

## The Constituents of *Siegesbeckia orientalis*

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**Abstract** – Two new diterpenoids, orientalin A (1), and B (2), have been isolated together with six known compounds, kirenol (3), ent-16 $\beta$ ,17-dihydroxykauran-19-oic acid (4), ent-16 $\beta$ ,17-dihydroxykauran-19-oic acid-16 $\beta$ ,17-acetonide (5), 3,7-dimethylquercetin (6),  $\beta$ -sitosterol (7), and daucosterol (8) from the ethanol extract of *Siegesbeckia orientalis* (Compositae). Their chemical structures have been elucidated as ent-15-acetoxy-2 $\alpha$ ,16,19-trihydroxypimar-8(14)-ene (1), ent-16-acetoxy-2 $\alpha$ ,15,19-trihydroxypimar-8(14)-ene (2), respectively, on the basis of chemical and spectral evidence.

**Keywords** – *Siegesbeckia orientalis*, orientalin A and B, Compositae.

### Introduction

Plants of the genus *Siegesbeckia* are annual herbs widely distributed in tropical and temperate zones and they have been used as a traditional medicine to treat rheumatic arthritis, hypertension, malaria, neurasthenia and snake-bite in China. Modern pharmacological experiments showed that the extracts and constituents of *Siegesbeckia* have analgesic, antiinflammatory (Yamatomo *et al.*, 1987), antihypertensive (Kim *et al.*, 1980), antioxidative (Su *et al.*, 1986), and infertile activities (Dong *et al.*, 1989; Yang *et al.*, 1976). A series of ent-kaurane and ent-pimarane diterpenoids and sesquiterpene lactones from *Siegesbeckia* have been reported (Baruah *et al.*, 1979; 1980; Bohlmann *et al.*, 1979; Kim *et al.*, 1979; Liu and Roder, 1991; Zdero *et al.*, 1991). In our continuing search for biologically active constituents from *Siegesbeckia* plants, three new diterpenoids have been reported previously (Xiong *et al.*, 1992). The present communications describes the isolation, struc-

tural elucidation and identification of other two novel compounds.

### Experiment

**General** – Kofler melting points were uncorrected; Optical rotations were taken on a Jasco-20C digital polarimeter. IR were recorded on KBr discs with a Perkin-Elmer 577 spectrometer. UV were obtained in EtOH on a UV-210A spectrometer. EIMS (positive) were measured on a VG Auto Spec-3000 spectrometer with direct inlet 70 or 20 eV. NMR were run on a Bruker AM-400 ( $^1\text{H}$ : 400 MHz,  $^{13}\text{C}$ : 100 MHz) spectrometer using TMS as internal standard; chemical shift values are reported in  $\delta$  (ppm) units (pyridine- $d_5$  and CDC  $l_3$ ). Coupling constants (J) were expressed in Hz.

**Plant Material** – *Siegesbeckia orientalis* were collected in Western Mountain, Kunming, Yunnan, China in Sept, 1992 and identified by Prof. Yanhui Li. A voucher specimen was deposited in the Herbarium of Kunming

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**Extraction and isolation** – Dried and powdered herbs (3.5 kg) were extracted with EtOH under reflux. Evapn of the solvent yielded a residue which was dissolved in EtOH-H<sub>2</sub>O (1:9) and shaken, in order, in petrol (x3), Et<sub>2</sub>O (x4), and n-BuOH (x4). The petrol soln was evapd in vacuo to obtain a residue which was decoloured with activated charcoal in EtOH, filtered and evapd to yield 196 g yellow gum. The Et<sub>2</sub>O and n-BuOH soln were also evapd in

vacuo to yield 12 g and 63 g yellow gums, respectively. The petrol extract was subjected to CC over Al<sub>2</sub>O<sub>3</sub> eluting with increasing proportions of acetone-petrol to yield **7** (1280 mg), **3** (65 mg). The ether extract was submitted to CC (silica gel) eluting with acetone-petrol (1:9-: 3) to afford **5** (20 mg), **4** (47 mg), **6** (175 mg), **3** (140 mg), **8** (30 mg). The n-BuOH extract was chromatographed on a silica gel column eluting with 3-15% MeOH-CHCl<sub>3</sub> to obtain **2** (100 mg), **1** (30 mg), **3** (2144 mg). Some components were further purified by recrystallization and prep. TLC (silica gel).

Orientalin A (**1**), C<sub>22</sub>H<sub>36</sub>O<sub>5</sub>, colourless needles (CHCl<sub>3</sub>-cyclohexane), mp. 158-159°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -57.5° (MeOH, c 0.348); no UV absorption; IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3340, 1725, 1650, 1450, 1365, 1240, 1035; EIMS (20 eV) m/z (%): 381[M+1]<sup>+</sup>(2), 363[M-OH]<sup>+</sup>(7), 345[M-H<sub>2</sub>O-OH]<sup>+</sup>(1), 321[M+1-AcOH]<sup>+</sup>(9), 277[M-CH(OAc)CH<sub>2</sub>OH]<sup>+</sup>(100), 259 [277-H<sub>2</sub>O]<sup>+</sup>(67), 241[277-2H<sub>2</sub>O]<sup>+</sup>(12), 151(25), 133 (14), 121(51), 109(51), 95(29), 81(15), 55(3), 43 (16); <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1 and 2.

Orientalin B (**2**), C<sub>22</sub>H<sub>36</sub>O<sub>5</sub>, colourless needles (CHCl<sub>3</sub>-cyclohexane), mp. 92.5-94°C; [ $\theta$ ]<sub>D</sub><sup>20</sup><sub>14.5</sub> -1352.448 (EtOH, c 0.0227); no UV absorption; IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3500, 3330, 1705, 1650, 1450, 1375, 1260, 1095, 1040, 1015; EIMS (20 eV) m/z (%): 381[M+1]<sup>+</sup>(1), 363[M-OH]<sup>+</sup>(3), 345[M-H<sub>2</sub>O-OH]<sup>+</sup>(1), 320[M-AcOH]<sup>+</sup>(5), 277[M-

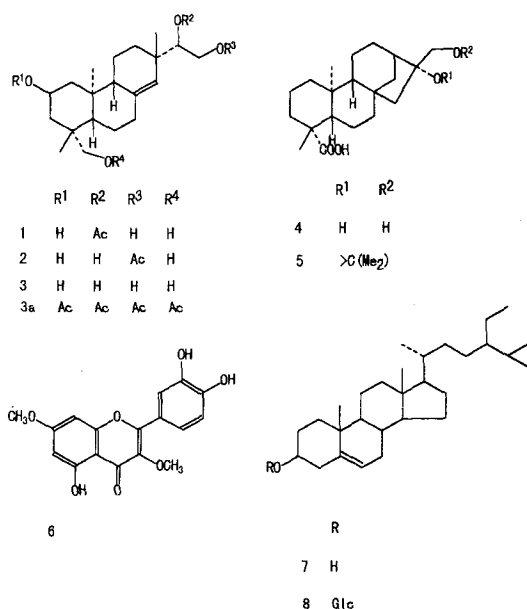


Fig. 1. The Structures of Compounds 1-8.

Table 1. <sup>1</sup>H NMR chemical shifts of **1** and **2** in CDCl<sub>3</sub>, **3** and **3a** in C<sub>5</sub>D<sub>5</sub>N

| Proton     | 1                  | 2                  | 3                  | 3a                 |
|------------|--------------------|--------------------|--------------------|--------------------|
| 2 $\alpha$ | 3.51 tt(11.2, 3.6) | 3.80 tt(11.5, 3.8) | 4.25 tt(11.3, 3.9) | 5.14 tt(9.6, 3.8)  |
| 14         | 5.05 br s          | 5.13 br s          | 5.43 br s          | 5.30 br s          |
| 15         | 4.87 dd(9.9, 2.2)  | 3.68 dd(9.2, 2.2)  |                    | 5.42 dd(8.9, 2.5)  |
| 16         | 3.75 dd(10.6, 2.2) | 4.21 dd(11.3, 2.2) | 4.07-3.98(3H, m)   | 4.61 dd(11.7, 2.5) |
| 16'        | 3.65 dd(10.6, 9.9) | 4.02 dd(11.3, 9.2) |                    | 4.29 dd(11.7, 8.9) |
| 19         | 3.68 d(11.2)       | 3.69 d(10.8)       | 4.17 d(10.3)       | 4.34 d(11.5)       |
| 19'        | 3.08 d(11.2)       | 3.35 d(10.8)       | 3.65 d(10.3)       | 4.02 d(11.5)       |
| 17-Me      | 0.90 s             | 0.89 s             | 1.17 s             | 1.02 s             |
| 18-Me      | 0.98 s             | 1.00 s             | 1.28 s             | 1.02 s             |
| 20-Me      | 0.85 s             | 0.74 s             | 0.82 s             | 0.94 s             |
| OAc        | 2.11 s             | 2.06 s             |                    | 2.11 s             |
|            |                    |                    |                    | 2.06 s             |
|            |                    |                    |                    | 2.03 s             |
|            |                    |                    |                    | 2.00 s             |

**Table 2.**  $^{13}\text{C}$  NMR chemical shifts of **1** and **2** in  $\text{CDCl}_3$ , **3** and **3a** in  $\text{C}_5\text{D}_5\text{N}$ 

| Carbon | 1                    | 2                    | 3                    | 3a                   |
|--------|----------------------|----------------------|----------------------|----------------------|
| 1      | 47.85 t              | 47.92 t              | 49.50 t              | 44.06 t              |
| 2      | 65.33 d              | 64.62 d              | 64.05 d              | 66.91 d              |
| 3      | 44.26 t              | 44.27 t              | 45.82 t              | 41.49 t              |
| 4      | 40.65 s <sup>a</sup> | 40.44 s <sup>a</sup> | 41.08 s <sup>a</sup> | 39.78 s <sup>a</sup> |
| 5      | 54.67 d              | 55.09 d              | 55.71 d              | 55.03 d              |
| 6      | 22.05 t              | 22.06 t              | 22.78 t              | 22.37 t              |
| 7      | 36.14 t              | 36.15 t              | 36.93 t              | 36.21 t              |
| 8      | 139.18 s             | 139.21 s             | 138.18 s             | 139.60 s             |
| 9      | 50.52 d              | 50.80 d              | 51.48 d              | 50.67 d              |
| 10     | 39.77 s <sup>a</sup> | 39.52 s <sup>a</sup> | 39.90 s <sup>a</sup> | 38.93 s <sup>a</sup> |
| 11     | 18.63 t              | 18.58 t              | 19.20 t              | 18.92 t              |
| 12     | 32.52 t              | 31.47 t              | 32.94 t              | 32.30 t              |
| 13     | 36.92 s              | 37.45 s              | 38.10 s              | 37.25 s              |
| 14     | 127.25 d             | 127.97 d             | 129.94 d             | 127.60 d             |
| 15     | 78.78 d              | 73.73 d              | 76.77 d              | 74.82 d              |
| 16     | 61.81 t              | 66.60 t              | 64.08 t              | 63.93 t              |
| 17     | 23.38 q              | 22.70 q              | 23.40 q              | 23.38 q              |
| 18     | 27.11 q              | 27.26 q              | 28.40 q              | 27.80 q              |
| 19     | 64.52 t              | 65.52 t              | 64.99 t              | 68.30 t              |
| 20     | 16.67 q              | 16.88 q              | 17.10 q              | 16.20 q              |
| OAc    | 172.27 s             | 171.52 s             |                      | 170.72 s             |
|        | 21.40 q              | 20.99 q              |                      | 170.58 s             |
|        |                      |                      |                      | 170.58 s             |
|        |                      |                      |                      | 170.12 s             |
|        |                      |                      |                      | 21.28 q              |
|        |                      |                      |                      | 20.79 q              |
|        |                      |                      |                      | 20.67 q              |
|        |                      |                      |                      | 20.67 q              |

a: Assignments may be interchangeable.

$\text{CH}(\text{OH})\text{CH}_2\text{OAc}]^+(100)$ ,  $259[277\text{-H}_2\text{O}]^+(98)$ ,  $241[277\text{-}2\text{H}_2\text{O}]^+(26)$ ,  $151(32)$ ,  $133(15)$ ,  $121(48)$ ,  $109(40)$ ,  $95(20)$ ,  $81(12)$ ,  $55(3)$ ,  $43(18)$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1 and 2.

Kirenol (**3**),  $\text{C}_{20}\text{H}_{34}\text{O}_4$ , colourless cubics (MeOH), mp.  $201\text{-}203^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{26}\text{-}36^\circ(\text{C}_5\text{H}_5\text{N}, c\ 0.606)$ ; no UV absorption; IR  $\nu_{\text{max}}^{\text{KBr}}\text{cm}^{-1}$ :  $3400$ ,  $1655$ ,  $1463$ ,  $1380$ ,  $1361$ ,  $1060$ ,  $1035$ ; EIMS ( $70\ \text{eV}$ )  $m/z$  (%):  $289[\text{M-CH}_2\text{OH-H}_2\text{O}]^+(1)$ ,  $277[\text{M-CH}(\text{OH})\text{CH}_2\text{OH}]^+(53)$ ,  $259[277\text{-H}_2\text{O}]^+(41)$ ,  $241[277\text{-}2\text{H}_2\text{O}]^+(10)$ ,  $151(17)$ ,  $133(10)$ ,  $121(68)$ ,  $109(79)$ ,  $95(39)$ ,  $81(31)$ ,  $61(18)$ ,  $55(53)$ ,  $43(100)$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1 and 2.

tetraacetylkirenol (**3a**),  $\text{C}_{22}\text{H}_{42}\text{O}_8$ , white powder (MeOH), mp.  $92\text{-}93^\circ\text{C}$ ; no UV absorption; IR  $\nu_{\text{max}}^{\text{KBr}}\text{cm}^{-1}$ :  $1740$ ,  $1655$ ,  $1452$ ,  $1370$ ,  $1250$ ,  $1040$ ,  $1030$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1 and 2.

ent- $16\beta,17$ -Dihydroxykauran- $19$ -oic acid (**4**),  $\text{C}_{20}\text{H}_{32}\text{O}_4$ , colourless plates (MeOH- $\text{CHCl}_3$ ), mp.  $266\text{-}268^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{25}\text{-}89.1^\circ(\text{C}_5\text{H}_5\text{N}, c\ 0.651)$ ; no UV absorption; IR  $\nu_{\text{max}}^{\text{KBr}}\text{cm}^{-1}$ :  $3430$ ,  $3250$ ,  $2940$ ,  $2920$ ,  $1690$ ,  $1462$ ,  $1438$ ,  $1225$ ,  $1171$ ,  $1160$ ,  $1060$ ,  $1026$ ,  $867$ ,  $787$ ; The mp, mmp,  $[\alpha]_{\text{D}}$ , IR, and  $R_f$  value (TLC) of **4** are in agreement with those of authentic sample (Xiong *et al.*, 1992).  $^1\text{H}$  and  $^{13}\text{C}$  NMR data see Table 1 and 2.

ent- $16\beta,17$ -Dihydroxykauran- $19$ -oic acid- $16\beta,17$ -acetone (**5**),  $\text{C}_{22}\text{H}_{36}\text{O}_4$ , colourless needles (acetone-cyclohexane), mp.  $225\text{-}225.5^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{25}\text{-}58.2^\circ(\text{CHCl}_3, c\ 0.208)$ ; no UV absorption; IR  $\nu_{\text{max}}^{\text{KBr}}\text{cm}^{-1}$ :  $3400$ ,  $2925$ ,  $1685$ ,  $1455$ ,  $1445$ ,  $1361$ ,  $1245$ ,  $1210$ ,  $1055$ ,  $890$ ; It was possible that **5** is an artifact produced in the process of isolation. The mp, mmp,  $[\alpha]_{\text{D}}$ , IR, and  $R_f$  value

(TLC) of **5** are in agreement with those of authentic sample (Xiong *et al.*, 1992).

3,7-Dimethylquercetin (**6**),  $C_{17}H_{14}O_7$ , yellow needles (acetone), mp. 232-234°C;  $[\alpha]_D^{25}$  -41.1° (MeOH, c 0.503); UV  $\lambda_{max}$ nm(log $\epsilon$ ): (1) EtOH: 257, 301sh, 360; (2) MeOH: 256.5(4.335), 296sh(3.925), 356.5(4.299); (3) MeOH+NaOAc: 260, 290sh, 384sh, 406; (4) MeOH+NaOAc+H<sub>3</sub>BO<sub>3</sub>: 260.5, 289sh, 378; (5) MeOH+AlCl<sub>3</sub>: 276, 300sh, 334.5sh, 442; (6) MeOH+AlCl<sub>3</sub>+HCl: 268.5, 298sh, 363sh, 401; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3170, 3080, 3000, 2935, 1656, 1588, 1492, 1429, 1342, 1309, 1237, 1208, 1160, 1110, 1004, 915, 805; EIMS (70 eV) m/z (%): 331[M+1]<sup>+</sup>(16), 330[M]<sup>+</sup>(100), 329[M-1]<sup>+</sup>(92), 313[M-OH]<sup>+</sup>(10), 312[M-H<sub>2</sub>O]<sup>+</sup>(16), 301[M-HCO]<sup>+</sup>(13), 287[M-MeCO]<sup>+</sup>(33), 167[A<sub>1</sub>+H]<sup>+</sup>(17), 151[A<sub>1</sub>-Me]<sup>+</sup>(21), 137[B<sub>2</sub>]<sup>+</sup>(19); <sup>1</sup>H NMR (C<sub>5</sub>D<sub>5</sub>N) $\delta$ : 8.17 (1H, br s, 2'-H), 7.81 (1H, d, J=8.62 Hz, 6'-H), 7.36 (1H, d, J=8.62 Hz, 5'-H), 6.58, 6.57 (each 1H, d, J=2.6 Hz, 6, 8-H), 3.89 (3H, s, 3-OMe), 3.77 (3H, s, 7-OMe); <sup>13</sup>C NMR (C<sub>5</sub>D<sub>5</sub>N) $\delta$ : 156.93 (s, c-2), 138.98(s, C-3), 179.12(s, C-4), 162.54(s, C-5), 98.33(d, C-6), 165.86(s, C-7), 92.44 (d, C-8), 157.19 (s, C-9), 106.47 (s, C-10), 122.10 (s, C-1'), 116.71 (d, C-2'), 147.31 (s, C-3'), 150.98 (s, C-4'), 116.81 (d, C-5'), 121.69 (d, C-6'), 59.86 (q, 3-OMe), 55.97 (q, 7-OMe) (Dong *et al.*, 1989).

$\beta$ -Sitosterol (**7**),  $C_{29}H_{50}O$ , colourless needles (acetone-petrol); mp. 136-138°C; no UV absorption; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3420, 3300, 2950, 2930, 2860, 1650, 1445, 1435, 1375, 1365, 1060, 1050, 967, 955; EIMS (70 eV) m/z (%): 414[M]<sup>+</sup>(8), 396[M-H<sub>2</sub>O]<sup>+</sup>(1), 351(2), 329(3), 271(15), 213(17), 159(22), 145(28), 133(23), 119(18), 107(29), 95(30), 83(40), 69(47), 55(84), 43(100); The mp, mmp, IR, MS, and R<sub>f</sub> value (TLC) of **7** are in agreement with those of authentic sample (Xiong *et al.*, 1992).

Dacosterol (**8**),  $C_{35}H_{60}O_6$ , white powder, no UV absorption; IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3400, 2950, 2930, 2860, 1650, 1460, 1375, 1360, 1165, 1105, 1070, 1050, 1020; The IR, and R<sub>f</sub> value (TLC) of **8** are in agreement with those of authentic sample (Xiong *et al.*, 1992).

## Results and Discussion

Orientalin A (**1**),  $C_{22}H_{36}O_5$ , M 380, was obtained as colorless needles. Its IR spectrum revealed that an OH (3340 cm<sup>-1</sup>), an ester (1725 and 1240 cm<sup>-1</sup>), and double bond (1650 cm<sup>-1</sup>) were present as functional groups. **1** was hydrolyzed with 5%KOH-MeOH under reflux for 15 minutes to afford kirenol (**3**). **1** was acetylated with the usual manner to give tetraacetate of kirenol (**3a**). A comparison of <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** with those of **3** showed the presence of another acetoxy [ $\delta$ 2.11 (3H, s), 21.40 (q) and 172.27 (s)] in **1** in place of a hydroxy in **3** and downfield shift of 15-H and 15-C signals from about  $\delta$ 4.03 and 76.77 in **3** to  $\delta$ 4.87 and 78.78 in **1** and upfield shift of 16-H<sub>2</sub>, 16-C and 13-C signals from about  $\delta$ 4.03, 64.08 and 38.10 in **3** to about  $\delta$ 3.70, 61.81 and 36.92 in **1**. On the basis of the above evidence, we assigned **1** as 15-acetoxykirenol, namely ent-15-acetoxy-2 $\alpha$ ,16,19-trihydroxypimar-8(14)-ene (**1**).

Orientalin B (**2**),  $C_{22}H_{36}O_5$ , M 380, was obtained as colourless needles. Its IR spectrum revealed that an OH (3500,3330 cm<sup>-1</sup>), an ester (1705 and 1260 cm<sup>-1</sup>), and double bond (1650 cm<sup>-1</sup>) were present as functional groups. **2** was hydrolysed with 5% KOH-MeOH under reflux for 15 minutes to afford Kirenol (**3**). **2** was acetylated with the usual manner to give tetraacetate of kirenol **3a**. A comparison of <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** with those of **2** showed the downfield shift of 16-H<sub>2</sub> and 16-C signals from about  $\delta$ 3.70 and 61.81 in **1** to about  $\delta$ 4.12 and 66.60 in **2** and upfield shift of 15-H and 15-C signals from  $\delta$ 4.87 and 78.78 in **1** to  $\delta$ 3.68 and 73.73 in **2**. Therefore, **2** was determined as 16-acetoxykirenol, namely ent-16-acetoxy-2 $\alpha$ ,15,19-trihydroxy-pimar-8(14)-ene (**2**).

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