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

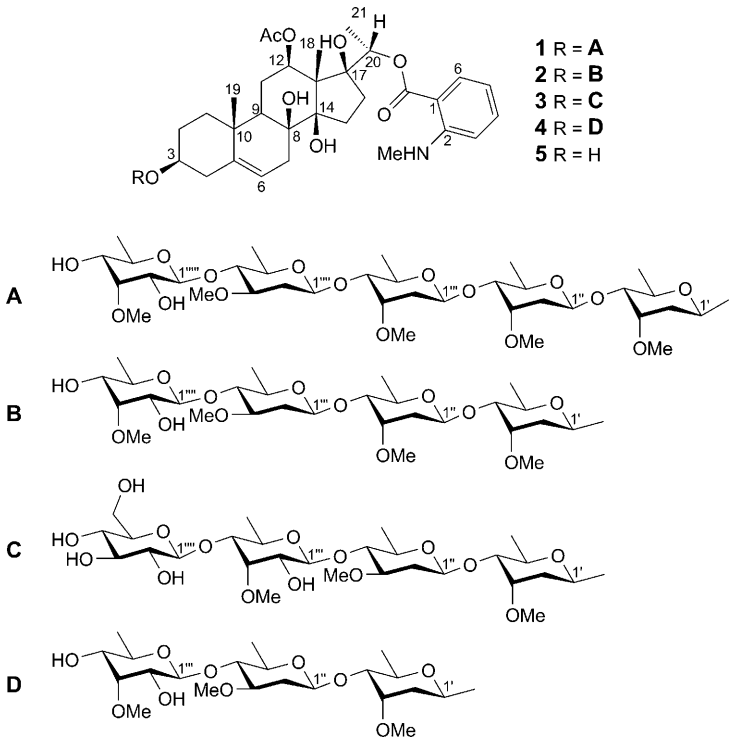
Three new pregnane glycosides, tinctorosides A–C (**1**–**3**, resp.), together with one known pregnane glycoside, stephanoside B (**4**), were isolated from the stems of *Marsdenia tinctoria* R. BR. (Asclepiadaceae). Their structures were elucidated by extensive spectral methods, especially 2D-NMR experiments (^{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 δ(C) 96.5, 100.5, 100.6, 101.9, and 102.0, correlating with the anomeric CH signals at δ(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 δ(C) and δ(H) values for the unidentified sugar as shown in *Table 2*, starting from the anomeric H- and C-signal at δ(H) 5.28 (*d*, J = 8.0) and δ(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; δ in ppm, J in Hz[a]).

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(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-β-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; δ in ppm, J in Hz[a]).

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(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*),	37.3	1.84–1.90 (*m*),	37.3	1.82–1.91 (*m*),	37.3
	2.25–2.36 (*m*)		2.24–2.30 (*m*)		2.25–2.32 (*m*)	
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*),	37.0	1.78–1.84 (*m*),	36.1	1.68–1.74 (*m*),	37.3
	2.25–2.36 (*m*)		2.35–2.41 (*m*)		2.44–2.50 (*m*)	
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‴)	1.76–1.83 (*m*),	36.1	1.70–1.75 (*m*),	37.7	3.83 (*dd*, *J* = 8.0, 3.0)	72.7
or H–C(2‴)	2.36–2.45 (*m*)		2.43–2.49 (*m*)			
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⁗)	1.67–1.78 (*m*),	37.7	3.81–3.87 (*m*)	72.7	3.99–4.02 (*m*)	75.5
or H–C(2⁗)	2.45–2.52 (*m*)					
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	1.61 (*d*, *J* = 5.0)	19.0	1.47 (*d*, *J* = 6.0)	18.9	4.36 (*dd*, *J* = 12.0, 5.0),	63.1
CH_2(6⁗)					4.55 (*dd*, *J* = 12.0, 3.0)	
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′) (δ(C) 37.3), C(2″) (δ(C) 37.0), and C(2‴) (δ(C) 36.1) of the three cymarose units as well as C(2⁗) (δ(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⁗) (δ(H) 5.28) and C(4⁗) (δ(C) 83.1), H–C(1‴) (δ(H) 4.67) and C(4‴) (δ(C) 83.2), H–C(1‴) (δ(H) 5.10) and C(4″) (δ(C) 83.4), H–C(1″) (δ(H) 5.10) and C(4′) (δ(C) 83.4), and between H–C(1′) (δ(H) 5.26) and C(3) (δ(C) 77.7) of the aglycone. This indicated the sequence of the sugar chain as 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.

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-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-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 δ(H) 5.26 (H–C(1′)) and the signal at δ(H) 3.78–3.86 (H–C(3)), and other key correlation peaks between the signals at δ(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*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-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; λ_{max} (log ε) 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); δ 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