Macrocyclic Glycosides from *Clematis hexapetala*

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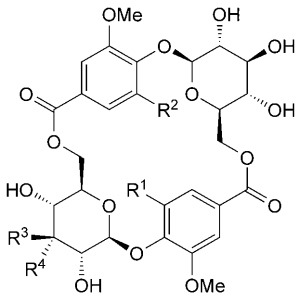
Two new macrocyclic glycosides, clemahexapetoside A (**1**) and B (**2**), along with the known compound clemochinenoside A (**3**), were isolated from the roots and rhizomes of *Clematis hexapetala*. Their structures were elucidated on the basis of chemical, physicochemical, and spectroscopic evidence.

Introduction. – The genus *Clematis* (Ranunculaceae) is a large genus within Dicotyledoneae, with *ca*. 300 species being known worldwide. The roots and rhizomes of *C. chinensis* OSBECK, *C. mandshurica* RUPR., and *C. hexapetala* PALL. are all recorded in the Chinese Pharmacopoeia. They are called '*weilingxian*' and are commonly being used as an anti-inflammatory, antitumor, and analgesic agents. Previous investigations were mainly directed toward *C. chinensis*, from the roots and rhizomes of which more than 40 triterpene saponins and lignans have been isolated [1–9]. In contrast, there are only few phytochemical investigations concerning *C. mandshurica* and *C. hexapetala*. We have already isolated and elucidated many phenolic glycosides from the roots and rhizomes of *C. mandshurica* [10].

Investigation on the chemical constituents of the roots and rhizomes of *C. hexapetala* now resulted in the isolation and characterization of two new macrocyclic glycosides, compounds **1** and **2**, together with the known constituent clemochinenoside A (**3**) [11]. Herein, we report their isolation and structure elucidation.



	R^1	R^2	R^3	R^4
1	MeO	H	H	OH
2	H	H	H	OH
3	MeO	MeO	OH	H

Results and Discussion. – Compound **1** was obtained as a colorless, amorphous powder, with the molecular formula $C_{29}H_{34}O_{17}$, as deduced from the $[M+Na]^+$ peak

at *m/z* 677.1695 by HR-FAB-MS. Acid hydrolysis afforded vanillic acid (=4-hydroxy-3-methoxybenzoic acid), syringic acid (=4-hydroxy-3,5-dimethoxybenzoic acid), D-allose (D-All), and D-glucose (D-Glc). The IR spectrum of **1** showed absorptions of OH groups (3400–3500), an ester C=O group (1722), and an aromatic ring (1608, 1510 cm^{-1}). The UV spectrum of **1** showed the typical absorption maxima of an aromatic ring at 218, 253, and 291 nm.

The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*)[1]) showed the signals of vanilloyl moiety [δ(H) 7.34 (*d*, *J*=2.0 Hz, 1 H), 6.80 (*dd*, *J*=2.0, 8.5 Hz, 1 H), 6.97 (*d*, *J*=8.5 Hz, 1 H), 3.77 (*s*, 3 H); δ(C) 122.4 (C(1a)), 111.9 (C(2a)), 148.4 (C(3a)), 150.1 (C(4a)), 114.3 (C(5a)), 122.4 (C(6a)), 165.1 (C(7a)), 55.5 (3a-MeO)] and a syringoyl moiety [δ(H) 7.07 (*d*, *J*=2.0 Hz, 1 H), 7.49 (*d*, *J*=2.0 Hz, 1 H), 3.96 (*s*, 3 H), 3.60 (*s*, 3 H); δ(C) 124.6 (C(1b)), 107.0 (C(2b)), 152.0 (C(3b)), 137.6 (C(4b)), 153.1 (C(5b)), 106.7 (C(6b)), 164.9 (C(7b)), 56.5 (3b-MeO), 55.9 (5b-MeO)]. Moreover, two hexapyranosyl units were observed in the ^{1}H- and ^{13}C-NMR spectra of **1**, the anomeric H-atoms resonating at δ(H) 5.31 (*d*, *J*=7.5 Hz, 1 H) and 5.13 (*d*, *J*=7.5 Hz, 1 H), respectively. The large coupling constant of the anomeric H-atoms (*J*=7.5 Hz) suggested that both sugar units were β-configured.

Analysis of the ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMQC spectra of **1** allowed us to unambiguously assign all H- and C-atoms of the two hexapyranosyl units (*Table*). Comparison of the ^{13}C-NMR chemical shifts with those of reference methyl glycosides [12], taking into account the known effects of *O*-glycosylation and the results of acid hydrolysis, revealed that **1** contained a D-allopyranosyl and a D-glucopyranosyl unit. In the HMBC spectrum of **1** (*Figure*), the following long-range correlations were observed: H–C(1′b)/C(4b), H–C(1′a)/C(4a), H–C(6′a)/C(7b), and H–C(6′b)/C(7a). In the NOESY spectrum, 3a-MeO (δ(H) 3.77 (*s*)) showed a cross-peak with H–C(2a) (δ(H) 7.34 (*d*, *J*=2.0 Hz)), and H–C(1′a) (δ(H) 5.31 (*d*, *J*=7.5 Hz)) showed a correlation with H–C(5a) (δ(H) 6.97 (*d*, *J*=8.5 Hz)). These data indicated that **1** was a macrocyclic glycoside, and enabled us to establish the orientation of the vanilloyl moiety.

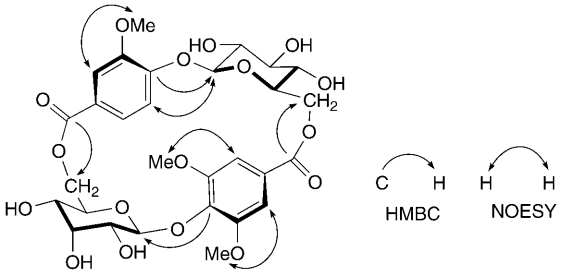


Figure. *Key HMBC and NOESY correlations for* **1**

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part*.

The ^{13}C-NMR spectroscopic data of the syringoyl moiety in **1** did not show four aromatic resonances, as expected for a symmetrically substituted benzene ring, but six resonance lines. The same phenomenon was observed in the ^{1}H-NMR spectrum of **1**, where the syringoyl moiety showed two *meta*-coupled H-atoms at δ(H) 7.07 (*d*, $J = 2.0$ Hz, 1 H) and 7.49 (*d*, $J = 2.0$ Hz, 1 H). These results point to a restricted mobility of the aromatic rings after cyclization, preventing their free rotation. Thus, the structure of **1** was elucidated as '4-({6-*O*-[(4-*O*-β-D-allopyranosyl)syringoyl]-β-D-glucopyranosyl}oxy)vanillic acid inner ester', and named *clemahexapetoside A*[1]).

Compound **2** was obtained as a colorless, amorphous powder, with the molecular formula $C_{28}H_{32}O_{16}$, as deduced from the $[M+H]^+$ peak at *m/z* 625.1749 by HR-FAB-MS and confirmed by ^{13}C-NMR spectroscopy. Acid hydrolysis afforded vanillic acid, D-allose and D-glucose. Comparison of the NMR data of **2** with those of **1** revealed that both compounds shared the same skeleton. The only difference was one MeO group less in **2** compared to **1**. The orientation of the vanillic acid moieties in **2** was established through the following NOESY correlations: 3a-MeO/H–C(2a), H–C(1′a)/H–C(5a), 3b-MeO/H–C(2b), and H–C(1′b)/H–C(5b). Detailed analyses of the NMR spectra of **2** resulted in the unambiguous assignments of all H- and C-atom resonances (*Table*). Thus, the structure of **2** was identified as '4-({6-*O*-[(4-*O*-β-D-allopyranosyl)vanilloyl]-β-D-glucopyranosyl}oxy)vanillic acid inner ester'[1]), and named *clemahexapetoside B*. Note that **2** differs from berchemolide [13] only in the orientation of a vanillic acid residue and the sugar moieties.

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Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Marine Chemical Industry*). Prep. HPLC: *Waters-Delta-600* pump, *ODS* column (250×10 mm, 5 μm; *Alltech*), with *Waters-2996* photodiode-array detector (280 nm); flow rate 2.5 ml/min. GC: *Agilent-6890N* gas chromatograph, with a *HP-5* capillary column (28 m×0.32 mm) and an FID detector operated at 260° (column temp. 180°), 1.0 ml/min N_2 as carrier gas. Melting points (m.p.): *X-4* micro-melting-point apparatus; uncorrected. UV Spectra: *TU-1901* spectrometer; λ_{max} in nm. IR spectra: *AVATAR-360* spectrometer. Optical rotations: *Perkin-Elmer-243B* digital polarimeter. NMR Spectra: *Varian-Inova-500* spectrometer; at 500 (^{1}H) or 125 MHz (^{13}C) in (D_6)DMSO at r.t.; δ in ppm rel. to Me_4Si, *J* in Hz. HR-FAB-MS (pos.): *Autospec-UltimaETOF* spectrometer; in *m/z*.

Plant Material. The roots and rhizomes of *C. hexapetala* were collected in Inner Mongolia of China. The plant was identified by Prof. *Peng-Fei Tu*. A voucher specimen (CH200409011) was deposited at the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The dried roots and rhizomes (9 kg) of *C. hexapetala* were extracted with 95% EtOH (3×60 l) for 2, 1, and 0.5 h, resp. After removal of the solvent under reduced pressure at 60°, the residue (1 kg) was suspended in H_2O (5 l) and defatted with petroleum ether (8 l). The aq. layer was further extracted with AcOEt (10 l) to afford an AcOEt-soluble extract (280 g). A portion of this extract (100 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 50:1, 20:1, 5:1): fractions *Fr. 1–Fr. 3*. *Fr. 3* (18 g) was purified by CC (SiO_2; $CHCl_3$/MeOH 6:1), followed by prep. HPLC (MeOH/H_2O 32:68, 2.5 ml/min, detection at 280 nm) to afford **1** (20 mg), **2** (18 mg), and **3** (58 mg).

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 500 and 125 MHz, resp., in (D_6)DMSO; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
Vanilloyl:				
1a		122.4		123.1
2a	7.34 (*d*, *J* = 2.0)	111.9	7.41 (*d*, *J* = 1.8)	112.1
3a		148.4		148.5
4a		150.1		150.1
5a	6.97 (*d*, *J* = 8.5)	114.3	7.38 (*d*, *J* = 8.7)	114.4
6a	6.80 (*dd*, *J* = 2.0, 8.5)	122.4	7.79 (*dd*, *J* = 1.8, 8.7)	122.5
7a		165.1		165.3
3a-MeO	3.77 (*s*)	55.5	3.78 (*s*)	55.5
D-Glc:				
1′a	5.31 (*d*, *J* = 7.5)	100.9	5.35 (*d*, *J* = 7.5)	98.2
2′a	3.44–3.49 (*m*)	73.8	3.33–3.47 (*m*)	72.8
3′a	3.20–3.40 (*m*)	76.7	3.33–3.47 (*m*)	76.9
4′a	3.10–3.14 (*m*)	71.2	3.15–3.21 (*m*)	70.6
5′a	3.20–3.40 (*m*)	73.9	3.33–3.47 (*m*)	73.5
6′a	4.39 (*dd*, *J* = 5.0, 11.5)	65.0	4.13 (*dd*, *J* = 5.0, 11.5)	65.5
	3.90 (br. *d*, *J* = 11.5)		3.92–3.96 (*m*)	
Syringoyl:				
1b		124.6		123.1
2b	7.07 (*d*, *J* = 2.0)	107.0	7.40 (*d*, *J* = 1.8)	112.1
3b		152.0		148.4
4b		137.6		149.8
5b		153.1	7.33 (*d*, *J* = 8.7)	114.3
6b	7.49 (*d*, *J* = 2.0)	106.7	7.77 (*dd*, *J* = 2.0, 8.7)	122.4
7b		164.9		165.2
3b-MeO	3.96 (*s*)	56.5	3.78 (*s*)	55.5
5b-MeO	3.60 (*s*)	55.9		
D-Allo:				
1′b	5.13 (*d*, *J* = 7.5)	97.5	5.20 (*d*, *J* = 6.5)	96.7
2′b	3.20–3.40 (*m*)	71.2	3.33–3.47 (*m*)	71.7
3′b	3.55 (*t*, *J* = 2.5)	69.9	3.57 (*t*, *J* = 2.5)	69.8
4′b	3.44–3.49 (*m*)	68.2	3.33–3.47 (*m*)	68.2
5′b	3.92–3.98 (*m*)	71.8	3.92–3.96 (*m*)	71.5
6′b	4.45 (*dd*, *J* = 5.5, 11.5)	64.8	4.41 (*dd*, *J* = 5.5, 11.5)	65.1
	4.26 (br. *d*, *J* = 11.5)		4.21 (br. *d*, *J* = 11.5)	

Clemahexapetoside A (='*4-({6-*O*-[(4-*O*-β-*D*-Allopyranosyl)syringoyl]-β-*D*-glucopyranosyl}oxy)vanillic Acid Inner Ester*'; **1**[2]). Colorless, amorphous powder. M.p. 320–322°. UV (MeOH): 291, 253, 218. $[\alpha]_D^{20} = +108$ ($c = 0.5$, pyridine). IR (KBr): 3400–3500, 1722, 1608, 1510. ^{1}H- and ^{13}C-NMR: see *Table*. HR-FAB-MS: 677.1695 ($[M+Na]^+$, $C_{29}H_{34}NaO_{17}^+$; calc. 677.1694).

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[2]) Systematic name: (3*S*,4*R*,5*R*,6*S*,7*R*,16*S*,17*R*,18*S*,19*S*,20*R*)-4,5,6,17,18,19-hexahydroxy-13,26,27-trimethoxy-2,9,15,22,29,32-hexaoxapentacyclo[22.2.2.2^{11,14}.1^{3,7}.1^{16,20}]dotriaconta-1(26),11,13,24,27,30-hexaene-10,23-dione.

Clemahexapetoside B (='*4-({6-*O*-[(4-*O*-β-*D*-Allopyranosyl)vanilloyl]-β-*D*-glucopyranosyl}oxy)vanillic Acid Inner Ester*'; **2**)[3]). Colorless, amorphous powder. M.p. 316–318°. UV (MeOH): 291, 253, 218. $[\alpha]_D^{20} = +98$ ($c = 0.6$, pyridine). IR (KBr): 3400–3500, 1720, 1610, 1508. ^{1}H- and ^{13}C-NMR: see *Table*. HR-FAB-MS: 625.1749 ($[M+H]^+$, $C_{28}H_{33}O_{16}^+$; calc. 625.1769).

Acid Hydrolysis of **1** *and* **2**. Each compound (5 mg) was hydrolyzed and analyzed as reported before [10]. Vanillic acid was detected by TLC after hydrolysis of **1** and **2**, while syringic acid was detected only in the case of **1**. D-Allose and D-Glucose were detected from **1** and **2** by GC after derivatization with L-cystein. The retention times were 11.37 and 11.63 min for D-All and D-Glc, resp.

REFERENCES

[1] H. Kizu, T. Tomimori, *Chem. Pharm. Bull.* **1979**, *27*, 2388.
[2] H. Kizu, T. Tomimori, *Chem. Pharm. Bull.* **1980**, *28*, 2827.
[3] H. Kizu, T. Tomimori, *Chem. Pharm. Bull.* **1980**, *28*, 3555.
[4] H. Kizu, T. Tomimori, *Chem. Pharm. Bull.* **1982**, *30*, 859.
[5] H. Kizu, T. Tomimori, *Chem. Pharm. Bull.* **1982**, *30*, 3340.
[6] B.-P. Shao, G.-W. Qin, R.-S. Xu, H.-M. Wu, K. Ma, *Phytochemistry* **1995**, *38*, 1473.
[7] B.-P. Shao, G.-W. Qin, R.-S. Xu, H.-M. Wu, K. Ma, *Phytochemistry* **1996**, *42*, 821.
[8] M. He, J.-H. Zhang, C.-Q. Hu, *Acta Pharm. Sin.* **2001**, *36*, 278.
[9] Y. Mimaki, A. Yokosuka, M. Hamanaka, C. Sakuma, T. Yamori, Y. Sashida, *J. Nat. Prod.* **2004**, *67*, 1511.
[10] S.-P. Shi, D. Jiang, C.-X. Dong, P.-F. Tu, *Helv. Chim. Acta* **2006**, *89*, 1023.
[11] C.-Q. Song, R.-S. Xu, *Chin. Chem. Lett.* **1992**, *3*, 119.
[12] E. Breitmaier, W. Voelter, 'Carbon-13 NMR Spectroscopy', VCH, Weinheim, 1987, 3rd edn., pp. 381–391.
[13] S. Nobuko, K. Masato, S. Atsushi, I. Takao, *Chem. Pharm. Bull.* **1992**, *40*, 851.

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[3]) Systematic name: (3*S*,4*R*,5*R*,6*S*,7*R*,16*S*,17*R*,18*S*,19*S*,20*R*)-4,5,6,17,18,19-hexahydroxy-13,26-dimethoxy-2,9,15,22,29,32-hexaoxapentacyclo[22.2.2.2^{11,14}.1^{3,7}.1^{16,20}]dotriaconta-1(26),11,13,24,27,30-hexaene-10,23-dione.