Polyhydroxypregnane Glycosides from the Roots of *Cynanchum otophyllum*

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Six new polyhydroxypregnane glycosides, namely cynotophyllosides A – F (**1** – **6**), together with five known steroids, were isolated from the roots of *Cynanchum otophyllum*. Their structures were elucidated by extensive spectroscopic methods (especially 2D-NMR techniques) and acid-catalyzed hydrolysis. Furthermore, high field 1D- and 2D-NMR experiments were employed to elucidate the structure of **8** previously deduced only on the basis of LC/MS data.

Introduction. – Pregnane glycosides, one of the chemotaxonomic markers of the Asclepiadaceae family [1 – 7], have been demonstrated to possess a wide range of bioactivities with potential to be employed as cytotoxic [8], multidrug-resistance modulating [3], immunosuppressive [9 – 12], and antivirus agents [13]. *Cynanchum otophyllum* SCHNEID is a perennial weed widely distributed in Southwest China. Its root has been used in traditional Chinese medicine to treat epilepsy and rheumatism [14]. Previous chemical and pharmacological investigations of this plant showed the presence of a number of pregnane-type steroid glycosides with anti-epilepsy activity [15 – 17]. In the current project, six new pregnane glycosides, cynotophyllosides A – F (**1** – **6**), together with the five known pregnanes **7** – **11**, were isolated from the roots of *C. otophyllum* (*Fig.*). The structures of the new steroidal glycosides were elucidated by spectroscopic and chemical methods.

Results and Discussion. – The crude extract of *C. otophyllum* was suspended in H_2O and partitioned successively with cyclohexane, $CHCl_3$, and BuOH. The $CHCl_3$ extract was then subjected to extensive column chromatography to give two steroids and nine steroidal glycosides. The structures of the five known compounds, namely caudatin 3-β-D-digitoxopyranoside (**7**) [18], caudatin 3-*O*-α-L-diginopyranosyl-(1 $\rightarrow$ 4)-β-D-cymaropyranoside (**8**) [19], deacetylmetaplexigenin (=(3β,12β,14β,17α)-3,8,12,14,17-pentahydroxypregn-5-en-20-one; **9**) [20], sarcostin (=(3β,12β,14β,17α,20*S*)-pregn-5-ene-3,8,12,14,17,20-hexol; **10**) [11], and kidjoranin 3-β-D-digitoxopyranoside (**11**) [8], were corroborated by comparison of their spectroscopic data with those reported in the literature.

All the new compounds showed positive *Lieberman–Burchard* and *Keller–Kiliani* reactions, suggesting that they were steroidal glycosides containing 2-deoxysugar units. The absolute configurations (D or L) of the 2,6-dideoxysugars in the new compounds, obtained from the acidic hydrolyzates, were established by TLC comparison with authentic sugar samples and comparison of the optical rotation of the 2,6-dideoxy-

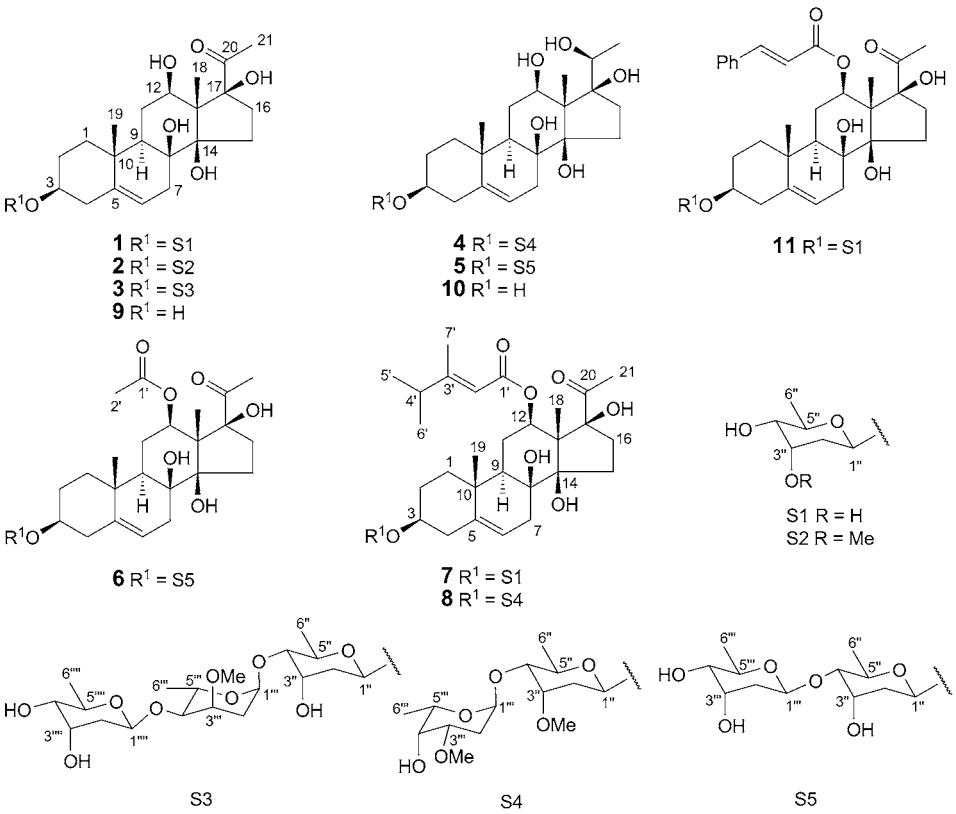

Figure. *Compounds* **1**–**11** *isolated from* Cynanchum otophyllum

sugars with those reported in the literature (see *Exper. Part*). A survey of closely related glycosides from the Asclepiadaceae family suggests that all the β-configured 2,6-dideoxysugars have the D-configuration, while the α-configured sugars are most likely the L-sugars [21]. In addition, C(2) of a 2-deoxysugar (cymarose (=2,6-dideoxy-3-*O*-methyl-*ribo*-hexose), digitoxose (=2,6-dideoxy-ribo-hexose), or diginose (=2,6-dideoxy-3-*O*-methyl-*lyxo*-hexose) that possesses an α-L-configuration usually appears in the ^{13}C-NMR spectrum at δ(C) *ca.* 32.0 or less, while that of a β-configured 2-deoxysugar normally resonates at a lower field with a δ(C) larger than 34.0 [22][23]. All the sugar units in the steroidal glycosides obtained in this study were thus tentatively established by ^{1}H- and ^{13}C-NMR analysis during the initial structure elucidation and were finally determined by optical-rotation analysis of the sugars obtained from their acidic hydrolyzates.

Comparison of the ^{1}H- and ^{13}C-NMR data of **1**–**3** (*Tables 1* and *2*) with those of deacetylmetaplexigenin (**9**) indicated that **1**–**3** shared the same aglycone identified as deacetylmetaplexigenin. Co-TLC of their acid hydrolyzates with deacetylmetaplexigenin (**9**) further supported this conclusion. Compound **1** had a molecular formula $C_{27}H_{42}O_9$, as indicated by the negative-ion-mode HR-ESI-MS (*m*/*z* 509.2744

($[M-H]^-$). Observation of an anomeric H-atom at δ(H) 4.96 (*dd*, $J=9.6$, 1.6 Hz, H–C(1″)), a CH at δ(H) 3.14 (*dd*, $J_{a,a}=9.4$ Hz, $J_{a,e}=3.0$ Hz), and a secondary Me at δ(H) 1.23 (*d*, $J=6.2$ Hz) in the ^{1}H-NMR spectrum indicated the presence of a β-digitoxopyranose unit in **1** [8], while the downfield shift of C(3) from δ(C) 71.6 in **9** to δ(C) 79.4 suggested that the β-digitoxopyranose unit was located at C(3). This was confirmed by the HMBC cross-peak H–C(1″)/C(3). The structure of **1** was therefore established as deacetylmetaplexigenin 3-β-D-digitoxopyranoside. Full assignments (*Tables 1* and *2*) of the ^{1}H- and ^{13}C-NMR data of **1** were achieved by a combination of ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY analyses.

Cynotophylloside B (**2**) was assigned the molecular formula $C_{28}H_{44}O_9$ on the basis of *m*/*z* 523.2910 ($[M-H]^-$) in the HR-ESI-MS, which was 14 amu higher than that of **1**. The NMR spectra of **2** bore a resemblance to those of **1**, except for the presence of an additional MeO signal (δ(H) 3.44; δ(C) 57.2) in **2** (*Tables 1* and *2*). The downfield shift of C(3″) from δ(C) 69.2 in **1** to 77.5 in **2** suggested the attachment of the MeO group to C(3″) and thus the presence of a β-cymaropyranoside unit was confirmed. HMBC analysis and co-TLC of the acid hydrolysate of **2** with **9** and cymarose further confirmed this conclusion. The structure of **2** was therefore established as deacetylmetaplexigenin 3-β-D-cymaropyranoside.

Cynotophylloside C (**3**) was given the molecular formula $C_{40}H_{64}O_{15}$ based on HR-ESI-MS. In the ^{13}C- and ^{1}H-NMR spectra (*Tables 1* and *2*), three anomeric CH groups (δ(H) 4.93 (*d*, $J=9.7$ Hz, H–C(1″), H–C(1⁗)) and 4.93 (*d*, $J=3.2$ Hz, H–C(1‴); δ(C) 94.5, 95.6, and 97.8) were easily identified, while signals assignable to the aglycone were similar to those of **9** with a glycosylation shift (from δ(C) 71.6 to 77.9) observed for C(3). Compound **3** was therefore a deacetylmetaplexigenin 3-triglycoside. Identification of the three sugar units as two β-digitoxopyranose units (δ(H) 4.93 (*d*, $J=9.7$ Hz, 2 H)) and an α-cymaropyranose unit (δ(H) 4.93 (*d*, $J=3.2$ Hz)) was accomplished by ^{1}H- and ^{13}C-NMR analysis and co-TLC of the acid hydrolyzate with the two standard sugars. Interpretation of the 2D-NMR data (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY) not only confirmed the presence of a three-sugar unit at C(3) but established the sugar sequence as 3-*O*-β-digitoxopyranosyl-(1 → 4)-*O*-α-cymaropyranosyl-(1 → 4)-β-digitoxopyranoside. In particular, the HMBCs H–C(1‴)/C(4″) and H–C(1⁗)/C(4‴) suggested the connection of the three sugars *via* two (1 → 4) linkages. The structure of **3** was thus determined as depicted.

The molecular formula of cynotophylloside D (**4**) was determined to be $C_{35}H_{58}O_{12}$ by the positive-ion-mode HR-ESI-MS. Inspection of the NMR data of **4** (*Tables 1* and *2*) showed the presence of two sugar units and a sarcostin aglycone [11]. The aglycone was confirmed to be **10** by co-TLC of the acid hydrolyzate, while the two sugar units were determined to be a cymaropyranose and a diginopyranose unit by NMR analysis (especially 2D-NMR). The two spin systems (C(1″) to C(6″) and C(1‴) to C(6‴)) of the sugar units were identified by analysis of the ^{1}H,^{1}H-COSY plot, the two MeO groups at δ(H) 3.42 and 3.39 were then fixed at C(3″) (δ(H) 77.0) and C(3‴) (δ(H) 74.3), respectively, on the basis of the HMBCs MeO–C(3″)/C(3″) and MeO–C(3‴)/C(3‴). The NOESY correlations H–C(4″)/Me(6″), H–C(4″)/H–C(3″), H–C(1″)/H–C(5″), H–C(4‴)/H–C(5‴), and H–C(4‴)/H–C(3‴), as well as the coupling constants of the anomeric H-atoms ($J=9.0$ Hz for H–C(1″) and $J=3.0$ for H–C(1‴)) further suggested that the two sugar units were a β-cymarose and α-diginose unit, respectively. The

Table 1. *^{1}H-NMR Data of Compounds* **1**–**5**

	1[a])	**2**[b])	**3**[b])	**4**[c])	**5**[a])
CH_2(1)	1.10–1.11, 1.85–1.87 (2*m*)	1.08–1.10, 1.79–1.81 (2*m*)	1.05–1.08, 1.85–1.87 (2*m*)	1.05–1.07, 1.85–1.18 (2*m*)	1.08–1.10, 1.84–1.87 (2*m*)
CH_2(2)	1.89–1.91, 1.62–1.64 (2*m*)	1.94–1.96, 1.66–1.69 (2*m*)	1.92–1.95, 1.67–1.69 (2*m*)	1.91–1.94, 1.66–1.68 (2*m*)	1.75–1.78, 1.63–1.65 (2*m*)
H–C(3)	3.51–3.53 (*m*)	3.55–1.57 (*m*)	3.55–3.57 (*m*)	3.66–3.69 (*m*)	3.50–3.54 (*m*)
CH_2(4)	2.20–2.22 (*m*), 2.38 (*dd*, *J* = 12.6, 3.7)	2.24–2.26 (*m*), 2.39 (*dd*, *J* = 12.7, 4.3)	2.26–2.28 (*m*), 2.37 (*dd*, *J* = 12.8, 4.1)	2.32–2.35, 2.36–2.38 (2*m*)	2.23–2.25 (*m*), 2.38 (*dd*, *J* = 12.7, 3.6)
H–C(6)	5.33 (*t*, *J* = 2.2)	5.37 (br. *s*)	5.35 (br. *s*)	5.37 (br. *s*)	5.33 (br. *s*)
CH_2(7)	2.09–2.11, 2.04–2.07 (2*m*)	2.30–2.33, 2.29–2.31 (2*m*)	2.16–18, 2.14–2.16 (2*m*)	2.17–2.19, 2.13–2.15 (2*m*)	2.14–2.16, 2.09–2.11 (2*m*)
H–C(9)	1.48 (*dd*, *J* = 13.1, 3.0)	1.46 (*dd*, *J* = 13.3, 3.3)	1.44 (*dd*, *J* = 13.1, 2.9)	1.44 (*d*, *J* = 11.3)	1.44 (*dd*, *J* = 13.2, 2.8)
C(10)	–	–	–	–	–
CH_2(11)	1.50–1.53, 1.49–1.51 (2*m*)	1.89–1.91, 1.58–61 (2*m*)	1.88–1.91, 1.58–1.61 (2*m*)	2.03–2.05, 1.73–1.75 (2*m*)	2.00–2.03, 1.61–1.63 (2*m*)
H–C(12)	3.49 (*dd*, *J* = 11.6, 3.9)	3.67–3.69 (*m*)	3.68–3.70 (*m*)	3.68–3.71 (*m*)	3.58 (*dd*, *J* = 11.6, 4.0)
CH_2(15)	1.80–1.84, 1.92–1.94 (2*m*)	1.94–1.96, 1.92–1.95 (2*m*)	1.94–1.97, 1.91–1.94 (2*m*)	1.84–1.86, 1.70–1.73 (2*m*)	1.85–1.87, 1.71–1.73 (2*m*)
CH_2(16)	2.87–2.89, 1.69–1.71 (2*m*)	2.73–2.75, 1.92–1.95 (2*m*)	2.75–2.77, 1.90–1.93 (2*m*)	1.84–1.87, 1.70–1.72 (2*m*)	1.81–1.83, 1.76–1.79 (2*m*)
Me(18)	1.34 (*s*)	1.26 (*s*)	1.27 (*s*)	1.35 (*s*)	1.33 (*s*)
Me(19)	1.17 (*s*)	1.16 (*s*)	1.15 (*s*)	1.19 (*s*)	1.18 (*s*)
H–C(20)	–	–	–	4.06 (*q*, *J* = 6.5)	3.96 (*q*, *J* = 6.5)
Me(21)	2.23 (*s*)	2.34 (*s*)	2.34 (*s*)	1.19 (*d*, *J* = 6.5)	1.16 (*d*, *J* = 6.5)
	D-Digit[d])	D-Cym[e])	D-Digit	D-Cym	D-Digit
H–C(1″)	4.96 (*dd*, *J* = 9.6, 1.6)	4.79 (*dd*, *J* = 9.5, 1.5)	4.93 (*d*, *J* = 9.7)	4.84 (*d*, *J* = 9.0)	4.95 (*dd*, *J* = 11.1, 1.4)
CH_2(2″)	1.93–1.96, 1.63–1.65 (2*m*)	2.27–2.29, 1.68–1.71 (2*m*)	2.05–2.07, 1.67–1.71 (2*m*)	2.18–2.21, 1.59–1.62 (2*m*)	1.94–1.96, 1.63–1.66 (2*m*)
H–C(3″)	4.01 (*d*, *J* = 3.0)	3.63–3.65 (*m*)	4.03–4.04 (*m*)	3.71–3.74 (*m*)	4.23 (*d*, *J* = 3.0)
H–C(4″)	3.14 (*dd*, *J* = 9.4, 3.0)	3.22 (*dt*, *J* = 9.8, 3.3)	3.23 (*dd*, *J* = 9.3, 2.8)	3.26 (*dd*, *J* = 9.5, 3.0)	3.22 (*dd*, *J* = 9.4, 2.8)
H–C(5″)	3.75 (*dq*, *J* = 9.6, 6.3)	3.57–3.59 (*m*)	3.78–3.81 (*m*)	3.83 (*dq*, *J* = 6.5, 3.0)	3.80–3.82 (*m*)
Me(6″)	1.23 (*d*, *J* = 6.2)	1.28 (*d*, *J* = 6.2)	1.22 (*d*, *J* = 5.4)	1.22 (*d*, *J* = 6.5)	1.20 (*d*, *J* = 6.3)
MeO–C(3″)	–	3.44 (*s*)	–	3.42 (*s*)	–

Table 1 (cont.)

	1[a])	**2**[b])	**3**[b])	**4**[c])	**5**[a])
			L-Cym	L-Dig[f])	D-Digit
H–C(1‴)			4.93 (*d*, *J* = 3.2)	4.99 (*d*, *J* = 3.0)	4.93 (*dd*, *J* = 9.8, 1.5)
CH_2(2‴)			2.22 – 2.25, 1.73 – 1.75 (2*m*)	1.93 – 1.95, 1.87 – 1.89 (2*m*)	2.00 – 2.03, 1.68 – 1.71 (2*m*)
H–C(3‴)			3.77 – 3.80 (*m*)	3.67 – 4.70 (*m*)	4.01 (*d*, *J* = 3.0)
H–C(4‴)			3.55 – 3.57 (*m*)	3.80 (br. *s*)	3.15 (*dd*, *J* = 9.6, 3.0)
H–C(5‴)			4.07 – 4.10 (*m*)	4.01 (*d*, *J* = 6.5)	3.75 – 3.77 (*m*)
Me(6‴)			1.24 (*d*, *J* = 5.8)	1.31 (*d*, *J* = 6.5)	1.24 (*d*, *J* = 6.3)
MeO–C(3‴)			3.41 (*s*)	3.39 (*s*)	–
			D-Digit		
H–C(1⁗)			4.93 (*d*, *J* = 9.7)		
CH_2(2⁗)			2.09 – 2.11, 1.70 – 1.73 (2*m*)		
H–C(3⁗)			4.05 – 4.08 (*m*)		
H–C(4⁗)			3.31 – 3.33 (*m*)		
H–C(5⁗)			3.64 – 3.67 (*m*)		
Me(6⁗)			1.28 (*d*, *J* = 6.2)		

[a]) Measured in CD_3OD at 400 MHz. [b]) Measured in $CDCl_3$ at 400 MHz. [c]) Measured in $CDCl_3$ at 500 MHz. [d]) Digit = digitoxopyranosyl. [e]) Cym = cymaropyranosyl. [f]) Dig = diginopyranosyl.

Table 2. *^{13}C-NMR Data of Compounds* **1**–**5**

	1[a])	**2**[b])	**3**[b])	**4**[c])	**5**[a])
C(1)	40.1	38.8	38.8	38.9	40.2
C(2)	30.2	28.9	28.9	29.0	30.4
C(3)	79.4	77.7	77.9	77.9	79.7
C(4)	39.8	38.7	38.7	38.7	40.1
C(5)	140.3	140.4	140.6	139.6	140.5
C(6)	119.8	117.9	117.7	118.5	120.2
C(7)	35.3	34.2	34.2	34.6	35.7
C(8)	74.9	74.2	74.3	73.8	75.1
C(9)	45.5	44.1	44.1	43.7	45.4
C(10)	38.0	37.0	37.1	37.0	38.3
C(11)	29.2	28.0	28.0	28.5	29.4
C(12)	70.0	69.4	69.3	70.8	71.8
C(13)	61.0	61.3	60.9	57.8	59.3
C(14)	89.8	87.8	87.8	87.9	89.4
C(15)	35.3	33.3	33.2	33.5	35.1
C(16)	34.4	32.4	32.5	32.5	34.7
C(17)	93.0	91.8	91.9	88.0	89.9
C(18)	8.9	7.7	7.7	10.1	10.9
C(19)	18.7	18.6	18.7	18.3	18.9
C(20)	212.3	213.6	214.0	72.4	73.9
C(21)	27.8	28.2	28.3	16.9	17.3
	D-Digit	D-Cym	D-Digit	D-Cym	D-Digit
C(1″)	97.2	95.6	95.6	95.6	97.3
C(2″)	39.8	34.0	37.0	34.2	39.1
C(3″)	69.2	77.5	67.6	77.0	68.6
C(4″)	74.3	70.7	80.1	81.7	84.0
C(5″)	70.8	72.5	68.3	68.8	69.8
C(6″)	18.6	18.2	18.1	18.3	18.8
*Me*O–C(3″)	–	57.2	–	56.9	–
			L-Cym	L-Dig	D-Digit
C(1‴)			97.8	100.4	100.9
C(2‴)			31.7	29.9	39.7
C(3‴)			72.7	74.3	69.4
C(4‴)			76.8	67.6	74.5
C(5‴)			64.4	66.0	71.1
C(6‴)			18.1	17.0	18.9
*Me*O–C(3‴)			57.0	55.5	
			D-Digit		
C(1⁗)			94.5		
C(2⁗)			37.8		
C(3⁗)			68.1		
C(4⁗)			73.0		
C(5⁗)			69.4		
C(6⁗)			17.9		

[a]) Measured in CD_3OD at 100 MHz. [b]) Measured in $CDCl_3$ at 100 MHz. [c]) Measured in $CDCl_3$ at 125 MHz.

presence of a diginose unit in **4** was also deduced from the δ(C) of the MeO group at C(3‴), which appeared at δ(C) *ca.* 55.5 as in similar diginose-containing pregnane glycosides [23], whereas it resonated at a lower field with a chemical shift larger than δ(C) 57.0 in cymarose- and oleandrose-containing glycosides [24][25]. This can be well explained by the γ-gauche effect: in the diginose unit, MeO–C(3‴) is in the same face with OH–C(4‴) and Me–C(5‴); as a result, the MeO is shielded more than that in the other two sugar units. Linkage of the two sugar units and the aglycone was solved by HMBC analysis, where the correlations H–C(1″)/C(3) and H–C(1‴)/C(4″) fixed the two-sugar moiety at C(3) and allowed the establishment of the structure of **4** as sarcostin 3-*O*-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside.

Cynotophylloside E (**5**) had the molecular formula $C_{33}H_{54}O_{12}$ as revealed by the HR-ESI-MS. The aglycone was also deduced to be sarcostin by NMR analysis and chemical hydrolysis. The NMR data (*Tables 1* and *2*) showed that it contained two β-configured sugar units (δ(H) 4.95 (*dd*, J = 11.1, 1.4 Hz) and 4.93 (*dd*, J = 9.8, 1.5); δ(C) 97.3 and 100.9). However, acid hydrolysis of **5** afforded only digitoxose, suggesting the presence of two digitoxose units in **5**. ^{13}C-NMR Data comparison between **5** and **1** showed a glycosylation shift for C(4″) in **5**, while the HMBC cross-peaks H–C(1″)/C(3) and H–C(1‴)/C(4″) allowed the assignment of **5** as sarcostin 3-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Cynotophylloside F (**6**) exhibited the molecular formula $C_{35}H_{54}O_{13}$ based on the negative-ion-mode HR-ESI-MS (*m*/*z* 705.3456 ($[M+Na]^+$, $C_{35}H_{54}NaO_{13}^+$). The ^{1}H- and ^{13}C-NMR spectra of **6** (*Table 3*) showed similarity with those of **1**, except that signals for an additional sugar unit and an Ac group were observed in the NMR spectra of **6**. The NMR data assignable to the sugar units of **6** were similar to those of **5**, suggesting that **6** had the same sugar sequence as **5**. The remaining NMR data were then assigned to metaplexigenin with the AcO group located at C(12) [9]. The conclusion was then confirmed by 2D-NMR data. Thus, compound **6** was established as metaplexigenin 3-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-digitoxopyranoside.

Compound **8** had the molecular formula $C_{42}H_{66}O_{13}$, as evidenced by its positive-ion-mode HR-ESI-MS (*m*/*z* 801.4408 ($[M+Na]^+$). Its ^{1}H- and ^{13}C-NMR spectra (*Table 3*) were almost superimposable on those of **7**, except for the presence of signals for an additional sugar unit [20]. Compound **8** was therefore considered to be a caudatin 3-dioside. Comparison of the ^{1}H- and ^{13}C-NMR data of **8** with those of **4** suggested that both compounds shared the same sugar sequence. Compound **8** was thus elucidated as caudatin 3-*O*-α-L-diginopyranosyl-(1 → 4)-β-D-cymaropyranoside. The structure of **8** was first reported as a metabolite of wilfoside C3N *in vivo*, and determined only by the LC/MS/MS method [19][26]. Now the complete assignment of the NMR signals of **8** is established by 1D- and 2D- NMR experiments (*Table 3*). This is the first time that compound **8** is reported as a natural product.

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Table 3. *^{1}H- and ^{13}C-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **6** *and* **8**. δ in ppm, J in Hz.

	6		**8**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.10–1.14, 1.84–1.87 (2*m*)	38.7	1.08–1.10, 1.89–1.91 (2*m*)	38.8
CH_2(2)	1.92–1.94, 1.66–1.69 (2*m*)	28.8	1.91–1.93, 1.64–1.66 (2*m*)	28.9
H–C(3)	3.57–3. 60 (*m*)	77.7	3.55–3.58 (*m*)	77.9
CH_2(4)	2.40–2.42, 2.52–2.54 (2*m*)	38.7	2.39–2.41, 2.31–2.35 (2*m*)	38.8
C(5)	–	140.9	–	140.6
H–C(6)	5.35 (br. *s*)	117.4	5.37 (*t*, *J* = 2.5)	117.7
CH_2(7)	2.17–2.20 (*m*)	34.0	2.17–2.19 (*m*)	34.2
C(8)	–	74.4	–	74.3
H–C(9)	1.52 (*dd*, *J* = 12.5, 4.0)	43.6	1.53–1.56 (*m*)	43.7
C(10)	–	37.0	–	37.1
CH_2(11)	1.76–1.78 (*m*)	24.2	1.89–1.91 (*m*)	24.2
H–C(12)	4.51 (*t*, *J* = 5.0)	72.5	4.56 (*t*, *J* = 7.8)	71.6
C(13)	–	57.6	–	57.9
C(14)	–	88.2	–	88.0
CH_2(15)	1.90–1.92, 1.93–1.96 (2*m*)	32.5	1.93–1.96, 1.92–1.94 (2*m*)	33.1
CH_2(16)	2.84–2.88, 1.81–1.83 (2*m*)	32.2	2.86–2.88, 1.84–1.86 (2*m*)	31.9
C(17)	–	91.7	–	91.5
Me–C(18)	1.43 (*s*)	9.2	1.41 (*s*)	9.4
Me–C(19)	1.12 (*s*)	18.6	1.13 (*s*)	18.6
C(20)	–	209.7	–	208.8
Me–C(21)	2.25 (*s*)	27.3	2.17 (*s*)	27.2
Substituent at C(12)				
C(1′)	–	170.0	–	165.9
Me(2′) or H–C(2′)	1.95 (*s*, AcO)	20.7	5.52 (*s*)	113.0
C(3′)			–	166.8
H–C(4′)			2.34–2.36 (*m*)	38.2
Me(5′)			1.06 (*d*, *J* = 6.9)	20.8
Me(6′)			1.06 (*d*, *J* = 6.9)	20.9
Me(7′)			2.13 (*s*)	16.5
	D-Digit		D-Cym	
H–C(1″)	4.92 (*d*, *J* = 8.5)	95.8	4.84 (*dd*, *J* = 9.3, 1.4)	95.7
CH_2(2″)	2.08–2.11, 1.71–1.73 (2*m*)	37.1	2.19–2.21, 1.58–1.61 (2*m*)	34.2
H–C(3″)	4.25 (br. *s*)	66.5	3.71–3.74 (*m*)	77.2
H–C(4″)	3.22 (*dd*, *J* = 9.5, 3.0)	82.6	3.27 (*dd*, *J* = 9.5, 2.9)	81.8
H–C(5″)	3.77–3.80 (*m*)	68.1	3.83–3.85 (*m*)	68.9
Me(6″)	1.23 (*d*, *J* = 6.0)	18.2	1.24 (*d*, *J* = 6.3)	18.3
*Me*O–C(3″)	–	–	3.42 (*s*)	56.9
	D-Digit		L-Dig	
H–C(1‴)	4.90 (*d*, *J* = 8.5)	98.3	5.00 (*d*, *J* = 3.3)	100.5
CH_2(2‴)	2.11–2.14, 1.73–1.75 (2*m*)	37.9	1.94–1.96, 1.85–1.89 (2*m*)	29.9
H–C(3‴)	4.10–4.12 (*m*)	68.1	3.67–3.70 (*m*)	74.3
H–C(4‴)	3.27–3.29 (*m*)	72.7	3.80 (*d*, *J* = 2.3)	67.6
H–C(5‴)	3.76–3.78 (*m*)	69.5	4.02 (*q*, *J* = 6.4)	66.0
Me(6‴)	1.28 (*d*, *J* = 7.5)	18.2	1.31 (*d*, *J* = 6.6)	17.0
*Me*O–C(3‴)	–	–	3.39 (*s*)	55.5

Experimental Part

General. All solvents were of anal. grade (*Hangzhou Gaojing Fine Chemical Plant*). TLC: precoated SiO_2 *G* F_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*). Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *RP-*C_{18} (SiO_2, 50 μm; *Merck*), *MCI* gel *CHP 20P* (75–150 μm; *Mitsubishi Chemical Industries Ltd.*), and *Toyopearl HW-40C* gel (50–100 μm; *Tosoh*); detection by UV light (254 and/or 366 nm) and 5% H_2SO_4/EtOH. Optical rotations: *AutoPolIV* polarimeter. IR Spectra: *Nicolet-Avatar-370* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-400* and *Bruker-AM-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Agilent-1100/LCQ-Advantage* mass spectrometers; in *m*/*z*.

Plant Material. The roots of *C. otophyllum* (10 kg) were collected from Guangxi Province of P. R. China in August 2009, and authenticated by Prof. *Shi-Man Huang*, Department of Biology, Hainan University of P. R. China. A voucher specimen was deposited with the Zhejiang University of Technology (accession No. ZJUT-CO-0908).

Extraction and Isolation. The dried plant material of *C. otophyllum* was ground and then extracted three times with 95% EtOH (50 l, 5 d each) at r.t. The solvent was evaporated to give a crude extract (688 g). The EtOH extract was suspended in H_2O (5.0 l) and partitioned successively with cyclohexane (15 l), $CHCl_3$ (15 l), and BuOH (15 l). The $CHCl_3$ fraction (89 g) was then subjected to CC (*MCI* gel, MeOH/H_2O 45:55 → 80:20): *Fractions A–D. Fr. B* (3.2 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 40:1 → 30:1): **9** (200 mg), **10** (67 mg), and **1** (45 mg). *Fr. C* (16.2 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 40:1 → 20:1), then to CC (*RP-*C_{18}, MeOH/H_2O 45:55 → 70:30): **11** (48 mg), **2** (66 mg), **7** (26 mg), and **8** (71 mg). *Fr. D* (3.8 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 40:1 → 10:1): *Frs. D1* and *D2*. *Fr. D1* (302 mg) was subjected to CC (*Toyopearl HW-40C*, MeOH): **3** (45 mg) and **5** (45 mg). *Fr. D2* (1.2 g) was subjected to CC (*RP-*C_{18}, MeOH/H_2O 50:50 → 65:35): **4** (200 mg) and **6** (35 mg).

Acidic Hydrolysis. To a soln. of each compound (1.0 mg) in MeOH (1 ml) was added 0.05N H_2SO_4 (1 ml). The soln. was kept at 60° for 2 h and then diluted with H_2O (3 ml). The soln. was neutralized with sat. aq. $Ba(OH)_2$ soln. The precipitation was filtered off, the filtrate evaporated, and the residue (the mixture of aglycone and sugars) subjected to CC (*Toyopearl HW-40C* gel, MeOH) to give fractions of sugars and aglycone. The aglycone fractions of **1**–**3** and of **4** and **5** showed the same TLC behavior as deacetylmetaplexigenin (**9**; R_f ($CHCl_3$/MeOH 10:1) 0.51) and sarcostin (**10**; R_f ($CHCl_3$/MeOH 10:1) 0.38), resp. Constituents of each sugar fraction were identified by co-TLC with authentic sugars: cymarose (R_f ($CHCl_3$/MeOH 8:1) 0.50), diginose (R_f ($CHCl_3$/MeOH 8:1) 0.46), and digitoxose (R_f ($CHCl_3$/MeOH 8:1) 0.40).

Determination of the Absolute Configuration of the Sugars. One of the glycosides **1**, **5**, or **6** (15 mg) was hydrolyzed by the above method to afford digitoxose, whose positive optical rotation $[\alpha]_D^{20} = +43.0$ ($c = 0.3$, H_2O) was indicative of a D-configuration ([26]: $[\alpha]_D^{20} = +48.4$). By the same method, the cymarose obtained from **2** was determined to be the D-isomer. Similarly, the cymarose and diginose obtained from **4** were determined to be the D- and L-form, resp. (D-cymarose: $[\alpha]_D^{20} = +50.2$ ($c = 0.22$, H_2O) [26]: $[\alpha]_D^{20} = +55.7$); L-diginose: $[\alpha]_D^{20} = -53.4$ ($c = 0.25$, H_2O) [24]: $[\alpha]_D^{20} = -60.6$). By the same method, the cymarose and digitoxose obtained from **3** was determined to be the L- and L-isomer, resp. (L-cymarose: $[\alpha]_D^{20} = -47.3$ ($c = 0.12$, H_2O)).

Cynotophylloside A (=*(3β,12β,14β,17α)-3-[(2,6-Dideoxy-β-*D*-ribo-hexopyranosyl)oxy]-8,12,14,17-tetrahydroxypregn-5-en-20-one*; **1**): Colorless amorphous powder. $[\alpha]_D^{20} = +23.6$ ($c = 0.25$, MeOH). IR (KBr): 3433, 2937, 1699, 1382, 1350, 1072, 1004, 910. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 509 ($[M-H]^-$). HR-ESI-MS (neg.): 509.2744 ($[M-H]^-$, $C_{27}H_{41}O_9^-$; calc.509.2751).

Cynotophylloside B (=*(3β,12β,14β,17α)-3-[(2,6-Dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl)oxy]-8,12,14,17-tetrahydroxypregn-5-en-20-one*; **2**): Colorless amorphous powder. $[\alpha]_D^{20} = +31.3$ ($c = 5.1$, $CHCl_3$). IR (KBr): 3447, 2935, 1700, 1375, 1352, 1083, 1000, 911. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 523 ($[M-H]^-$). HR-ESI-MS (neg.): 523.2910 ($[M-H]^-$, $C_{29}H_{43}O_9^-$; calc. 523.2907).

Cynotophylloside C (=*(3β,12β,14β,17α)-3-{[*O*-2,6-Dideoxy-β-*D*-ribo-hexopyranosyl-(1 → 4)-O-2,6-dideoxy-3,O-methyl-α-*L*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-β-*D*-ribo-hexopyranosyl]oxy}-8,12,14,17-tetrahydroxypregn-5-en-20-one* (**3**): Colorless amorphous powder. $[\alpha]_D^{20} = +41.8$ ($c = 1.5$, $CHCl_3$). IR (KBr): 3446, 2932, 1702, 1377, 1077, 1003, 974. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 807

($[M+Na]^+$), 1591 ($[2M+Na]^+$). ESI-MS (neg.): 783 ($[M-H]^-$). HR-ESI-MS (pos.): 807.4138 ($[M+Na]^+$, $C_{40}H_{64}NaO_{15}^+$; calc. 807.4143).

Cynotophylloside D (=*(3β,12β,14β,17α,20*S*)-3-{[2,6-Dideoxy-4*-O-*(2,6-dideoxy-3*-O-*methyl-α*-L-lyxo-*hexopyranosyl)-3*-O-*methyl-β*-D-ribo-*hexopyranosyl]oxy}pregn-5-ene-8,12,14,17,20-pentol*; **4**): Colorless, amorphous powder. $[\alpha]_D^{20} = -2.3$ ($c = 0.26$, $CHCl_3$). IR (KBr): 3438, 2970, 2934, 1448, 1371, 1096, 1073, 998, 755. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 669 ($[M-H]^-$). HR-ESI-MS (neg.): 669.3848 ($[M-H]^-$, $C_{35}H_{57}O_{12}^-$; calc. 669.3850).

Cynotophylloside E (=*(3β,12β,14β,17α,20*S*)-3-{[2,6-Dideoxy-4*-O-*(2,6-dideoxy-β*-D-ribo-*hexopyranosyl)-β*-D-ribo-*hexopyranosyl]oxy}pregn-5-ene-8,12,14,17,20-pentol*; **5**): Colorless, amorphous powder. $[\alpha]_D^{20} = +33.3$ ($c = 0.32$, MeOH). IR (KBr): 3369, 2979, 2904, 1639, 1407, 1373, 1074, 1001, 720. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 641 ($[M-H]^-$), 1283 ($[2M-H]^-$). HR-ESI-MS (neg.): 641.3530 ($[M-H]^-$, $C_{33}H_{53}O_{12}$; calc. 641.3537).

Cynotophylloside F (=*(3β,12β,14β,17α)-12-(Acetyloxy)-3-{[2,6-dideoxy-4*-O-*(2,6-dideoxy-β*-D-ribo-*hexopyranosyl)-β*-D-ribo-*hexopyranosyl]oxy}-8,14,17-trihydroxypregn-5-en-20-one*; **6**). Colorless, amorphous powder. $[\alpha]_D^{20} = +3.0$ ($c = 2.5$, $CHCl_3$). IR (KBr): 3459, 2970, 2933, 1712, 1372, 1237, 1067, 1011, 988, 866, 753. ^{1}H- and ^{13}C-NMR: *Table 3*. ESI-MS (pos.): 705 ($[M+Na]^+$); 1387 ($[2M+Na]^+$). ESI-MS (neg.): 717 ($[M+Cl]^-$); 1363 ($[2M-H]^-$). HR-ESI-MS (pos.): 705.3456 ($[M+Na]^+$, $C_{35}H_{54}NaO_{13}^+$; calc. 705.3462).

Caudatin 3-O-*α*-L-*Diginopyranosyl-(1→4)-β*-D-*cymaropyranoside* (=*(3β,12β,14β,17α)-3-{[2,6-Dideoxy-4*-O-*(2,6-dideoxy-3-O-methyl-α*-L-lyxo-*hexopyranosyl)-3*-O-*methyl-β*-D-ribo-*hexopyranosyl]oxy}-12-{[(2E)-3,4-dimethyl-1-oxopent-2-en-1-yl]oxy}-8,14,17-trihydroxypregn-5-en-20-one*; **8**): Colorless, amorphous powder. $[\alpha]_D^{20} = -6.3$ ($c = 6.5$, $CHCl_3$). UV (MeOH): 225 (3.78). IR (KBr): 3467, 2934, 2872, 1711, 1643, 1458, 1389, 1223, 1166, 1095, 995, 938, 863. ^{1}H- and ^{13}C-NMR see *Table 3*. ESI-MS (pos.): 801 ($[M+Na]^+$). HR-ESI-MS (pos.): 801.4408 ($[M+Na]^+$, $C_{42}H_{66}NaO_{13}^+$; calc. 801.4401).

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