoxicity [5–8]. In our present investigation, six new sesquiterpenoid lactones, eupalinolides F–K[1]) (**1**–**6**; *Fig. 1*), were isolated from the whole plant of *Eupatorium lindleyanum* DC. Their structures and configurations were elucidated mainly by spectroscopic methods, especially 2D-NMR techniques; and their cytotoxic activities against NB4 and K562 leukemia cell lines were evaluated.

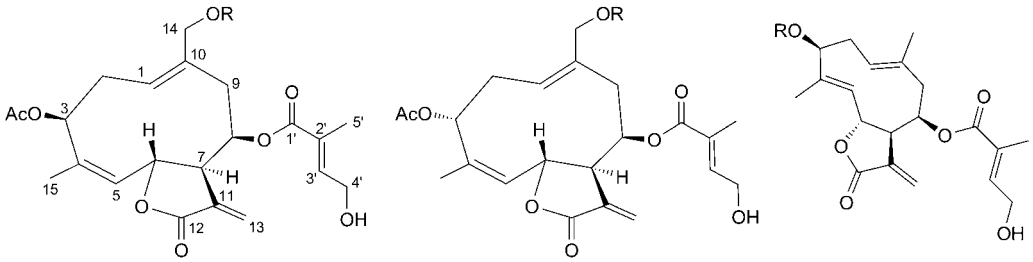


Fig. 1. *Compounds* **1**–**6** *isolated from* Eupatorium lindleyanum

**Results and Discussion.** – *Structure Elucidation*. Eupalinolide F (**1**) was obtained as colorless gum. The molecular formula $C_{22}H_{28}O_8$ was assigned on the basis of the $[M+Na]^+$ peak at *m*/*z* 443.1673 ($C_{22}H_{28}NaO_8^+$) in the HR-ESI-MS. The $^1$H- and

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

$^{13}$C-NMR spectrum of **1** (*Table 1*) displayed 22 C-atoms, which were assigned by HSQC, HMBC, and DEPT experiments to the resonances of three Me groups and five secondary, seven tertiary, and seven quaternary C-atoms. These characteristic features implied that **1** is a germacrane type sesquiterpenoid [5]. In the $^{13}$C-NMR, the signal at $\delta$(C) 169.3 was assignable to the CO of a lactone group, and the signals at $\delta$(C) 137.9 and 124.7 were attributable to a terminal C=C bond, indicating the presence of an $\alpha$-methylidene-$\gamma$-lactone moiety, which was confirmed by the corresponding HMBCs (*Fig. 2*). The H-atom at $\delta$(H) 5.09 and the C-atom at $\delta$(C) 76.2 were assigned to H–C(3) and C(3), respectively, of an O-bearing CH group. The downfield shifted H–C(3) signal indicated that an AcO group was attached to C(3), which was confirmed by HMBCs. The signals at $\delta$(H) 1.70 (br. *s*, Me(5′)), 4.09–4.17 (*m*, $CH_2$(4′)), and 6.64 (br. *s*, H–C(3′)) and at $\delta$(C) 165.6 (ester CO) and 125.9 and 143.4 (C=C bond) obviously pointed to the presence of a (4-hydroxytigloyl)oxy moiety (tiglic acid = (2*E*)-2-methylbut-2-enoic acid), which was confirmed by the pertinent HMBCs and ROESY

Table 1. *NMR Data of Compounds* **1**–**3**. $\delta$ in ppm, *J* in Hz.

| Position | **1**[a]) | | **2**[b]) | | **3**[b]) | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| H–C(1) | 5.24–5.32 (*m*) | 125.7 | 5.46–5.52 (*m*) | 129.9 | 5.22–5.26 (*m*) | 126.5 |
| $CH_2$(2) | 2.60–2.70, 2.33–2.42 (2*m*) | 28.5 | 2.73–2.80, 2.40–2.47 (2*m*) | 29.3 | 2.82–2.88, 2.12–2.18 (2*m*) | 30.1 |
| H–C(3) | 5.09 (br. *s*) | 76.2 | 5.27–5.31 (*m*) | 76.1 | 5.45 (*dd*, *J* = 11.5, 4.5) | 71.7 |
| C(4) | | 135.7 | | 136.5 | | 135.3 |
| H–C(5) | 5.19–5.24 (*m*) | 126.2 | 5.23 (br. *s*) | 126.2 | 5.19–5.24 (*m*) | 125.1 |
| H–C(6) | 5.65–5.73 (*m*) | 75.4 | 5.85 (br. *d*, *J* = 14) | 75.4 | 5.37 (br. *d*, *J* = 10.5) | 74.3 |
| H–C(7) | 3.17–3.21 (*m*) | 47.1 | 3.02 (br. *s*) | 48.1 | 3.06 (br. *s*) | 48.1 |
| H–C(8) | 5.16–5.19 (*m*) | 78.8 | 5.25 (br. *s*) | 78.5 | 5.28 (br. *s*) | 78.9 |
| $CH_2$(9) | 2.98–3.07, 2.26–2.33 (2*m*) | 36.3 | 3.13 (br. *d*, *J* = 13.5), 2.33 (br. *d*, *J* = 14) | 37.6 | 3.10 (br. *d*, *J* = 13), 2.25 (br. *d*, *J* = 13.5) | 37.6 |
| C(10) | | 139.6 | | 134.2 | | 138.4 |
| C(11) | | 137.9 | | 137.1 | | 137.1 |
| C(12) | | 169.3 | | 169.8 | | 169.7 |
| $CH_2$(13) | 6.17, 5.98 (2 br. *s*) | 124.7 | 6.36 (*d*, *J* = 2.5), 5.82 (br. *s*) | 125.1 | 6.33, 5.85 (2 br. *s*) | 124.9 |
| $CH_2$(14) | 4.36, 3.80 (2*d*, *J* = 12.6) | 59.4 | 4.94 (br. *d*, *J* = 12.5), 4.65 (br. *d*, *J* = 13) | 63.2 | 4.57 (br. *d*, *J* = 12.5), 4.20 (br. *d*, *J* = 13) | 60.2 |
| Me(15) | 1.77 (*s*) | 22.5 | 1.84 (br. *s*) | 23.0 | 1.77 (br. *s*) | 17.7 |
| C(1′) | | 165.6 | | 166.0 | | 166.4 |
| C(2′) | | 125.9 | | 127.1 | | 127.6 |
| H–C(3′) | 6.64 (br. *s*) | 143.4 | 6.76 (br. *s*) | 142.7 | 6.75 (br. *t*, *J* = 5.5) | 141.4 |
| $CH_2$(4′) | 4.09–4.17 (*m*) | 58.0 | 4.28 (*d*, *J* = 6) | 59.3 | 4.24 (br. *d*, *J* = 6) | 58.8 |
| Me(5′) | 1.70 (*s*) | 12.3 | 1.77 (*s*) | 12.4 | 1.77 (br. *s*) | 12.4 |
| MeCOO | 2.02 (*s*) | 20.9, 169.0 | 2.01 (*s*) | 20.7, 169.4 | 2.08 (*s*) | 20.8, 170.3 |
| MeCOO | | | 2.17 (*s*) | 21.1, 171.2 | | |

[a]) Measured in ($D_6$)DMSO. [b]) Measured in $CDCl_3$.

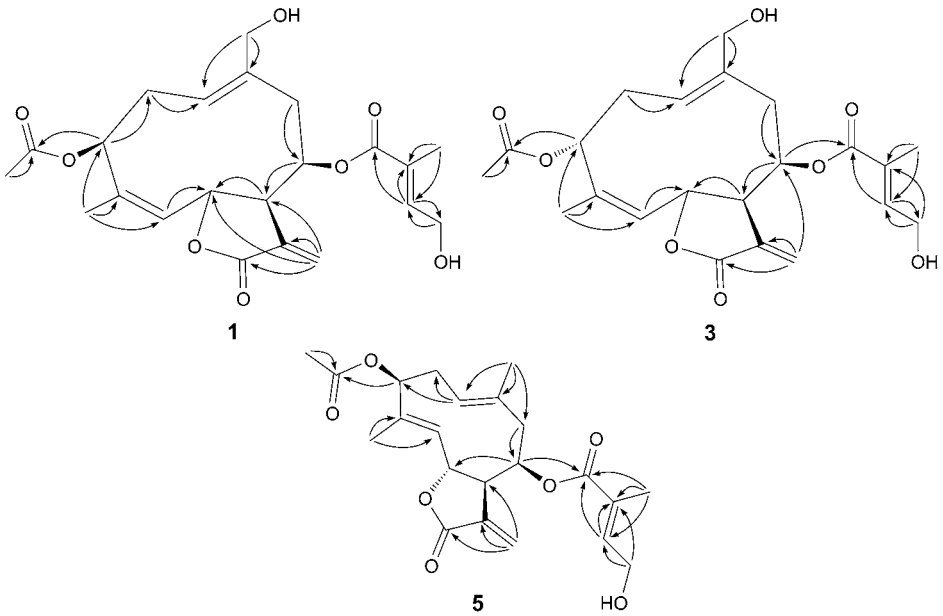


Fig. 2. *Key HMBC features of* **1**, **3**, *and* **5**

correlations (*Fig. 3*) [5]. The position of this unsaturated ester moiety was not indicated by the HMBC spectrum but was assumed to be C(8), because H–C(8) resonated at $\delta$(H) 5.16–5.19 in the lower-field area. A C=C bond inferred by $\delta$(C) 125.7 (C(1)) and 139.6 (C(10)) and $\delta$(H) 5.24–5.32 (*m*, H–C(1)) was assignable to a C(1)=C(10) bond, which was confirmed by the correlations $CH_2$(14)/C(1) and $CH_2$(14)/C(10) and additional important correlations in the HMBC plot. The other C=C bond inferred by $\delta$(C) 135.7 (C(4)) and 126.2 (C(5)) and $\delta$(H) 5.19–5.24 (*m*, H–C(5)) was assignable to C(4)=C(5), which was confirmed by the correlations between Me(15) with C(4) and C(5) and the COSY cross-peaks with the nearby H-atom signals. The relative configuration of **1** was determined *via* a ROESY experiment (*Fig. 2*), in which the correlations between H–C(7) with H–C(5) and H–C(8) indicated that H–C(5) and H–C(8) were $\alpha$-oriented, if H–C(7) adopted an $\alpha$-orientation, which we assume on the basis of biogenetic considerations [5]. The geometry of the C(4)=C(5) bond was (*Z*) according to the ROESY correlation H–C(5)/Me(15). In addition, the correlation H–C(3)/Me(15) indicated that H–C(3) was $\alpha$-oriented, since H–C(5) also adopted an $\alpha$-orientation. The unsaturated ester was determined to be (4-hydroxytigloyl)oxy because the correlations $CH_2$(4′)/H–C(3′) and $CH_2$(4′)/Me(5′) were observed. Based on the above discussion, compound **1** was elucidated to be (1*Z*,3$\beta$,4*Z*,6$\alpha$,7$\beta$,8$\beta$)-3-(acetyloxy)-14-hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

Eupalinolide G (**2**) showed a molecular formula $C_{24}H_{30}O_9$ as determined by HR-ESI-MS (*m*/*z* 485.1789 ($[M+Na]^+$, $C_{24}H_{30}NaO_9^+$). The $^1$H- and $^{13}$C-NMR spectrum of **2** (*Table 1*) displayed 24 C-atoms, which were assigned by HSQC, HMBC, and DEPT

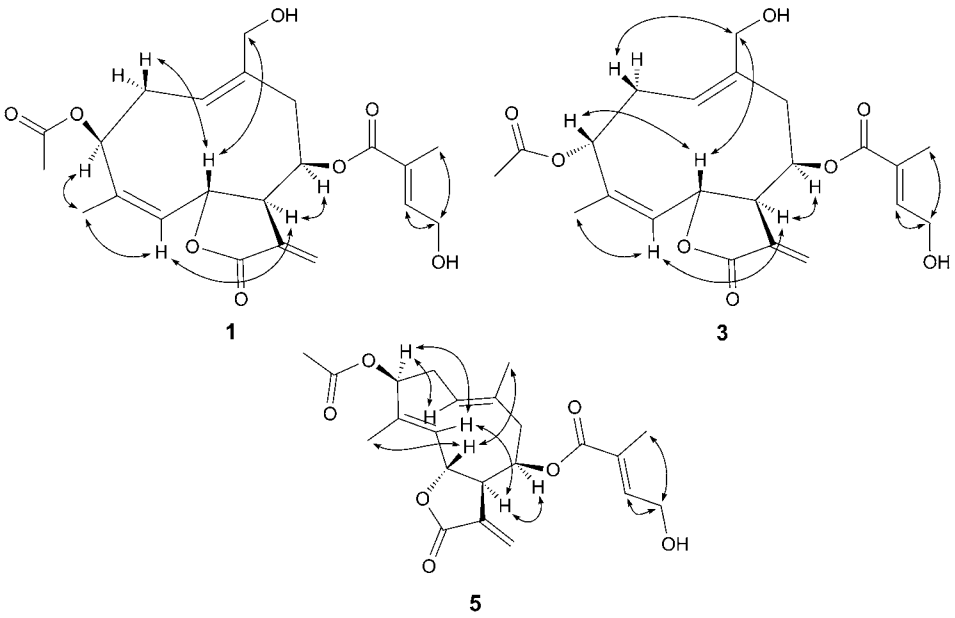


Fig. 3. *Key ROESY correlations of* **1**, **3**, *and* **5**

experiments to the resonances of four Me groups and five secondary, seven tertiary, and eight quaternary C-atoms. Comparison of the $^1$H- and $^{13}$C-NMR data of **2** and **1** revealed that they were very similar, except for the signals of $CH_2$(14) and C(14), which in **2** showed a downfield shift, suggesting that C(14) ($\delta$(C) 63.2) bears an ester group. An *AB* pattern at $\delta$(H) 4.65 and 4.94 was assigned to $CH_2$(14) [4]. HMBC and ROESY (*Fig. 2*) experiments were run to assign all H- and C-atom signals and the relative configuration of **2**. The latter was also confirmed by comparison with the NMR data of hiyodorilactone B (=(1*E*,3$\beta$,4*Z*,6$\alpha$,7$\beta$,8$\beta$)-3-(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone) [9]. Thus, compound **2** was identified as the 14-(acetyloxy) derivative of **1**, namely as (1*Z*,3$\beta$,4*Z*,6$\alpha$,7$\beta$,8$\beta$)-3,14-bis(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

Eupalinolide H (**3**) gave the molecular formula $C_{22}H_{28}O_8$ as determined by HR-ESI-MS (*m*/*z* 443.1673 ($[M+Na]^+$, $C_{22}H_{28}NaO_8^+$)) inferring the presence of nine degrees of unsaturation. The $^1$H- and $^{13}$C-NMR spectrum of **3** (*Table 1*) displayed 22 C-atoms, which were assigned by HSQC, HMBC (*Fig. 2*), and DEPT experiments to the resonances of three Me groups and five secondary, seven tertiary, and seven quaternary C-atoms. According to the NMR data, compound **3** was similar to compound **1**, the difference being H–C(3) of compound **3**, which had a pronounced downfield shift. The ROESY correlation between H–C(3) and H–C(6) indicated that H–C(3) was $\beta$-oriented [7] (*Fig. 3*). HMBC and ROESY experiments were run to assign all H- and C-atom signals and the relative configuration of **3**. Compound **3** was identified as (1*Z*,3$\alpha$,4*Z*,6$\alpha$,7$\beta$,8$\beta$)-3-(acetyloxy)-14-hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

Eupalinolide I (**4**) was formulated as $C_{24}H_{30}O_9$ by HR-ESI-MS (*m/z* 485.1789 ($[M+Na]^+$, $C_{24}H_{30}NaO_9^+$)). The $^1$H- and $^{13}$C-NMR spectrum of **4** (*Table 2*) implied 24 C-atoms, which were assigned by HSQC, HMBC, and DEPT experiments to the resonances of four Me groups and five secondary, seven tertiary, and eight quaternary C-atoms. Comparison of the $^1$H- and $^{13}$C-NMR data of **4** and **3** revealed their similarity, except for those of $CH_2$(14) and C(14) of **4** which were downfield shifted, suggesting that C(14) ($\delta$(C) 62.2) bears an ester group. An *AB* pattern at $\delta$(H) 4.67 and 4.96 was assigned to $CH_2$(14). HMBC and ROESY experiments were run to assign all H- and C-atom signals and the relative configuration of **4**. Thus, compound **4** was identified as (1*Z*,3$\alpha$,4*Z*,6$\alpha$,7$\beta$,8$\beta$)-3,14-bis(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

Table 2. *NMR Data of Compounds* **4**–**6**. $\delta$ in ppm, *J* in Hz.

| Position | **4**[a]) | | **5**[a]) | | **6**[b]) | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| H–C(1) | 5.42–5.44 (*m*) | 129.9 | 4.95 (br. *d*, *J* = 12.9) | 127.7 | 4.96–4.99 (*m*) | 129.0 |
| $CH_2$(2) | 2.83–2.90, 2.19–2.27 (2*m*) | 30.2 | 2.53–2.61, 2.35–2.39 (2*m*) | 32.2 | 2.27–2.33, 2.14–2.22 (2*m*) | 35.4 |
| H–C(3) | 5.52 (*dd*, *J* = 11.5, 5) | 71.3 | 5.20–5.26 (*m*) | 79.0 | 4.15–4.18 (2*m*) | 76.6 |
| C(4) | | 135.4 | | 139.8 | | 144.2 |
| H–C(5) | 5.25–5.29 (*m*) | 125.0 | 4.96 (br. *d*, *J* = 12.9) | 125.5 | 4.92–4.96 (*m*) | 123.0 |
| H–C(6) | 5.36 (br. *d*, *J* = 11) | 74.0 | 5.16–5.20 (*m*) | 74.9 | 5.14 (*t*, *J* = 9.5) | 75.1 |
| H–C(7) | 3.07 (br. *s*) | 48.0 | 2.96–3.00 (*m*) | 52.3 | 3.19–3.24 (*m*) | 50.9 |
| H–C(8) | 5.25 (br. *s*) | 78.9 | 5.79 (br. *s*) | 71.9 | 5.66–5.69 (*m*) | 72.1 |
| $CH_2$(9) | 3.00 (br. *d*, *J* = 13), 2.35 (br. *d*, *J* = 14) | 38.1 | 2.80–2.87, 2.31–2.37 (2*m*) | 44.0 | 2.59–2.66, 2.34–2.39 (2*m*) | 42.9 |
| C(10) | | 133.6 | | 136.1 | | 133.5 |
| C(11) | | 136.9 | | 136.2 | | 137.1 |
| C(12) | | 169.3 | | 169.6 | | 169.0 |
| $CH_2$(13) | 6.34 (*d*, *J* = 2), 5.85 (br. *s*) | 124.9 | 6.30, 5.64 (2*d*, *J* = 3.2) | 121.9 | 6.13, 5.66 (2*d*, *J* = 3.2) | 120.7 |
| $CH_2$(14) or Me(14) | 4.96, 4.67 (2 br. *d*, *J* = 13) | 62.2 | 1.52 (*s*) | 19.3 | 1.41 (*s*) | 18.7 |
| Me(15) | 1.80 (br. *s*) | 17.7 | 1.78 (*s*) | 12.8 | 1.67 (*s*) | 11.9 |
| C(1′) | | 166.3 | | 166.3 | | 165.7 |
| C(2′) | | 126.9 | | 127.3 | | 125.6 |
| H–C(3′) | 6.77 (br. *t*, *J* = 5.5) | 142.5 | 6.80 (br. *t*, *J* = 5.8) | 142.5 | 6.70 (*t*, *J* = 6) | 143.9 |
| $CH_2$(4′) | 4.29 (br. *d*, *J* = 6) | 59.0 | 4.35 (*d*, *J* = 5.8) | 59.6 | 4.12–4.16 (*m*) | 58.0 |
| Me(5′) | 1.78 (br. *s*) | 12.3 | 1.80 (*s*) | 12.9 | 1.74 (*s*) | 12.4 |
| MeCOO | 2.02 (*s*) | 20.5, 169.9 | 2.13 (*s*) | 21.1, 170.4 | | |
| MeCOO | 2.10 (*s*) | 20.7, 170.6 | | | | |

[a]) Measured in $CDCl_3$. [b]) Measured in (D)DMSO.

Eupalinolide J (**5**) had a molecular formula $C_{22}H_{28}O_7$ as deduced from the $[M+Na]^+$ peak at *m/z* 427.1730 ($C_{22}H_{28}NaO_7^+$) in the HR-ESI-MS. The $^1$H- and $^{13}$C-NMR spectrum of **5** (*Table 2*) displayed 22 C-atoms, which were assigned by HSQC, HMBC (*Fig. 2*), and DEPT experiments to the resonances of four Me groups and four secondary, seven

tertiary, and seven quaternary C-atoms. The $^1$H- and $^{13}$C-NMR data of **5** indicated that its structure was closely related to the known compound, (3*β*,8*β*)-3-(acetyloxy)-14-hydroxy-8-(sarracenyloxy)-costunolide = (1*Z*,3*β*,4*E*,6*α*,7*β*,8*β*)-3-(acetyloxy)-14-hydroxy-8-{[(2*E*)-2-(hydroxymethyl)-1-oxobut-2-en-1-yl]oxy}germacra-1(10),4,11(13)-trieno-12,6-lactone [10], and the two compounds likely possess the same sesquiterpenoid core. The differences were the presence of a Me group at C(10) and of a (4-hydroxytigloyl)oxy moiety at C(8) in compound **5**.

The relative configuration of **5** was demonstrated by the ROESY experiment (*Fig. 3*). The correlations H–C(7)/H–C(5) and H–C(8) indicated that H–C(5) and H–C(8) were *α*-oriented since H–C(7) adopted *α*-orientation [5][8]. Then the correlations H–C(3)/H–C(5) suggested that H–C(3) was also *α*-oriented. In addition, the correlations Me(15)/H–C(6) was consistent with the (*E*) configuration of C(4)=C(5) and the correlation H–C(5)/H–C(7). Therefore, the structure of **5** was established as depicted in the formula and elucidated to be (1*E*,3*β*,4*E*,6*α*,7*β*,8*β*)-3-(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

Eupalinolide K (**6**) showed a molecular-ion peak at *m*/*z* 385.1624 ($[M+Na]^+$, $C_{20}H_{26}NaO_6^+$) in its HR-ESI-MS, corresponding to the molecular formula $C_{20}H_{26}O_6$. The $^1$H- and $^{13}$C-NMR spectrum of **6** (*Table 2*) displayed 20 C-atoms, which were assigned by HSQC, HMBC, and DEPT experiments to the resonances of three Me groups and four secondary, seven tertiary, and six quaternary C-atoms. Comparison of the $^1$H- and $^{13}$C-NMR data of **6** and **5** revealed their similarity, except for those of H–C(3) and C(3) of **6** which were upfield shifted, suggesting that C(3) ($\delta$(C) 76.6) bears an OH group. HMBC and ROESY experiments were run to assign all H- and C-atom signals and the relative configuration of **6**. Thus, the structure of **6** was identified as (1*E*,3*β*,4*E*,6*α*,7*β*,8*β*)-3-hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone[1]).

*Biological Study.* The cytotoxic activities of the isolated compounds were determined against NB4 and K562 leukemia cell lines with the MTT method [11]. The $IC_{50}$ values (*Table 3*) showed that compounds **2** and **4** with an AcO group at C(14) had lower $IC_{50}$ values than compounds **1** and **3**, which indicated that the AcO group at C(14) may be essential for the cytotoxicity activities of this type of sesquiterpenoid lactones. Moreover, compound **6** with an OH group at C(3) had a higher $IC_{50}$ value

Table 3. *Cytotoxicity of Compounds* **1**–**6** *against NB4 and K562 Leukemia Cell Lines* ($IC_{50}$ [μM])

| Compound | $IC_{50}$ [μM] | |
|---|---|---|
| | NB4 | K562 |
| **1** | 12.54 | 17.88 |
| **2** | 7.27 | 8.42 |
| **3** | 26.44 | 26.44 |
| **4** | 9.77 | 12.46 |
| **5** | 5.27 | 3.03 |
| **6** | 17.34 | 19.59 |
| Adriamycin[a]) | 1.93 | 2.65 |

[a]) Adriamycin was used as positive control.

than compound **5**, which indicated that the AcO group at C(3) may be essential for the cytotoxic activities of this type of sesquiterpenoid lactones.

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## Experimental Part

*General*. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai). Prep. HPLC: *H&E* chromatograph, equipped with a *P3050* pump and a *QuikSep* UV detector (*UV50*); *YMC-Pack-ODS-A* column (5 μm; 250 × 20 mm i.d.); elution with MeOH/$H_2O$ mixtures; flow rate 5 ml/min; detection at 240 and 254 nm; $t_R$ in min. Column chromatography (CC): silica gel ($SiO_2$; 200–300 mesh), *Sephadex LH-20*, $C_{18}$ reversed-phase silica gel (250 mesh; *YMC*), and *Amberchrom*™ *CG161* (*Rohm and Haas*). TLC: precoated silica gel $GF_{254}$ plates (*Qingdao Haiyang Chemical Plant*, Qingdao, P. R. China). Optical rotations: in MeOH. *Rodolph-Autopol* polarimeter; IR Spectra: *Varian-ProStar-LC240* spectrometer; KBr disk; in $cm^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *Varian-Unity-Inova-400* and *Bruker-AV III-500 MHz* spectrometer; $Me_4Si$ as internal standard. HR-ESI-MS: *Agilent-6520B*-Q-TOF spectrometer; in *m*/*z*.

*Plant Material*. The whole plant of *Eupatorium lindleyanum* DC. was collected from Xuyu County of Jiangsu Province, P. R. China, and identified by Prof. *Dao-Feng Chen*, Department of Pharmacognosy, Fudan University, Shanghai, P. R. China. A voucher specimen (DFC-YMZ2010091201) has been deposited with the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University.

*Extraction and Isolation*. The dried powder of the whole plant (20 kg) of *Eupatorium lindleyanum* DC. was treated with 80% EtOH under reflux. The EtOH extract (2.5 kg), suspended in hot $H_2O$, was successively extracted with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$ extract was concentrated to give a residue (110 g), which was subjected to CC ($SiO_2$, MeOH/$CHCl_3$ 100 : 0 → 0 : 100): *Fractions 1–6*. *Fr. 3* (10.8 g) was fractionated by CC (*Amberchrom*™, 60% $H_2O$/MeOH) and then by CC ($SiO_2$, $CHCl_3$/MeOH 50 : 1 → 2 : 1): *Frs. 3a–3f*. *Fr. 3b* (51 mg) was purified by semi-prep. reversed-phased HPLC (70% $H_2O$/MeOH, **5** (24 mg; $t_R$ 42.6 min). *Fr. 3e* (253 mg) was subjected to semi-prep. reversed-phased HPLC (60% $H_2O$/MeOH: **1** (87 mg; $t_R$ 40.2), **2** (21 mg; $t_R$ 47.5), **3** (17 mg; $t_R$ 43.5), **4** (15 mg; $t_R$ 52.4), and **6** (24 mg; $t_R$ 45.9).

*Eupalinolide F* (= *(1Z,3β,4Z,6α,7β,8β)-3-(Acetyloxy)-14-hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone* = *(2E)-4-Hydroxy-2-methylbut-2-enoic Acid (3aR,4R,6Z,9S,10Z,11aR)-9-(Acetyloxy)-2,3,3a,4,5,8,9,11a-octahydro-6-(hydroxymethyl)-10-methyl-3-methylene-2-oxocyclodeca[b]furan-4-yl Ester*; **1**). Colorless gum. $[\alpha]_D^{25} = -134.8$ ($c = 0.4$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1H$- and $^{13}C$-NMR: *Table 1*. HR-ESI-MS: 443.1673 ($[M + Na]^+$, $C_{22}H_{28}NaO_8^+$; calc. 443.1676).

*Eupalinolide G* (= *(1Z,3β,4Z,6α,7β,8β)-3,14-Bis(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone* = *(2E)-4-Hydroxy-2-methylbut-2-enoic Acid (3aR,4R,6Z,9S,10Z,11aR)-9-(Acetyloxy)-6-[(acetyloxy)methyl]-2,3,3a,4,5,8,9,11a-octahydro-10-methyl-3-methylene-2-oxocyclodeca[b]furan-4-yl Ester*; **2**): Colorless gum. $[\alpha]_D^{25} = -110.5$ ($c = 1.0$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1H$- and $^{13}C$-NMR: *Table 1*. HR-ESI-MS: 485.1789 ($[M + Na]^+$, $C_{24}H_{30}NaO_9^+$; calc. 485.1782).

*Eupalinolide H* (= *(1Z,3α,4Z,6α,7β,8β)-3-(Acetyloxy)-14-hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone* = *(2E)-4-Hydroxy-2-methylbut-2-enoic Acid (3aR,4R,6Z,9R,10Z,11aR)-9-(Acetyloxy)-2,3,3a,4,5,8,9,11a-octahydro-6-(hydroxymethyl)-10-methyl-3-methylene-2-oxocyclodeca[b]furan-1-yl Ester*; **3**): Colorless gum. $[\alpha]_D^{25} = -109.4$ ($c = 1.3$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1H$- and $^{13}C$-NMR: *Table 1*. HR-ESI-MS: 443.1673 ($[M + Na]^+$, $C_{22}H_{28}NaO_8^+$; calc. 443.1676).

*Eupalinolide I* (=*(1*Z*,3α,4*Z*,6α,7β,8β)-3,14-Bis(acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone*=(2*E*)-4-Hydroxy-2-methylbut-2-enoic Acid (3a*R*,4*R*,6*Z*,9*R*,10*Z*,11a*R*)-9-(Acetyloxy)-6-[(acetyloxy)methyl]-2,3,3a,4,5,8,9,11a-octahydro-10-methyl-3-methylene-2-oxocyclodeca[*b*]furan-1-yl Ester*; **4**): Colorless gum. $[\alpha]_D^{25} = -133.6$ ($c = 1.2$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1$H- and $^{13}$C-NMR: *Table 2*. HR-ESI-MS: 485.1789 ($[M+Na]^+$, $C_{24}H_{30}NaO_9^+$; calc. 485.1782).

*Eupalinolide J* (=*(1*E*,3β,4*E*,6α,7β,8β)-3-(Acetyloxy)-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone*=(2*E*)-4-Hydroxy-2-methylbut-2-enoic Acid (3a*R*,4*R*,6*E*,9*S*,10*E*,11a*R*)-9-(Acetyloxy)-2,3,3a,4,5,8,9,11a-octahydro-6,10-dimethyl-3-methylene-2-oxocyclodeca[*b*]furan-4-yl Ester*; **5**): Colorless gum. $[\alpha]_D^{25} = +79$ ($c = 0.2$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1$H- and $^{13}$C-NMR: *Table 2*. HR-ESI-MS: 427.1730 ($[M+Na]^+$, $C_{22}H_{28}NaO_7^+$; calc. 427.1727).

*Eupalinolide K* (=*(1*E*,3β,4*E*,6α,7β,8β)-3-Hydroxy-8-[(4-hydroxytigloyl)oxy]germacra-1(10),4,11(13)-trieno-12,6-lactone*=(2*E*)-4-Hydroxy-2-methylbut-2-enoic Acid (3a*R*,4*R*,6*E*,9*S*,10*E*,11a*R*)-2,3,3a,4,5,8,9,11a-Octahydro-9-hydroxy-6,10-dimethyl-3-methylene-2-oxocyclodeca[*b*]furan-4-yl Ester*; **6**): Colorless gum. $[\alpha]_D^{25} = +85.6$ ($c = 0.3$, MeOH). IR: 3460, 2976, 1653, 1558, 1418, 1260, 1020. $^1$H- and $^{13}$C-NMR: *Table 2*. HR-ESI-MS: 385.1624 ($[M+Na]^+$, $C_{20}H_{26}NaO_6^+$; calc. 385.1622).

*Cytotoxicity Assays.* The cytotoxic assay toward NB4 and K562 leukemia cell lines was carried out by the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) method [11].

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