# Triterpenoid Ester Saponins from *Dipteronia dyeriana*

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The phytochemical investigation of *Dipteronia dyeriana* (Aceraceae) resulted in the isolation and identification of five new triterpenoid ester saponins, dipterosides A–E (**1**–**5**). Their structures were elucidated by spectroscopic methods and chemical evidence. The inhibitory activities of these compounds against human leukemia K562 and human hepatoma HepG2 cells were also evaluated.

**Introduction.** – Aceraceae comprises two genera, *Acer* and *Dipteronia* [1]. The plants of *Acer* contain various bioactive substances, such as triterpenoid saponins with antitumor activity [2], stilbene glycosides with hepatoprotective and antioxidative activity [3][4], diarylheptanoids as inhibitors of nitric oxide production [5], and flavonoids with the inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase [6]. *Dipteronia* is a genus endemic to China and includes only two species, *Dipteronia sinensis* OLIV. and *Dipteronia dyeriana* HENRY. To the best of our knowledge, the chemical constituents and bioactivity of the genus have not been reported yet. In our program to search antitumor agents from natural products, we investigated the branches of *D. dyeriana* phytochemically, which led to the isolation of five new triterpenoid ester saponins, dipterosides A–E (**1**–**5**) (*Fig.*). In this article, the structure elucidation of these new compounds along with their inhibitory effects on human leukemia K562 and human hepatoma HepG2 cells are reported.

**Results and Discussion.** – Dipteroside A (**1**), a white amorphous powder, was assigned the molecular formula $C_{54}H_{84}O_{22}$, as deduced from its HR-ESI-MS (*m*/*z* 1083.5370 ($[M-H]^-$)). The IR spectrum of **1** exhibited absorption bands at 1725 and 1641 $cm^{-1}$ assignable to an $\alpha,\beta$-unsaturated C=O group, along with bands at 3426 and 1073 $cm^{-1}$ that are characteristic of an oligoglycosidic structure. After acid hydrolysis of **1**, D-glucopyranose and L-arabinopyranose were obtained and identified by TLC and by their optical rotation. The $^1$H- and $^{13}$C-NMR (*Tables 1* and *2*), HMBC, $^1$H,$^1$H-COSY, HSQC, and ROESY data established the structure of **1** as 22-*O*-acetyl-21-*O*-angeloyl-3-*O*-{*O*-$\alpha$-L-arabinopyranosyl-(1 → 4)-*O*-[$\beta$-D-glucopyranosyl-(1 → 2)]-$\beta$-D-

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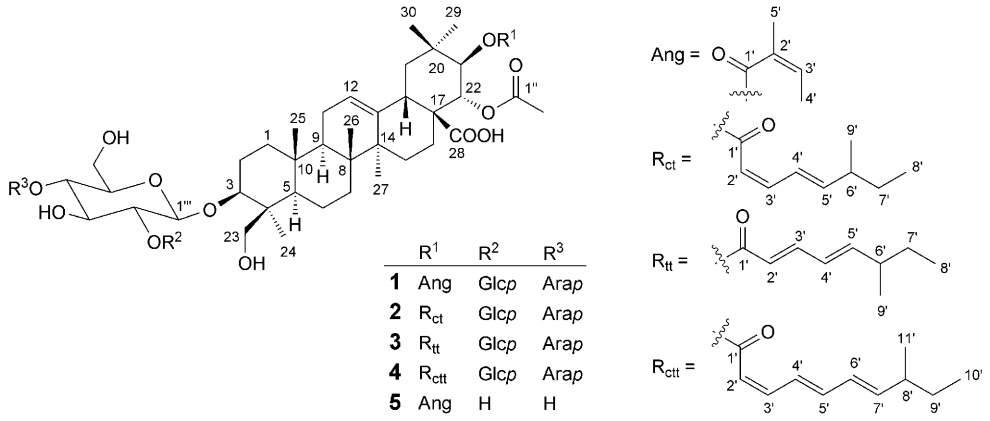


Figure. *Structures of dipterosides A – E* (**1**–**5**) *isolated from* Dipteronia dyeriana

glucopyranosyl}-23-hydroxyacerogenic acid. (acerogenic acid = (3$\beta$,21$\beta$,22$\alpha$)-3,21,22-trihydroxyolean-12-en-28-oic acid; angelic acid = (2*Z*)-2-methylbut-2-enoic acid).

The $^1$H- and $^{13}$C-NMR spectra of **1** displayed the signals for six Me groups connected to tertiary C-atoms ($\delta$(H) 0.76 (*s*), 0.85 (*s*), 0.90 (*s*), 1.11 (*s*), and 1.20 (*s*, 2 ×), an olefinic H-atom ($\delta$(H) 5.33 (br. *s*)), a $CH_2OH$ group ($\delta$(H) 4.09 (*d*, *J* = 11.2) and 3.20 (*d*, *J* = 11.2); $\delta$(C) 64.2) and a COOH group ($\delta$(C) 177.1), which are the features of the oleanolic acid skeleton [7]. Extensive analysis and comparison of the NMR spectra of **1** with those of (4$\beta$)-23-hydroxyacerogenic acid [2] suggested that compound **1** was an ester saponin of the latter. The anomeric H-atoms at $\delta$(H) 4.47 (*d*, *J* = 8.0, H–C(1‴)), 4.80 (*d*, *J* = 8.0, H–C(1⁗)), and 4.29 (*d*, *J* = 7.2, H–C(1⁗′)), and the anomeric C-atoms at $\delta$(C) 104.8 (C(1‴)), 104.0 (C(1⁗)), and 105.3 (C(1⁗′)) suggested the presence of three sugar units in the molecule. The key HMBC cross-peaks of H–C(1‴)/C(3), H–C(1⁗)/C(2‴), and H–C(1⁗′)/C(4‴), and other 2D-NMR data ($^1$H,$^1$H-COSY, HSQC, and ROESY) established the sequence of the sugar chain as 3-*O*-{*O*-$\alpha$-L-arabinopyranosyl-(1 → 4)-*O*-[$\beta$-D-glucopyranosyl-(1 → 2)]-$\beta$-D-glucopyranosyl}.

The remaining signals showed the presence of an angeloyl group ($\delta$(H) 6.14 (*dq*, *J* = 1.2, 7.2, H–C(3′)), 1.94 (*dd*, *J* = 7.2, 1.2, Me(4′)), and 1.82 (*t*, *J* = 1.2, Me(5′)); $\delta$(C) 168.6 (*s*, C(1′)), 128.8 (*s*, C(2′)), 139.9 (*d*, C(3′)), 16.0 (*q*, C(4′)), and 20.7 (*q*, C(5′))) and an Ac group ($\delta$(H) 1.90 (*s*, Me); $\delta$(C) 171.6 (*s*) and 20.7 (*q*)) in **1**. The angeloyloxy group at C(21) and the AcO group at C(22) were established by the HMBC cross-peaks correlations of H–C(21)/C(1′) and H–C(22)/C(1″), resp.

Dipteroside B (**2**) was obtained as a white amorphous powder. The HR-ESI-MS of **2** exhibited the $[M-H]^-$ signal at *m*/*z* 1137.5867, in accordance with the molecular formula $C_{58}H_{90}O_{22}$. The IR spectrum of **2** also showed absorption bands for the $\alpha$,$\beta$-unsaturated C=O group and the oligoglycosidic structure. An extensive 1D- and 2D-NMR spectroscopic study suggested that **2** and **1** differ structurally only in the acyloxy substituent at C(21). In **2**, the angeloyloxy group of **1** was replaced by a (2*Z*,4*E*)-6-methylocta-2,4-dienoyl moiety, which was identified by comparison of the $^1$H-NMR data of **2** with those reported in [2] and by the 2D-NMR data of **2**. Therefore, the structure of **2** was elucidated as 22-*O*-acetyl-3-*O*-{*O*-$\alpha$-L-arabinopyranosyl-(1 → 4)-*O*-[$\beta$-D-glucopyranosyl-(1 → 2)]-$\beta$-D-glucopyranosyl}-23-hydroxy-21-*O*-[(2*Z*,4*E*)-6-methylocta-2,4-dienoyl]acerogenic acid.

Table 1. *$^1$H-NMR Data of Compounds* **1**–**5**. $\delta$ in ppm, $J$ in Hz.

| | **1**[a]) | **2**[b]) | **3**[c]) | **4**[b]) | **5**[a]) |
|---|---|---|---|---|---|
| $CH_2$(1) | 1.62, 0.99 (2*m*) | 1.62, 0.99 (2*m*) | 1.33, 0.79 (2*m*) | 1.60, 0.97 (2*m*) | 1.62, 1.00 (2*m*) |
| $CH_2$(2) | 2.07, 1.81 (2*m*) | 2.07, 1.81 (2*m*) | 2.20, 1.81 (2*m*) | 2.06, 1.80 (2*m*) | 1.94, 1.78 (2*m*) |
| H–C(3) | 3.37 (*dd*, *J* = 12.0, 4.5) | 3.36 (*dd*, *J* = 12.0, 4.0) | 3.33 (*dd*, *J* = 11.5, 4.5) | 3.33 (*m*) | 3.40 (*dd*, *J* = 11.5, 4.5) |
| H–C(5) | 0.94 (*d*, *J* = 12.0) | 0.94 (*d*, *J* = 12.0) | 0.83 (*d*, *J* = 12.0) | 0.92 (*d*, *J* = 11.6) | 0.93 (*d*, *J* = 12.0) |
| $CH_2$(6) | 1.62, 1.35 (2*m*) | 1.62, 1.35 (2*m*) | 1.52, 1.18 (2*m*) | 1.59, 1.32 (2*m*) | 1.64, 1.38 (2*m*) |
| $CH_2$(7) | 1.52 (br. *d*, *J* = 12.0), 1.32 (*m*) | 1.52 (*d*, *J* = 12.0), 1.34 (*m*) | 1.36, 1.22 (2*m*) | 1.48 (*d*, *J* = 11.6), 1.31 (*m*) | 1.50 (br. *d*, *J* = 12.0), 1.34 (*m*) |
| H–C(9) | 1.58 (*m*) | 1.58 (*m*) | 1.54 (*m*) | 1.56 (*m*) | 1.57 (*m*) |
| $CH_2$(11) | 1.89 (*m*) | 1.90 (*m*) | 1.84, 1.79 (2*m*) | 1.90, 1.87 (2*m*) | 1.88 (*m*) |
| H–C(12) | 5.33 (br. *s*) | 5.33 (br. *s*) | 5.45 (br. *t*, *J* = 3.5) | 5.29 (br. *s*) | 5.31 (br. *t*, *J* = 3.5) |
| $CH_2$(15) | 1.83, 1.15 (2*m*) | 1.85, 1.14 (2*m*) | 2.24, 1.20 (2*m*) | 1.82, 1.11 (2*m*) | 1.91, 1.10 (2*m*) |
| $CH_2$(16) | 2.15, 1.88 (2*m*) | 2.13, 1.85 (2*m*) | 2.60 (br. *d*, *J* = 12.0), 2.06 (*m*) | 2.11, 1.85 (2*m*) | 2.14, 1.81 (2*m*) |
| H–C(18) | 3.07 (*dd*, *J* = 14.0, 4.0) | 3.07 (*dd*, *J* = 14.0, 3.5) | 3.52 (*dd*, *J* = 14.0, 3.5) | 3.03 (*dd*, *J* = 14.0, 3.6) | 3.10 (br. *d*, *J* = 13.0) |
| $CH_2$(19) | 2.07, 1.32 (2*m*) | 2.06, 1.32 (2*m*) | 2.22, 1.45 (2*m*) | 2.04, 1.30 (2*m*) | 2.04, 1.30 (2*m*) |
| H–C(21) | 5.08 (*d*, *J* = 10.4) | 5.04 (*d*, *J* = 10.5) | 5.56 (*d*, *J* = 11.0) | 5.02 (*d*, *J* = 10.4) | 5.05 (*d*, *J* = 10.5) |
| H–C(22) | 5.33 (*d*, *J* = 10.4) | 5.31 (*d*, *J* = 10.5) | 6.02 (*d*, *J* = 11.0) | 5.28 (*d*, *J* = 10.4) | 5.36 (*d*, *J* = 10.5) |
| $CH_2$(23) | 4.09 (*d*, *J* = 11.2), 3.20 (*d*, *J* = 11.2) | 4.08 (*d*, *J* = 11.0), 3.20 (*d*, *J* = 11.0) | 4.26 (*m*), 3.30 (*d*, *J* = 11.5) | 4.05 (*d*, *J* = 11.2), 3.18 (*d*, *J* = 11.2) | 4.01 (*d*, *J* = 11.0), 3.20 (*m*) |
| Me(24) | 1.20 (*s*) | 1.20 (*s*) | 1.33 (*s*) | 1.16 (*s*) | 1.20 (*s*) |
| Me(25) | 0.85 (*s*) | 0.85 (*s*) | 0.59 (*s*) | 0.81 (*s*) | 0.86 (*s*) |
| Me(26) | 0.76 (*s*) | 0.76 (*s*) | 0.85 (*s*) | 0.72 (*s*) | 0.80 (*s*) |
| Me(27) | 1.20 (*s*) | 1.20 (*s*) | 1.25 (*s*) | 1.16 (*s*) | 1.20 (*s*) |
| Me(29) | 0.90 (*s*) | 0.90 (*s*) | 1.08 (*s*) | 0.89 (*s*) | 0.89 (*s*) |
| Me(30) | 1.11 (*s*) | 1.10 (*s*) | 1.27 (*s*) | 1.06 (*s*) | 1.11 (*s*) |
| | Angeloyl: | $R_{ct}$: | $R_{tt}$: | $R_{ctt}$: | Angeloyl: |
| H–C(2′) | | 5.51 (*d*, *J* = 12.0) | 6.08 (*d*, *J* = 15.0) | 5.50 (*d*, *J* = 11.2) | |
| H–C(3′) | 6.14 (*dq*, *J* = 1.2, 7.2) | 6.66 (*t*, *J* = 12.0) | 7.60 (*dd*, *J* = 15.0, 11.0) | 6.66 (*t*, *J* = 11.2) | 6.12 (*dq*, *J* = 1.5, 7.5) |
| Me(4′) or H–C(4′) | 1.94 (*dd*, *J* = 7.2, 1.2) | 7.25 (*dd*, *J* = 15.0, 12.0) | 6.18 (*dd*, *J* = 15.0, 11.0) | 7.29 (*dd*, *J* = 15.2, 11.2) | 1.94 (*dd*, *J* = 7.5, 1.5) |
| Me(5′) or H–C(5′) | 1.82 (*t*, *J* = 1.2) | 6.02 (*dd*, *J* = 15.0, 7.5) | 5.98 (*dd*, *J* = 15.0, 8.0) | 6.52 (*dd*, *J* = 15.2, 10.8) | 1.81 (*t*, *J* = 1.5) |
| H–C(6′) | | 2.20 (*m*) | 2.04 (*m*) | 6.16 (*dd*, *J* = 15.2, 10.8) | |
| $CH_2$(7′) or H–C(7′) | | 1.40 (*m*) | 1.24 (*m*) | 5.83 (*dd*, *J* = 15.2, 8.0) | |
| Me(8′) or H–C(8′) | | 0.88 (*t*, *J* = 7.5) | 0.74 (*t*, *J* = 7.5) | 2.12 (*m*) | |
| Me(9′) or $CH_2$(9′) | | 1.05 (*d*, *J* = 6.5) | 0.89 (*d*, *J* = 6.5) | 1.34 (*m*) | |
| Me(10′) | | | | 0.84 (*t*, *J* = 7.6) | |
| Me(11′) | | | | 0.99 (*d*, *J* = 6.8) | |
| Ac: | | | | | |
| Me(2″) | 1.90 (*s*) | 1.87 (*s*) | 2.05 (*s*) | 1.84 (*s*) | 1.90 (*s*) |

*Table 1* (cont.)

| | **1**[a]) | **2**[b]) | **3**[c]) | **4**[b]) | **5**[a]) |
|---|---|---|---|---|---|
| Glc*p*: | | | | | |
| H–C(1‴) | 4.47 (*d*, *J* = 8.0) | 4.47 (*d*, *J* = 7.5) | 4.74 (*d*, *J* = 8.0) | 4.43 (*d*, *J* = 8.0) | 4.40 (*d*, *J* = 8.0) |
| H–C(2‴) | 3.54 (*m*) | 3.54 (*m*) | 4.17 (*t*, *J* = 8.0) | 3.52 (*m*) | 3.14 (*t*, *J* = 8.0) |
| H–C(3‴) | 3.76 (*m*) | 3.75 (*m*) | 4.27 (*m*) | 3.72 (*m*) | 3.34 (*t*, *J* = 8.0) |
| H–C(4‴) | 3.54 (*m*) | 3.54 (*m*) | 4.27 (*m*) | 3.52 (*m*) | 3.26 (*m*) |
| H–C(5‴) | 3.40 (*m*) | 3.41 (*m*) | 3.78 (*m*) | 3.39 (*m*) | 3.26 (*m*) |
| $CH_2$(6‴) | 3.84, 3.76 (2*m*) | 3.85, 3.76 (2*m*) | 4.54 (*dd*, *J* = 11.0, 4.0), 4.47 (*m*) | 3.82, 3.72 (2*m*) | 3.84 (*dd*, *J* = 12.0, 2.0), 3.66 (*dd*, *J* = 12.0, 5.0) |
| Glc*p*: | | | | | |
| H–C(1⁗) | 4.80 (*d*, *J* = 8.0) | 4.80 (*d*, *J* = 8.0) | 5.64 (*d*, *J* = 8.0) | 4.77 (*d*, *J* = 8.0) | |
| H–C(2⁗) | 3.17 (*m*) | 3.17 (*m*) | 4.11 (*m*) | 3.14 (*m*) | |
| H–C(3⁗) | 3.34 (*t*, *J* = 9.5) | 3.34 (*t*, *J* = 9.0) | 4.27 (*m*) | 3.30 (*m*) | |
| H–C(4⁗) | 3.43 (*t*, *J* = 9.5) | 3.43 (*t*, *J* = 9.0) | 4.52 (*m*) | 3.41 (*t*, *J* = 9.2) | |
| H–C(5⁗) | 3.18 (*m*) | 3.18 (*m*) | 3.78 (*m*) | 3.15 (*m*) | |
| $CH_2$(6⁗) | 3.84 (*m*), 3.71 (*dd*, *J* = 12.4, 3.6) | 3.85 (*m*), 3.71 (*dd*, *J* = 12.5, 3.5) | 4.47 (*m*), 4.34 (br. *d*, *J* = 12.0) | 3.82 (*m*), 3.67 (*dd*, *J* = 12.4, 3.2) | |
| Ara*p*: | | | | | |
| H–C(1⁗) | 4.29 (*d*, *J* = 7.2) | 4.29 (*d*, *J* = 7.5) | 5.00 (*d*, *J* = 8.0) | 4.25 (*d*, *J* = 7.2) | |
| H–C(2⁗) | 3.54 (*m*) | 3.54 (*m*) | 4.49 (*m*) | 3.52 (*m*) | |
| H–C(3⁗) | 3.50 (*dd*, *J* = 9.5, 3.5) | 3.51 (*dd*, *J* = 9.0, 3.0) | 4.13 (*m*) | 3.48 (*dd*, *J* = 9.6, 3.2) | |
| H–C(4⁗) | 3.80 (*m*) | 3.80 (*m*) | 4.27 (*m*) | 3.77 (*m*) | |
| $CH_2$(5⁗) | 3.91 (*dd*, *J* = 12.5, 2.0), 3.60 (br. *d*, *J* = 12.5) | 3.91 (*dd*, *J* = 12.5, 2.0), 3.60 (*d*, *J* = 12.5) | 4.30, 3.78 (2*m*) | 3.87 (*dd*, *J* = 12.4, 1.6), 3.56 (*d*, *J* = 12.4) | |

[a]) At 400 MHz in $CD_3OD$. [b]) At 500 MHz in $CD_3OD$. [c]) At 500 MHz in $C_5D_5N$.

Dipteroside C (**3**), a white amorphous powder, had the same molecular formula $C_{58}H_{90}O_{22}$ as **2**, as deduced from the HR-ESI-MS (*m*/*z* 1137.5833 ($[M-H]^-$)). The $^1$H- and $^{13}$C-NMR spectra of **3** (*Tables 1* and *2*) showed high analogy with those of **2**. In **3**, a (2*E*,4*E*)-6-methylocta-2,4-dienoyl moiety was present [8] instead of the (2*Z*,4*E*)-6-methylocta-2,4-dienoyl group of **2**. In addition, an HMBC experiment demonstrated the identical acylation and sugar linkage patterns. Thus, the structure of **3** was elucidated as 22-*O*-acetyl-3-*O*{*O*-*α*-L-arabinopyranosyl-(1 → 4)-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*β*-D-glucopyranosyl]-23-hydroxy-21-*O*-[(2*E*,4*E*)-6-methylocta-2,4-dienoyl]-acerogenic acid.

Dipteroside D (**4**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{60}H_{92}O_{22}$ by HR-ESI-MS showing a pseudomolecular-ion peak at *m*/*z* 1163.6009 ($[M-H]^-$). The $^1$H- and $^{13}$C-NMR spectra of **4** (*Tables 1* and *2*) were also very similar to those of **2**. However, The C(21) ester group was deduced as (2*Z*,4*E*,6*E*)-8-methyldeca-2,4,6-trienoyl from the 1D- and 2D-NMR data of **4**. Therefore, **4** was determined as 22-*O*-acetyl-3-*O*-{*O*-*α*-L-arabinopyranosyl-(1 → 4)-*O*-

Table 2. *$^{13}$C-NMR Data of Compounds* **1**–**5**. $\delta$ in ppm.

| | **1**[a]) | **2**[a]) | **3**[b]) | **4**[a]) | **5**[a]) |
|---|---|---|---|---|---|
| C(1) | 39.3 (*t*) | 39.5 (*t*) | 38.4 (*t*) | 39.4 (*t*) | 39.5 (*t*) |
| C(2) | 27.2 (*t*) | 27.0 (*t*) | 26.5 (*t*) | 27.1 (*t*) | 27.2 (*t*) |
| C(3) | 92.3 (*d*) | 92.3 (*d*) | 91.1 (*d*) | 92.3 (*d*) | 90.4 (*d*) |
| C(4) | 44.4 (*s*) | 44.4 (*s*) | 43.7 (*s*) | 44.4 (*s*) | 44.8 (*s*) |
| C(5) | 57.4 (*d*) | 57.3 (*d*) | 56.2 (*d*) | 57.3 (*d*) | 57.3 (*d*) |
| C(6) | 19.4 (*t*) | 19.5 (*t*) | 18.6 (*t*) | 19.3 (*t*) | 19.5 (*t*) |
| C(7) | 34.0 (*t*) | 33.9 (*t*) | 33.2 (*t*) | 34.0 (*t*) | 34.2 (*t*) |
| C(8) | 43.0 (*s*) | 42.9 (*s*) | 42.2 (*s*) | 43.0 (*s*) | 43.0 (*s*) |
| C(9) | 48.8 (*d*) | 48.6 (*d*) | 47.7 (*d*) | 48.4 (*d*) | 48.4 (*d*) |
| C(10) | 37.5 (*s*) | 37.5 (*s*) | 36.5 (*s*) | 37.5 (*s*) | 37.6 (*s*) |
| C(11) | 24.9 (*t*) | 24.8 (*t*) | 24.0 (*t*) | 24.8 (*t*) | 24.8 (*t*) |
| C(12) | 125.2 (*d*) | 125.1 (*d*) | 123.1 (*d*) | 125.2 (*d*) | 124.6 (*d*) |
| C(13) | 142.7 (*s*) | 142.7 (*s*) | 142.4 (*s*) | 142.7 (*s*) | 143.4 (*s*) |
| C(14) | 40.6 (*s*) | 40.6 (*s*) | 39.7 (*s*) | 40.6 (*s*) | 40.6 (*s*) |
| C(15) | 28.2 (*t*) | 27.9 (*t*) | 27.7 (*t*) | 28.1 (*t*) | 28.4 (*t*) |
| C(16) | 19.2 (*t*) | 19.2 (*t*) | 19.0 (*t*) | 19.2 (*t*) | 19.5 (*t*) |
| C(17) | 53.2 (*s*) | 53.1 (*s*) | 52.4 (*s*) | 53.1 (*s*) | 53.7 (*s*) |
| C(18) | 43.0 (*d*) | 43.0 (*d*) | 42.3 (*d*) | 43.0 (*d*) | 43.4 (*d*) |
| C(19) | 46.5 (*t*) | 46.7 (*t*) | 45.9 (*t*) | 46.6 (*t*) | 47.2 (*t*) |
| C(20) | 37.0 (*s*) | 36.9 (*s*) | 36.5 (*s*) | 37.0 (*s*) | 37.0 (*s*) |
| C(21) | 76.7 (*d*) | 76.4 (*d*) | 76.2 (*d*) | 76.4 (*d*) | 77.1 (*d*) |
| C(22) | 74.8 (*d*) | 74.8 (*d*) | 74.3 (*d*) | 74.8 (*d*) | 75.6 (*d*) |
| C(23) | 64.2 (*t*) | 64.2 (*t*) | 63.4 (*t*) | 64.2 (*t*) | 64.1 (*t*) |
| C(24) | 22.9 (*q*) | 22.9 (*q*) | 22.6 (*q*) | 22.9 (*q*) | 23.2 (*q*) |
| C(25) | 15.9 (*q*) | 15.9 (*q*) | 15.3 (*q*) | 15.9 (*q*) | 15.8 (*q*) |
| C(26) | 17.6 (*q*) | 17.5 (*q*) | 17.2 (*q*) | 17.5 (*q*) | 17.8 (*q*) |
| C(27) | 26.5 (*q*) | 26.4 (*q*) | 26.2 (*q*) | 26.5 (*q*) | 26.6 (*q*) |
| C(28) | 177.1 (*s*) | 177.1 (*s*) | 176.3 (*s*) | 177.1 (*s*) | 178.5 (*s*) |
| C(29) | 29.1 (*q*) | 29.2 (*q*) | 29.1 (*q*) | 29.3 (*q*) | 29.5 (*q*) |
| C(30) | 20.1 (*q*) | 19.9 (*q*) | 19.8 (*q*) | 19.9 (*q*) | 20.1 (*q*) |
| | Angeloyl: | $R_{ct}$: | $R_{tt}$: | $R_{ctt}$: | Angeloyl: |
| C(1′) | 168.6 (*s*) | 167.5 (*s*) | 167.0 (*s*) | 167.6 (*s*) | 168.6 (*s*) |
| C(2′) | 128.8 (*s*) | 115.7 (*d*) | 119.6 (*d*) | 116.1 (*d*) | 128.9 (*s*) |
| C(3′) | 139.9 (*d*) | 147.6 (*d*) | 146.4 (*d*) | 147.3 (*d*) | 139.6 (*d*) |
| C(4′) | 16.0 (*q*) | 126.7 (*d*) | 127.3 (*d*) | 127.7 (*d*) | 16.0 (*q*) |
| C(5′) | 20.7 (*q*) | 152.9 (*d*) | 150.8 (*d*) | 144.3 (*d*) | 20.8 (*q*) |
| C(6′) | | 40.1 (*d*) | 38.9 (*d*) | 130.0 (*d*) | |
| C(7′) | | 30.4 (*t*) | 29.4 (*t*) | 147.7 (*d*) | |
| C(8′) | | 12.1 (*q*) | 11.8 (*q*) | 40.2 (*d*) | |
| C(9′) | | 20.0 (*q*) | 19.4 (*q*) | 30.6 (*t*) | |
| C(10′) | | | | 12.1 (*q*) | |
| C(11′) | | | | 20.2 (*q*) | |
| Ac: | | | | | |
| C(1″) | 171.6 (*s*) | 171.6 (*s*) | 170.3 (*s*) | 171.7 (*s*) | 171.9 (*s*) |
| C(2″) | 20.7 (*q*) | 20.8 (*q*) | 20.8 (*q*) | 20.7 (*q*) | 20.9 (*q*) |
| Glc*p*: | | | | | |
| C(1‴) | 104.8 (*d*) | 104.8 (*d*) | 104.5 (*d*) | 104.8 (*d*) | 106.1 (*d*) |
| C(2‴) | 80.2 (*d*) | 80.2 (*d*) | 80.2 (*d*) | 80.2 (*d*) | 75.6 (*d*) |
| C(3‴) | 77.0 (*d*) | 77.0 (*d*) | 76.7 (*d*) | 77.0 (*d*) | 78.2 (*d*) |

*Table 2* (cont.)

| | **1**[a]) | **2**[a]) | **3**[b]) | **4**[a]) | **5**[a]) |
|---|---|---|---|---|---|
| C(4‴) | 80.3 (*d*) | 80.3 (*d*) | 80.3 (*d*) | 80.3 (*d*) | 71.7 (*d*) |
| C(5‴) | 76.3 (*d*) | 76.3 (*d*) | 76.6 (*d*) | 76.3 (*d*) | 78.0 (*d*) |
| C(6‴) | 61.8 (*t*) | 61.8 (*t*) | 61.7 (*t*) | 61.8 (*t*) | 62.7 (*t*) |
| Glc*p:* | | | | | |
| C(1⁗) | 104.0 (*d*) | 104.0 (*d*) | 104.4 (*d*) | 104.0 (*d*) | |
| C(2⁗) | 75.7 (*d*) | 75.4 (*d*) | 75.8 (*d*) | 75.5 (*d*) | |
| C(3⁗) | 78.0 (*d*) | 78.0 (*d*) | 78.4 (*d*) | 78.0 (*d*) | |
| C(4⁗) | 70.1 (*d*) | 70.1 (*d*) | 69.8 (*d*) | 70.2 (*d*) | |
| C(5⁗) | 78.0 (*d*) | 77.9 (*d*) | 78.4 (*d*) | 78.0 (*d*) | |
| C(6⁗) | 61.8 (*t*) | 61.8 (*t*) | 61.6 (*t*) | 61.8 (*t*) | |
| Ara*p:* | | | | | |
| C(1⁗′) | 105.3 (*d*) | 105.3 (*d*) | 105.7 (*d*) | 105.3 (*d*) | |
| C(2⁗′) | 72.5 (*d*) | 72.5 (*d*) | 72.5 (*d*) | 72.5 (*d*) | |
| C(3⁗′) | 74.4 (*d*) | 74.3 (*d*) | 74.6 (*d*) | 74.3 (*d*) | |
| C(4⁗′) | 70.0 (*d*) | 70.0 (*d*) | 69.6 (*d*) | 69.9 (*d*) | |
| C(5⁗′) | 67.8 (*t*) | 67.6 (*t*) | 67.8 (*t*) | 67.8 (*t*) | |

[a]) At 100 MHz in $CD_3OD$. [b]) At 125 MHz in $C_5D_5N$.

[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-23-hydroxy-21-*O*-[(2*Z*,4*E*,6*E*)-8-methyldeca-2,4,6-trienoyl]acerogenic acid.

Dipteroside E (**5**) was isolated as a white amorphous powder. The FAB-MS spectrum of **5** showed a pseudomolecular-ion peak at *m/z* 789 ($[M-H]^-$), besides significant fragment peaks at *m/z* 627 ($[M-\text{hexose}]^-$). The molecular formula was determined to be $C_{43}H_{66}O_{13}$ on the basis of the HR-ESI-MS (*m/z* 789.4415 ($[M-H]^-$)). The $^1$H- and $^{13}$C-NMR spectra of **5** (*Tables 1* and *2*) showed close resemblance to those of **1**, implying the same triterpenoid skeleton, as well as the angeloyl and Ac moiety. However, in **5**, the substituent at C(3) was a glucosyloxy moiety instead of the trisaccharide moiety in **1**. Thus, the structure of compound **5** was determined to be 22-*O*-acetyl-21-*O*-angeloyl-3-*O*-β-D-glucopyranosyl-23-hydroxyacerogenic acid.

All compounds **1**–**5** were tested for the activities against K562 and HepG2 cells, and dipteroside C (**3**) showed weak inhibitory effects on K562 cells with an $IC_{50}$ value of 102.4 μM as compared to cisplatin ($IC_{50} = 3.15$ μM).

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## Experimental Part

*General.* Anal. TLC: precoated silica gel plates ($SiO_2$, $F_{254}$; *Qingdao Meigao Chemical Co.*); spots were visualized by spraying with 5% $H_2SO_4$ in EtOH, followed by heating. Prep. TLC: $SiO_2$ ($F_{254}$; *Qindao Meigao Chemical Co.*). Column chromatography (CC): $SiO_2$ (200–300 mesh; *Qingdao Meigao Chemical Co.*); *C18* $SiO_2$ (40–75 μm; *Fuji Silysia Chemical Ltd.*); $D_{101}$ macroporous resin (*Qingdao Meigao Chemical Co.*). HPLC: *Agilent 1200*, anal. column, *Zorbax SB-C*$_{18}$ (9.4 × 250 mm, 5 μm). Optical

rotations: *Jasco DIP370* automatic digital polarimeter. UV Spectra: *Shimadzu 210A* double-beam spectrometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Bio-Rad FTS135* spectrophotometer; KBr pellets, $\tilde{\nu}$ in $cm^{-1}$. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; $\delta$ in ppm rel. to $Me_4Si$, *J* in Hz. MS: *VG Autospec-3000* magnetic-sector instrument and *API-Qstar-Pulsar* instrument; in *m*/*z*.

*Plant Material*. *Dipteronia dyeriana* was collected from Pingbian County, Yunnan Province, P. R. China, in January 2007, and identified by Associate Prof. *Z. Q. Ouyang* of the *Yunnan Introduction and Propagation Center for Rare and Endangered Plants*, P. R. China. A voucher specimen (PB0701) was deposited with the Laboratory of Ethnobotany, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

*Extraction and Isolation*. The air-dried branches of *D. dyeriana* (11.5 kg) were extracted with 95% EtOH under reflux (3 ×, for 4, 3, and 3 h, resp.). The solvent was evaporated to afford a residue (1300 g), which was separated by CC ($D_{101}$) resin to afford two fractions, one with $H_2O$ (discarded) and one with EtOH (83 g). The EtOH fraction was subjected to CC ($SiO_2$, $CHCl_3$/MeOH 8:1, 4:1, 2:1, 1:1, and 0:1): *Fractions 1–9*. *Fr. 3* (10 g) was purified by CC (*C18*, MeOH/$H_2O$ 90:10; $SiO_2$, $CHCl_3$/MeOH 10:1), and then by prep. TLC ($SiO_2$, $CHCl_3$/MeOH/$H_2O$ 30:10:1): **5** (8 mg). *Fr. 5* (8.2 g) was subjected to CC (*C18*, MeOH/$H_2O$ 90:10) and semiprep. HPLC (*C18*, MeCN/0.1% $CF_3COOH$ soln. 70:30): **1** (98 mg), **4** (8 mg), and *Fr. 5.1*. *Fr. 5.1* was then separated by semiprep. HPLC (*C18*, MeOH/0.1% $CF_3COOH$ soln. 80:20): **2** (11 mg) and **3** (17 mg).

*Dipteroside A* (=*(3β,4β,21β,22α)-22-(Acetyloxy)-3-{{*O-*α*-L-*arabinopyranosyl-(1→4)*-O-*[β*-D-*glucopyranosyl-(1→2)]-β*-D-*glucopyranosyl}oxy}-23-hydroxy-21-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}-olean-12-en-28-oic Acid*; **1**): White amorphous powder. $[\alpha]_D^{21} = +10.5$ ($c = 0.35$, MeOH). UV (MeOH): 211 (3.83). IR: 3426, 2955, 1725, 1641, 1242, 1152, 1073, 1033. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS: 1083 ($[M-H]^-$). HR-ESI-MS: 1083.5370 ($[M-H]^-$, $C_{54}H_{83}O_{22}^-$; calc. 1083.5376).

*Acid Hydrolysis of* **1**. Compound **1** (50 mg) was hydrolyzed by refluxing in 10% HCl/EtOH for 3 h at 75°. The mixture was dried and subjected to CC ($SiO_2$, $CHCl_3$/MeOH/$H_2O$ 200:20:1 and 80:20:1): D-glucose ($[\alpha]_D^{20} = +70.0$ ($c = 0.15$, $H_2O$)) and L-arabinose ($[\alpha]_D^{20} = +60.0$ ($c = 0.05$, $H_2O$)), identified by TLC comparison with authentic samples and by their optical rotations.

*Dipteroside B* (=*(3β,4β,21β,22α)-22-(Acetyloxy)-3-{{*O-*α*-L-*arabinopyranosyl-(1→4)*-O-*[β*-D-*glucopyranosyl-(1→2)]-β*-D-*glucopyranosyl}oxy}-23-hydroxy-21-{[(2Z,4*E*)-6-methyl-1-oxoocta-2,4-dien-1-yl]oxy}olean-12-en-28-oic Acid*; **2**): White amorphous powder. $[\alpha]_D^{21} = +7.4$ ($c = 0.58$, MeOH). UV (MeOH): 264 (3.75). IR: 3423, 2963, 1723, 1679, 1638, 1246, 1072, 1031. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS: 1137 ($[M-H]^-$). HR-ESI-MS: 1137.5867 ($[M-H]^-$, $C_{58}H_{89}O_{22}^-$; calc. 1137.5845).

*Dipteroside C* (=*(3β,4β,21β,22α)-22-(Acetyloxy)-3-{{*O-*α*-L-*arabinopyranosyl-(1→4)*-O-*[β*-D-*glucopyranosyl-(1→2)]-β*-D-*glucopyranosyl}oxy}-23-hydroxy-21-{[(2*E*,4*E*)-6-methyl-1-oxoocta-2,4-dien-1-yl]oxy}olean-12-en-28-oic Acid*; **3**): White amorphous powder. $[\alpha]_D^{21} = +29.1$ ($c = 0.62$, pyridine). UV (MeOH): 262 (4.08). IR: 3416, 2964, 1719, 1678, 1642, 1259, 1139, 1072, 1029. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS: 1137 ($[M-H]^-$). HR-ESI-MS: 1137.5833 ($[M-H]^-$, $C_{58}H_{89}O_{22}^-$; calc. 1137.5845).

*Dipteroside D* (=*(3β,4β,21β,22α)-22-(Acetyloxy)-3-{{*O-*α*-L-*arabinopyranosyl-(1→4)*-O-*[β*-D-*glucopyranosyl-(1→2)]-β*-D-*glucopyranosyl}oxy}-23-hydroxy-21-{[(2Z,4*E*,6*E*)-8-methyl-1-oxodeca-2,4,6-trien-1-yl]oxy}olean-12-en-28-oic Acid*; **4**): White amorphous powder. $[\alpha]_D^{22} = +33.9$ ($c = 0.29$, $CHCl_3$/MeOH 1:2). UV (MeOH): 306 (4.13). IR: 3427, 2961, 2933, 1723, 1678, 1639, 1248, 1142, 1072, 1031. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-FAB-MS: 1163.6009 ($[M-H]^-$, $C_{60}H_{91}O_{22}^-$; calc. 1163.6002).

*Dipteroside E* (=*(3β,4β,21β,22α)-22-(Acetyloxy)-3-(β*-D-*glucopyranosyloxy)-24-hydroxy-21-{[(2Z)-2-methyl-1-oxobut-2-en-1-yl]oxy}olean-12-en-28-oic Acid*; **5**): White amorphous powder. $[\alpha]_D^{21} = +21.8$ ($c = 0.42$, MeOH). UV (MeOH): 306 (3.04), 264 (3.40). IR: 3426, 2957, 1725, 1638, 1578, 1459, 1380, 1256, 1156, 1074, 1034. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS: 789 ($[M-H]^-$), 627 ($[M-162-H]^-$). HR-ESI-MS: 789.4415 ($[M-H]^-$, $C_{43}H_{65}O_{13}^-$; calc. 789.4425).

*Cytotoxicity Assay*. K562 and HepG2 cells were maintained at 37° in *PRMI1640* medium (*Sigma*) containing 10% fetal bovine serum (*Hangzhou Sijiqing Biological Engineering Materials Co., Ltd.*, P. R. China) in an atmosphere of humidified 5% $CO_2$. Growth inhibition experiments were carried out in quintuplicate in 96-well microplates (*Corning*), and the amount of viable cells at the end of the incubation period was determined by using a modified MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-1,2,3,4-tetrazolium bromide) [9]. The $IC_{50}$ value was calculated by the GWBASIC

software. Cisplatin (*Yunnan Gejiu Bio-Pharmaceutical Co., Ltd.*, P. R. China) was used as a positive control.

## REFERENCES

[1] T. Z. Xu, *Acta Bot. Yunnan.* **1996**, *18*, 43.
[2] S. M. Kupchan, M. Takasugi, R. M. Smith, P. S. Steyn, *J. Org. Chem*. **1971**, *36*, 1972.
[3] H. Yang, S. H. Sung, Y. C. Kim, *J. Nat. Prod.* **2005**, *68*, 101.
[4] H. Yang, M. K. Lee, Y. C. Kim, *J. Agric. Food Chem*. **2005**, *53*, 4182.
[5] T. Morikawa, J. Tao, I. Toguchida, H. Matsuda, M. Yoshikawa, *J. Nat. Prod*. **2003**, *66*, 86.
[6] H. J. Kim, E.-R. Woo, C.-G. Shin, H. Park, *J. Nat. Prod.* **1998**, *61*, 145.
[7] Y. Mimaki, M. Kuroda, T. Asano, Y. Sashida, *J. Nat. Prod.* **1999**, *62*, 1279.
[8] A. Guerriero, M. D'Ambrosio, V. Cuomo, F. Vanzanella, F. Pietra, *Helv. Chim. Acta* **1989**, *72*, 438.
[9] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55; J. J. Zhou, X. F. Yue, J. X. Han, W. Y. Yang, *Zhongguo Yiyao Gongye Zazhi* **1993**, *24*, 455.

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