# Three New Pregnane Glycosides from *Marsdenia tinctoria*

by **Zhu-Lin Gao**[a][b][c], **Hong-Ping He**[a], **Ying-Tong Di**[a], **Xin Fang**[a], **Chun-Shun Li**[a], **Qiang Zhang**[a], **Pei-Ji Zhao**[a], **Shun-Lin Li**[a], and **Xiao-Jiang Hao***[a]

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China (phone: +86-871-5223263; fax: +86-871-5219684; e-mail: haoxj@mail.kib.ac.cn)

[b]) Key Laboratory of Medicinal Chemistry for Natural Resource (Yunnan University), Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan 650091, P. R. China

[c]) Graduate University of the Chinese Academy of Sciences, Beijing 100039, P. R. China

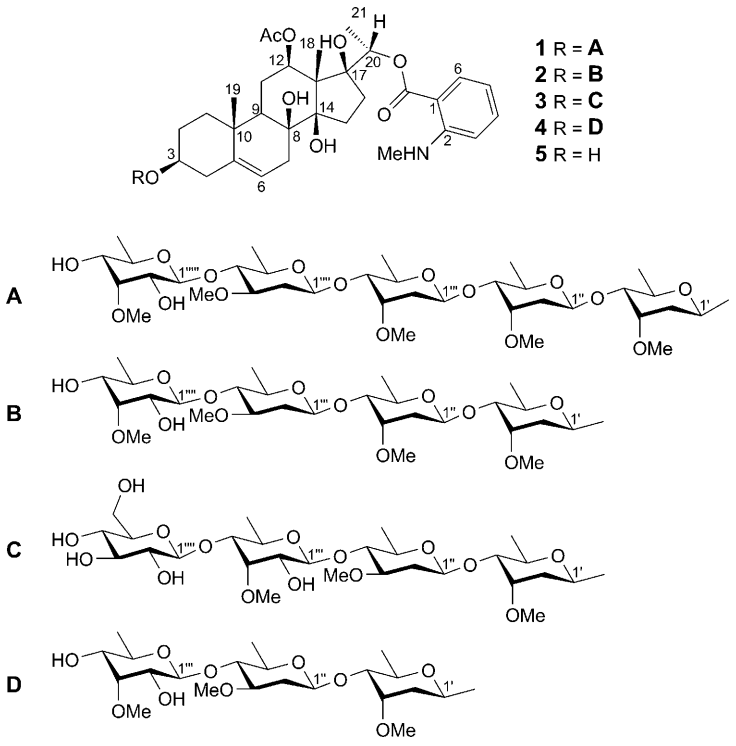
Three new pregnane glycosides, tinctorosides A–C (**1**–**3**, resp.), together with one known pregnane glycoside, stephanoside B (**4**), were isolated from the stems of *Marsdenia tinctoria* R. BR. (Asclepiadaceae). Their structures were elucidated by extensive spectral methods, especially 2D-NMR experiments ($^1$H,$^1$H-COSY, HSQC, HMBC, TOCSY, HSQC-TOCSY, and ROESY), and chemical evidence.

**Introduction.** – *Marsdenia tinctoria* R. BR. (Asclepiadaceae) is a perennial climber widely distributed in South China, Taiwan, and Tibet. Its stem has been used as folk medicine for the treatment of rheumatic pain and hepatomegaly in China [1]. Previous phytochemical investigation revealed that this plant contained a steroidal alkaloid and two steroids, but the glycosides were not studied yet [2][3].

In this article, we report the isolation and characterization of three new pregnane glycosides, tinctorosides A–C (**1**–**3**, resp.), together with one known pregnane glycoside, stephanoside B (**4**), from the stems of *M. tinctoria*.

**Results and Discussion.** – The EtOH extract of the stems of *M. tinctoria* was partitioned between $CHCl_3$ and $H_2O$. The $CHCl_3$-soluble portion was subsequently separated by column chromatography (silica gel, *RP-18*, *Sephadex HL-20*, and semi-preparative HPLC) to provide the four compounds **1**–**4**. All of these compounds showed positive *Liebermann–Burchard* and *Keller–Kiliani* reactions, indicating that they were all steroidal glycosides containing 2-deoxy-sugar moieties.

Tinctoroside A (**1**) was obtained as an amorphous powder. Its molecular formula was determined as $C_{66}H_{103}NO_{24}$ by HR-ESI-MS (negative-ion mode; *m/z* 1328.6572 ($[M + Cl]^-$); calc. 1328.6558). The IR spectrum of **1** showed absorptions at 3432 (OH), 1734 (C=O), 1677 (C=C), and 1084 (C–O–C) cm$^{-1}$. The assignments of the $^1$H- and $^{13}$C-NMR signals of **1** were successfully carried out with $^1$H,$^1$H-COSY, HSQC, HMBC, TOCSY, HSQC-TOCSY, and ROESY experiments (*Tables 1* and *2*). On the basis of its 1D- and 2D-NMR data and chemical evidence, the structure of **1** was established as stephanthraniline A 3-*O*-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1→




4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

The $^{13}$C-NMR and HSQC spectra of **1** showed the presence of 66 C-atoms comprising ten Me, five MeO, eleven $CH_2$, thirty CH, and ten quaternary C-atoms. In the $^{13}$C-NMR spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those of stephanthraniline A (**5**) [4] within the range of glycosylation shifts at C(2) ($\Delta\delta$(C) −2.0), C(3) (+7.1), and C(4) (−4.0), suggesting that the sugar moiety was linked at C(3) of the aglycone [5]. Furthermore, in the NMR spectra of **1**, five anomeric C-signals were identified at $\delta$(C) 96.5, 100.5, 100.6, 101.9, and 102.0, correlating with the anomeric CH signals at $\delta$(H) 5.26, 5.10, 5.10, 4.67, and 5.28, respectively, which indicated that compound **1** was a stephanthraniline A 3-*O*-pentoside. The β-linkages of each of the sugars were evident from the $^1$H-NMR coupling constants of the anomeric signals [6].

Mild acid hydrolysis of **1** afforded the aglycone stephanthraniline A (**5**), identified by comparison of its spectroscopic data with those in the literature [4]. In addition, the monosaccharides cymarose, oleandrose, and an unidentified sugar were detected in the hydrolysate by TLC. The HMBC and $^1$H,$^1$H-COSY experiments allowed the sequential assignments of the $\delta$(C) and $\delta$(H) values for the unidentified sugar as shown in *Table 2*, starting from the anomeric H- and C-signal at $\delta$(H) 5.28 (*d*, $J$ = 8.0) and $\delta$(C) 102.0. Those findings suggested that the unidentified sugar is 6-deoxy-3-*O*-methyl-β-D-allose

Table 1. *$^1$H- and $^{13}$C-NMR Spectroscopic Data of the Aglycones of* **1**–**3**. At 500/125 MHz, resp., in ($D_5$)Pyridine; $\delta$ in ppm, $J$ in Hz[a]).

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| $CH_2$(1) | 1.01 (*t*, *J* = 12.0), 1.68–1.75 (*m*) | 38.8 | 1.02 (*t*, *J* = 12.0), 1.68–1.75 (*m*) | 38.8 | 1.03 (*t*, *J* = 12.0), 1.68–1.77 (*m*) | 38.8 |
| $CH_2$(2) | 1.73–1.82 (*m*), 2.01–2.10 (*m*) | 29.9 | 1.72–1.82 (*m*), 2.00–2.07 (*m*) | 29.9 | 1.73–1.82 (*m*), 2.02–2.09 (*m*) | 29.9 |
| H–C(3) | 3.78–3.85 (*m*) | 77.7 | 3.78–3.85 (*m*) | 77.7 | 3.78–3.86 (*m*) | 77.7 |
| $CH_2$(4) | 2.38–2.44 (*m*), 2.50–2.59 (*m*) | 39.3 | 2.35–2.44 (*m*), 2.49–2.56 (*m*) | 39.3 | 2.37–2.46 (*m*), 2.49–2.57 (*m*) | 39.3 |
| C(5) | | 139.3 | | 139.3 | | 139.3 |
| H–C(6) | 5.31 (br. *s*) | 119.4 | 5.31 (br. *s*) | 119.4 | 5.31 (br. *s*) | 119.4 |
| $CH_2$(7) | 2.29–2.37 (*m*), 2.41–2.52 (*m*) | 34.9 | 2.28–2.35 (*m*), 2.41–2.49 (*m*) | 34.9 | 2.29–2.38 (*m*), 2.43–2.51 (*m*) | 34.9 |
| C(8) | | 74.4 | | 74.4 | | 74.4 |
| H–C(9) | 1.66–1.73 (*m*) | 44.1 | 1.64–1.71 (*m*) | 44.1 | 1.65–1.72 (*m*) | 44.1 |
| C(10) | | 37.3 | | 37.3 | | 37.3 |
| $CH_2$(11) | 1.87–1.98 (*m*), 2.20–2.34 (*m*) | 25.6 | 1.88–1.96 (*m*), 2.20–2.34 (*m*) | 25.6 | 1.90–1.98 (*m*), 2.22–2.32 (*m*) | 25.6 |
| H–C(12) | 5.15 (*dd*, *J* = 11.5, 4.5) | 74.6 | 5.14 (*dd*, *J* = 11.5, 4.5) | 74.6 | 5.14 (*dd*, *J* = 11.5, 4.5) | 74.6 |
| C(13) | | 57.0 | | 57.0 | | 57.0 |
| C(14) | | 88.9 | | 88.9 | | 88.9 |
| $CH_2$(15) | 2.08–2.15 (*m*) | 33.7 | 2.07–2.12 (*m*) | 33.7 | 2.09–2.14 (*m*) | 33.7 |
| $CH_2$(16) | 1.98–2.06 (*m*) | 33.9 | 1.96–2.06 (*m*) | 33.9 | 1.99–2.08 (*m*) | 33.9 |
| C(17) | | 87.7 | | 87.6 | | 87.6 |
| Me(18) | 2.01 (*s*) | 11.3 | 2.01 (*s*) | 11.3 | 2.01 (*s*) | 11.3 |
| Me(19) | 1.29 (*s*) | 18.1 | 1.28 (*s*) | 18.1 | 1.28 (*s*) | 18.1 |
| H–C(20) | 5.17 (*q*, *J* = 6.0) | 74.9 | 5.16 (*q*, *J* = 6.0) | 74.9 | 5.17 (*q*, *J* = 6.0) | 74.9 |
| Me(21) | 1.52 (*d*, *J* = 6.0) | 15.6 | 1.52 (*d*, *J* = 6.0) | 15.6 | 1.52 (*d*, *J* = 6.0) | 15.6 |
| 12-AcO: | | | | | | |
| C=O | | 171.4 | | 171.4 | | 171.4 |
| Me | 2.10 (*s*) | 22.0 | 2.10 (*s*) | 22.0 | 2.10 (*s*) | 22.0 |
| 20-AnthO: | | | | | | |
| C=O | | 168.3 | | 168.3 | | 168.3 |
| C(1) | | 111.1 | | 111.1 | | 111.1 |
| C(2) | | 152.6 | | 152.6 | | 152.6 |
| H–C(3) | 6.72 (*d*, *J* = 8.0) | 111.5 | 6.71 (*d*, *J* = 8.0) | 111.5 | 6.71 (*d*, *J* = 8.0) | 111.5 |
| H–C(4) | 7.40 (*t*, *J* = 8.0) | 135.1 | 7.40 (*t*, *J* = 8.0) | 135.1 | 7.40 (*t*, *J* = 8.0) | 135.1 |
| H–C(5) | 6.58 (*t*, *J* = 8.0) | 114.8 | 6.57 (*t*, *J* = 8.0) | 114.8 | 6.57 (*t*, *J* = 8.0) | 114.8 |
| H–C(6) | 8.33 (*d*, *J* = 8.0) | 132.6 | 8.34 (*d*, *J* = 8.0) | 132.6 | 8.34 (*d*, *J* = 8.0) | 132.6 |
| Me | 2.75 (*d*, *J* = 5.0) | 29.5 | 2.76 (*d*, *J* = 5.0) | 29.6 | 2.76 (*d*, *J* = 5.0) | 29.6 |

[a]) Assignments based on $^1$H,$^1$H-COSY, HSQC, HMBC, TOCSY, HSQC-TOCSY, and ROESY experiments.

(3-*O*-methyl-$\beta$-D-allomethylose) on the basis of its $^1$H- and $^{13}$C-NMR assignments, which are in agreement with those of similar compounds [4][7]. The existence of one D-oleandropyranosyl and three D-cymaropyranosyl units were confirmed by comparison of their spectroscopic data with those in the literature [4][8]. Further, the chemical-

Table 2. *$^1$H- and $^{13}$C-NMR Spectroscopic Data of the Sugar Moieties of* **1**–**3**. At 500/125 MHz, resp., in ($D_5$)Pyridine; $\delta$ in ppm, $J$ in Hz[a]).

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| | Cym | | Cym | | Cym | |
| H–C(1′) | 5.26 (br. *d*, *J* = 10.0) | 96.5 | 5.26 (br. *d*, *J* = 10.0) | 96.4 | 5.26 (br. *d*, *J* = 10.0) | 96.4 |
| $CH_2$(2′) | 1.87–1.96 (*m*), 2.25–2.36 (*m*) | 37.3 | 1.84–1.90 (*m*), 2.24–2.30 (*m*) | 37.3 | 1.82–1.91 (*m*), 2.25–2.32 (*m*) | 37.3 |
| H–C(3′) | 3.96–4.03 (*m*) | 77.8 | 3.99–4.03 (*m*) | 77.9 | 4.00–4.03 (*m*) | 77.9 |
| H–C(4′) | 3.50–3.58 (*m*) | 83.4 | 3.48–3.56 (*m*) | 83.4 | 3.44–3.50 (*m*) | 83.5 |
| H–C(5′) | 4.18–4.26 (*m*) | 69.2 | 4.17–4.25 (*m*) | 69.2 | 4.18–4.21 (*m*) | 69.0 |
| Me(6′) | 1.40 (*d*, *J* = 6.0) | 18.5 | 1.40 (*d*, *J* = 6.0) | 18.2 | 1.41 (*d*, *J* = 6.0) | 18.7 |
| 3′-MeO | 3.60 (*s*) | 58.9 | 3.53 (*s*) | 58.9 | 3.54 (*s*) | 58.9 |
| | Cym | | Cym | | Ole | |
| H–C(1″) | 5.10 (br. *d*, *J* = 10.0) | 100.5 | 5.12 (br. *d*, *J* = 10.0) | 100.6 | 4.66 (br. *d*, *J* = 9.5) | 101.9 |
| $CH_2$(2″) | 1.87–1.96 (*m*), 2.25–2.36 (*m*) | 37.0 | 1.78–1.84 (*m*), 2.35–2.41 (*m*) | 36.1 | 1.68–1.74 (*m*), 2.44–2.50 (*m*) | 37.3 |
| H–C(3″) | 4.05–4.12 (*m*) | 78.1 | 3.73–3.78 (*m*) | 78.9 | 3.53–3.59 (*m*) | 79.4 |
| H–C(4″) | 3.50–3.58 (*m*) | 83.4 | 3.44–3.49 (*m*) | 83.5 | 3.50–3.58 (*m*) | 83.0 |
| H–C(5″) | 4.16–4.20 (*m*) | 69.1 | 4.16–4.20 (*m*) | 69.0 | 3.51–3.58 (*m*) | 72.0 |
| Me(6″) | 1.37 (*d*, *J* = 5.5) | 18.2 | 1.41 (*d*, *J* = 6.0) | 18.7 | 1.59 (*d*, *J* = 5.0) | 18.9 |
| 3″-MeO | 3.54 (*s*) | 58.9 | 3.47 (*s*) | 58.2 | 3.50 (*s*) | 57.4 |
| | Cym | | Ole | | Alm | |
| H–C(1‴) | 5.10 (br. *d*, *J* = 10.0) | 100.6 | 4.67 (br. *d*, *J* = 9.5) | 101.9 | 5.25 (*d*, *J* = 8.0) | 101.9 |
| $CH_2$(2‴) or H–C(2‴) | 1.76–1.83 (*m*), 2.36–2.45 (*m*) | 36.1 | 1.70–1.75 (*m*), 2.43–2.49 (*m*) | 37.7 | 3.83 (*dd*, *J* = 8.0, 3.0) | 72.7 |
| H–C(3‴) | 3.75–3.78 (*m*) | 78.9 | 3.53–3.59 (*m*) | 79.4 | 4.49 (*t*, *J* = 3.0) | 83.2 |
| H–C(4‴) | 3.38–3.45 (*m*) | 83.2 | 3.52–3.60 (*m*) | 83.1 | 3.74 (*dd*, *J* = 9.0, 3.0) | 83.4 |
| H–C(5‴) | 4.12–4.19 (*m*) | 69.0 | 3.53–3.57 (*m*) | 72.0 | 4.24–4.29 (*m*) | 69.6 |
| Me(6‴) | 1.36 (*d*, *J* = 5.5) | 18.6 | 1.61 (*d*, *J* = 5.0) | 19.0 | 1.64 (*d*, *J* = 6.0) | 18.3 |
| 3‴-MeO | 3.47 (*s*) | 58.2 | 3.53 (*s*) | 57.4 | 3.82 (br. *s*) | 61.8 |
| | Ole | | Alm | | Glc | |
| H–C(1⁗) | 4.67 (br. *d*, *J* = 9.5) | 101.9 | 5.27 (*d*, *J* = 8.0) | 102.0 | 4.97 (*d*, *J* = 8.0) | 106.6 |
| $CH_2$(2⁗) or H–C(2⁗) | 1.67–1.78 (*m*), 2.45–2.52 (*m*) | 37.7 | 3.81–3.87 (*m*) | 72.7 | 3.99–4.02 (*m*) | 75.5 |
| H–C(3⁗) | 3.54–3.61 (*m*) | 79.4 | 4.38 (*t*, *J* = 3.0) | 83.1 | 4.21–4.27 (*m*) | 78.4 |
| H–C(4⁗) | 3.52–3.60 (*m*) | 83.1 | 3.50–3.54 (*m*) | 74.2 | 4.15–4.20 (*m*) | 72.0 |
| H–C(5⁗) | 3.52–3.61 (*m*) | 72.1 | 4.05–4.13 (*m*) | 71.0 | 3.98–4.01 (*m*) | 78.4 |
| Me(6⁗) or $CH_2$(6⁗) | 1.61 (*d*, *J* = 5.0) | 19.0 | 1.47 (*d*, *J* = 6.0) | 18.9 | 4.36 (*dd*, *J* = 12.0, 5.0), 4.55 (*dd*, *J* = 12.0, 3.0) | 63.1 |
| 3⁗-MeO | 3.54 (*s*) | 57.4 | 3.86 (*s*) | 61.7 | | |
| | Alm | | | | | |
| H–C(1⁗′) | 5.28 (*d*, *J* = 8.0) | 102.0 | | | | |
| H–C(2⁗′) | 3.82–3.91 (*m*) | 72.7 | | | | |
| H–C(3⁗′) | 4.38 (*t*, *J* = 3.0) | 83.1 | | | | |
| H–C(4⁗′) | 3.50–3.59 (*m*) | 74.2 | | | | |
| H–C(5⁗′) | 4.06–4.15 (*m*) | 71.0 | | | | |
| Me(6⁗′) | 1.48 (*d*, *J* = 6.0) | 18.9 | | | | |
| 3⁗′-MeO | 3.87 (*s*) | 61.7 | | | | |

[a]) Assignments based on $^1$H,$^1$H-COSY, HSQC, HMBC, TOCSY, HSQC-TOCSY, and ROESY experiments. Cym, Ole, Alm and Glc refer to cymaropyranosyl, oleandropyranosyl, 6-deoxy-3-*O*-methyl-allopyranosyl, and glucopyranosyl, resp.

shift values for C(2′) ($\delta$(C) 37.3), C(2″) ($\delta$(C) 37.0), and C(2‴) ($\delta$(C) 36.1) of the three cymarose units as well as C(2⁗) ($\delta$(C) 37.7) of the oleandrose unit present in **1** showed that they all have D-configuration [9].

The sequence of these five sugars in **1** was determined by a HMBC experiment. Long-rang correlations were observed between H–C(1⁗) ($\delta$(H) 5.28) and C(4⁗) ($\delta$(C) 83.1), H–C(1‴) ($\delta$(H) 4.67) and C(4‴) ($\delta$(C) 83.2), H–C(1‴) ($\delta$(H) 5.10) and C(4″) ($\delta$(C) 83.4), H–C(1″) ($\delta$(H) 5.10) and C(4′) ($\delta$(C) 83.4), and between H–C(1′) ($\delta$(H) 5.26) and C(3) ($\delta$(C) 77.7) of the aglycone. This indicated the sequence of the sugar chain as 6-deoxy-3-*O*-methyl-$\beta$-D-allopyranosyl-(1 → 4)-$\beta$-D-oleandropyranosyl-(1 → 4)-$\beta$-D-cymaropyranosyl-(1 → 4)-$\beta$-D-cymaropyranosyl-(1 → 4)-$\beta$-D-cymaropyranoside.

Tinctoroside B (**2**) was isolated as an amorphous powder with a molecular formula of $C_{59}H_{91}NO_{21}$, deduced from the HR-ESI-MS (positive-ion mode; *m*/*z* 1172.6010 ($[M+Na]^+$), calc. 1172.5981). On mild acid hydrolysis, **2** gave stephanthraniline A (**5**) as the aglycone, and the same sugar composition as compound **1**. Analysis of the NMR data (*Table 2*) of the sugar chain of compound **2** and the comparison with those of **1** showed that the signals for one cymaropyranosyl unit were absent in **2**. This conclusion was further confirmed by the 2D-NMR spectra. Therefore, the structure of **2** was deduced to be stephanthraniline A 3-*O*-6-deoxy-3-*O*-methyl-$\beta$-D-allopyranosyl-(1 → 4)-$\beta$-D-oleandropyranosyl-(1 → 4)-$\beta$-D-cymaropyranosyl-(1 → 4)-$\beta$-D-cymaropyranoside.

Tinctoroside C (**3**) was obtained as an amorphous power, and was assigned the molecular formula $C_{58}H_{89}NO_{23}$, as shown by its HR-ESI-MS (positive-ion mode; *m*/*z* 1190.5709 ($[M+Na]^+$), calc. 1190.5723). Mild acid hydrolysis of **3** yielded stephanthraniline A (**5**) as the aglycone, as well as cymarose, oleandrose, 3-*O*-methylallomethylose, and glucose as the sugar moieties. The comparison of the $^{13}$C-NMR spectral data of **3** (*Table 2*) with those of bouceroside indicated that **3** possessed the same sugar sequence in the oligosaccharide moiety as bouceroside [8]. The position of each sugar residue was further confirmed by a 2D-ROESY experiment, which showed a cross-peak between the signal at $\delta$(H) 5.26 (H–C(1′)) and the signal at $\delta$(H) 3.78–3.86 (H–C(3)), and other key correlation peaks between the signals at $\delta$(H) 4.66 (H–C(1″)) and 3.44–3.50 (H–C(4′)), 5.25 (H–C(1‴)) and 3.50–3.58 (H–C(4″)), 4.97 (H–C(1⁗)) and 3.74 (H–C(4‴)). The configuration of cymarose, oleandrose, and 3-*O*-methylallomethylose was deduced to be D, as in case of compound **1**. Meanwhile, the configuration of the glucose was tentatively assigned as D from biogenetic consideration. Comparing with stephanthraniline A (**5**), the glycosylation shifts were observed at C(2) ($\Delta\delta$(C) − 2.0), C(3) (+ 7.1), and C(4) (− 4.0) in the aglycone moiety, therefore, the sugar moiety was linked to C(3) of the aglycone. Based on the above evidence, compound **3** was determined to be stephanthraniline A 3-*O*-$\beta$-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-$\beta$-D-allopyranosyl-(1 → 4)-$\beta$-D-oleandropyranosyl-(1 → 4)-$\beta$-D-cymaropyranoside.

Finally, the known compound stephanoside B (**4**) was identified by comparison of the $^1$H- and $^{13}$C-NMR, and ESI-MS data with those reported in the literature. It has been previously found only in *Stephanotis lutchuensis*, another plant of the family Marsdeniaceae [4].

The authors are grateful to Prof. *Shishan Yu*, Key Laboratory of Bioactive Substance and Resources Utilization of Chinese Herbal Medicine, Ministry of Education & Institute of Materia Medica, Peking Union College and Chinese Academy of Medical Sciences, and Dr. *Xiaoxia Ma*, State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the authentic samples of deoxy sugars.

## Experimental Part

*General*. The MPLC instrument includes a *Büchi Pump Module C-605* and a *Büchi Pump Manager C-615*. Column chromatography (CC): silica gel *H* ($SiO_2$; 10–40 μm; *Qingdao Marine Chemical Ltd. Co.*), *RP-18* (40–75 μm; *Merck Co.*), and *Sephadex LH-20* (40–70 μm, *Pharmacia*). Semi-prep. HPLC: *Agilent 1100* chromatograph, with a diode-array detector and a *Zorbax SB-C-18* (*Agilent Co. Ltd.*, USA) column (9.4 × 250 mm, 10 μm), developed with MeOH/$H_2O$ 80:20 (30 min; flow rate, 3.0 ml/min) at 30°. TLC: on plates precoated with silica gel $GF_{254}$ (*Qingdao Marine Chemical Ltd. Co.*); visualization by spraying with 5% $H_2SO_4$ in EtOH, followed by heating. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Shimadzu 210A* double-beam spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets, in $cm^{-1}$. NMR Spectra: *Bruker AM-400* instrument (400/100 MHz) and *Bruker DRX-500* instrument (500/125 MHz); $\delta$ in ppm rel. to TMS as internal standard, *J* in Hz. ESI-MS: *Finnigan MAT 90* instrument; in *m/z*. HR-ESI-MS: *API Qstar Pulsar LC/TOF* instrument.

*Plant Material*. The stems of *M. tinctoria* were collected in March 2002 in Xishuangbanna of Yunnan Province, P. R. China and identified by Prof. *Dedin Tao*. A voucher specimen (NO. 20020329) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

*Extraction and Isolation*. The air-dried and powdered stems of *M. tinctoria* (1 kg) were extracted with 95% EtOH (2 l) under reflux three times (4, 4, and 2 h, resp.). After evaporation of the org. solvent, the residue was suspended in $H_2O$ (2 l) and extracted with petroleum ether (PE; 1 l × 3) and $CHCl_3$ (1 l × 3) successively. The $CHCl_3$-soluble fraction (20 g) was subjected to CC ($SiO_2$; $CHCl_3$/MeOH 100:0 → 50:50) to afford six fractions (*Frs. 1–6*). *Fr. 3* (3.0 g) was sequentially subjected to CC over $SiO_2$ ($CHCl_3$/MeOH 95:5 → 90:10), *Sephadex LH-20* (MeOH), and *RP-18* (MPLC, MeOH/$H_2O$ 75:25), and further purified by semi-prep. HPLC (MeOH/$H_2O$ 80:20) to afford **1** (21 mg, $t_R$ 16.8 min), **2** (17 mg, $t_R$ 12.9 min), and **4** (41 mg, $t_R$ 10.4 min). *Fr. 4* (3.5 g) on repeated CC ($SiO_2$; $CHCl_3$/MeOH 90:10 → 85:15) and *RP-18* (MeOH/$H_2O$ 70:30) yielded compound **3** (260 mg).

*Acid Hydrolysis of* **1**, **2**, *and* **3**. A soln. of **1**, **2**, or **3** (each 5 mg) in 3 ml 50% dioxane (dioxane/$H_2O$ 1:1) and 3 ml 0.05M $H_2SO_4$ was heated at 95° for 2 h. After dioxane was removed *in vacuo*, the soln. was extracted with $CHCl_3$. The $CHCl_3$ residue of the three compounds was separated by prep. TLC to afford **5** (2 mg). The $H_2O$ layer of each compound was neutralized with sat. aq. $Ba(OH)_2$ soln., and the precipitation was filtered off. The filtrate was evaporated, and the sugars identified by TLC comparison with authentic samples. In the hydrolysates of **1**–**3**, a spot which did not correspond to a reference sugar was attributed to 3-*O*-methylallomethylose. Cymarose, oleandrose, and 3-*O*-methylallomethylose were detected from **1**–**3**; glucose was detected from **3**. $R_f$ (D-cymarose) 0.41 ($CHCl_3$/MeOH 9:1) and 0.33 (PE/$Me_2CO$ 3:2); $R_f$ (D-oleandrose) 0.31 ($CHCl_3$/MeOH 9:1) and 0.23 (PE/$Me_2CO$ 3:2); $R_f$ (3-*O*-methyl-D-allomethylose) 0.18 ($CHCl_3$/MeOH 9:1) and 0.14 (PE/$Me_2CO$ 3:2); $R_f$ (D-glucose) 0.30 ($CHCl_3$/MeOH/$H_2O$ 7:3:0.5).

*Tinctoroside A* (=*(3β,9α,12β,14β,17α,20*S*)-12-(Acetyloxy)-3-{[6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-α-*D*-ribo-hexopyranosyl]oxy}-8,14,17-trihydroxypregn-5-en-20-yl 2-(Methylamino)benzoate*; **1**). White amorphous power. $[\alpha]_D^{19} = 0$ ($c = 0.15$, MeOH). UV (MeOH): 355 (3.93), 253 (4.05), 222 (4.57), 201 (4.38). IR (KBr): 3432, 1734, 1677, 1581, 1520, 1245, 1084. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-ESI-MS (neg.): 1328.6572 ($[M + Cl]^-$, $C_{66}H_{103}ClNO_{24}^-$; calc. 1328.6558).

*Tinctoroside B* (=*(3β,9α,12β,14β,17α,20*S*)-12-(Acetyloxy)-3-{[6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*arabino*-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ri-

bo-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-ribo-*hexopyranosyl]oxy}-8,14,17-trihydroxypregn-5-en-20-yl 2-(Methylamino)benzoate*; **2**). White amorphous power. $[\alpha]_D^{19} = 0$ ($c = 0.20$, MeOH). UV (MeOH): 355 (3.41), 253 (3.57), 222 (4.07), 201 (3.93). IR (KBr): 3440, 1732, 1678, 1582, 1520, 1245, 1082. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 1172.6010 ($[M + Na]^+$, $C_{59}H_{91}NNaO_{21}^+$; calc. 1172.5981).

*Tinctoroside C* (*= (3β,9α,12β,14β,17α,20*S*)-12-(Acetyloxy)-3-{[β*-D-*glucopyranosyl-(1 → 4)-6-deoxy-3*-O-*methyl-β*-D-*allopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-β*-D-arabino-*hexopyranosyl-(1 → 4)-2,6-dideoxy-3*-O-*methyl-α*-D-ribo-*hexopyranosyl]oxy}-8,14,17-trihydroxypregn-5-en-20-yl 2-(Methylamino)-benzoate*; **3**). White amorphous power. $[\alpha]_D^{19} = -3$ ($c = 0.20$, MeOH). UV (MeOH): 356 (3.60), 254 (3.71), 222 (4.22), 200 (4.03), 194 (3.96). IR (KBr): 3450, 1732, 1674, 1583, 1521, 1260, 1079. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-ESI-MS (pos.): 1190.5709 ($[M + Na]^+$, $C_{58}H_{89}NNaO_{23}^+$; calc. 1190.5723).

## REFERENCES


[1] Editorial Committee of Administration Bureau of Traditional Chinese Medicine, 'Chinese Materia Medica', Shanghai Scientific and Technical Press, 1999, Vol. 6, p. 3860.
[2] A. K. A. Chowdhury, B. C. Sen, M. F. Hashim, M. Ahmed, *Pharmazie* **1993**, *48*, 628.
[3] A. K. A. Chowdhury, M. F. Hashim, B. C. Sen, O. F. Khan, M. Ahmed, *Pure Appl. Chem.* **1994**, *66*, 2343.
[4] K. Yoshikawa, N. Okada, Y. Kann, S. Arihara, *Chem. Pharm. Bull.* **1996**, *44*, 1790.
[5] R. Kasai, M. Suzuo, J. Asakawa, O. Tanaka, *Tetrahedron Lett.* **1977**, *18*, 175.
[6] T. Nakagawa, K. Hayashi, K. Wada, H. Mitsuhashi, *Tetrahedron Lett.* **1982**, *23*, 5431.
[7] Y. Ye, X. Li, H. Sun, F. Chen, Y. Pan, *Helv. Chim. Acta* **2004**, *87*, 2378.
[8] K. Hayashi, I. Iida, Y. Nakao, Y. Nakao, K. Kaneko, *Phytochemistry* **1988**, *27*, 3919.
[9] R. Vleggaar, F. R. van Heerden, L. A. P. Anderson, G. L. Erasmus, *J. Chem. Soc., Perkin Trans. 1* **1993**, 483.


*Received March 2, 2009*