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TWO NEW MACROCYCLIC COMPOUNDS FROM THE STEMS OF *CLEMATIS ARMANDII*

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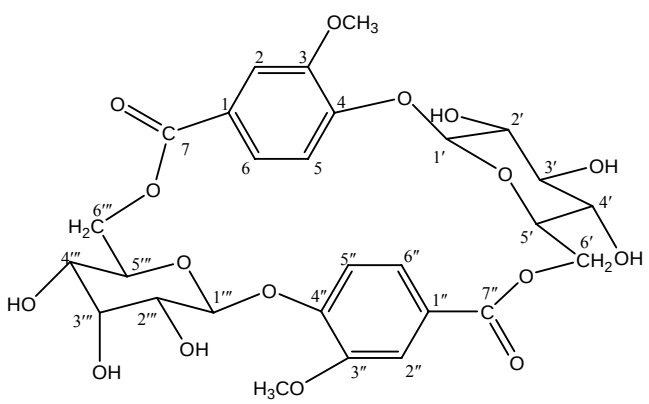
Abstract—Phytochemical investigation of *Clematis armandii* (Ranunculaceae) has resulted in the isolation of two new macrocyclic compounds named clemoarmanosides A (**1**) and B (**2**), along with two known macrocyclic compounds berchemolide (**3**) and clemochinenoside B (**4**). The structures of **1** and **2** were elucidated on the basis of spectroscopic data and chemical methods. In addition, several ^{13}C-NMR data of **4** were amended by 2D-NMR spectra analysis in this paper.

INTRODUCTION

The stems of *Clematis armandii* Franch. (Ranunculaceae) is a source of the traditional Chinese medicine "Chuan-Mu-Tong", which has been widely used as a diuretic, galactopoietic, emmenagogue as well as anti-inflammatory and antitumor agent. It is also used in the treatment of hydropsy, gonorrhea, and arthralgia.[1] *C. armandii* is an evergreen and perennial liana, and mainly distributes in southwest of China. Up to date, chemical and biological work on this plant have been limited to several flavonoids and lignans in a few previous studies.[2-4] As a result, the objective of the present study is to reveal more detailed information about its chemical constituents. In this paper, we report two new macrocyclic compounds named clemoarmanosides A (**1**) and B (**2**), along with two known constituents isolated from this plant.

The structures of two new compounds were elucidated on the basis of the spectroscopic analyses and chemical evidence, and two known constituents were identified by comparison of the spectral data with reported data as berchemolide (**3**)[5] and clemochinenoside B (**4**).[7]



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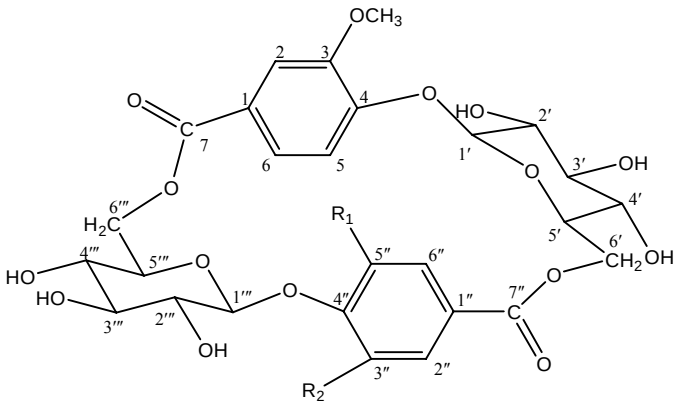

	2	**3**	**4**
R_1	H	H	OCH_3
R_2	H	OCH_3	OCH_3

Figure 1. Structures of compounds (**1-4**)

RESULTS AND DISCUSSION

Clemoarmanoside A (**1**) was obtained as white crystals. The molecular formula of **1** was established as $C_{28}H_{32}O_{16}$ with thirteen degrees of unsaturation by HR-ESI-MS (647.1558 $[M+Na]^+$). The IR spectrum of **1** showed a broad absorption band for hydroxyl groups at 3446 cm^{-1}, as well as absorption bands due to aromatic esters (1718, 1279, 1088 cm^{-1}) and benzene rings (1600, 1514 cm^{-1}). The UV spectrum of **1** exhibited absorption maxima at 219, 253, and 292 nm. The ^{1}H-NMR spectrum of **1** exhibited two series of ABX-type aromatic proton signals, one at δ_H7.33 (1H, d, J = 9.0 Hz), 7.75 (1H, dd, J = 9.0, 2.4 Hz) and 7.41 (1H, d, J = 2.4 Hz), and the other at δ_H7.38 (1H, d, J = 9.0 Hz), 7.78 (1H, dd, J = 9.0, 2.4 Hz) and 7.42 (1H, d, J = 2.4 Hz). There also existed two methoxy proton signals at δ_H3.79 (6H, s), in addition to two anomeric proton signals at δ_H 5.24 (1H, d, J = 7.2 Hz) and 5.38 (1H, d, J = 7.8 Hz) and other signals (δ_H4.41-3.20) due to sugars. The ^{13}C-NMR spectrum showed the presence of two carbonyl carbon signals at δ_C165.2 (C-7) and δ_C165.1 (C-7″). In addition, the presence of two substituted benzene rings, two methoxy groups, and two sugar moieties were confirmed by the ^{13}C-NMR data (Table 1). On the basis of

Table 1. ^{1}H- (600MHz) and ^{13}C-NMR (150 MHz) data for compounds (**1-4**), in DMSO-d_6 [a)]

No.	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C (× 2)	δ_H (× 2)	δ_C	δ_H
1	122.5		122.5		122.5		122.5	
2	112.1	7.41 (d, 2.4)	112.2	7.41 (d, 1.8)	112.1	7.42 (d, 1.8)	111.9	7.35 (d, 1.8)
3	148.4		148.4		148.4		148.3	
4	149.8		149.9		149.8		149.9	
5	114.4	7.33 (d, 9.0)	114.4	7.40 (d, 8.4)	114.5	7.37 (d, 8.4)	114.4	6.99 (d, 8.4)
6	123.1	7.75 (dd, 9.0,2.4)	122.9	7.78 (dd, 8.4, 1.8)	122.9	7.75 (dd, 8.4, 1.8)	122.3	6.80 (dd, 8.4, 1.8)
7	165.2		165.1		165.1		165.0	
3-OMe	55.5	3.79, s	55.5	3.79, s	55.5	3.80, s	55.5	3.78, s
1′	98.3	5.24 (d, 7.2)	98.2	5.25 (d, 7.8)	98.3	5.24 (d, 7.2)	99.0	5.04 (d, 7.8)
2′	72.8	3.39, m [b)]	72.8	3.37, m [b)]	72.8	3.39, m [b)]	73.0	3.37, m [b)]
3′	76.9	3.39, m [b)]	76.7	3.37, m [b)]	76.9	3.39, m [b)]	77.2	3.37, m [b)]
4′	70.6	3.20, m	70.6	3.18, m [b)]	70.6	3.19, m	70.6	3.20, m
5′	73.5	3.97, m [b)]	73.5	3.97, m [b)]	73.5	3.97, m	73.9	3.96, m [b)]
6′	65.1	4.09, m [b)] 4.41 (br d, 11.4)	65.0	4.10, m 4.39 (br d, 10.8)[b)]	65.1	4.09, m 4.41 (br d, 10.2)	64.2	4.44, m 4.47 (dd, 11.4, 2.4)
1″	122.4		122.5				124.5	
2″	112.1	7.42 (d, 2.4)	131.1	7.96 (d, 8.4)			107.0	7.08 (d, 1.8)
3″	148.4		115.9	7.19 (d, 8.4)			153.1	
4″	150.1		160.4				137.7	
5″	114.3	7.38 (d, 9.0)	115.9	7.19 (d, 8.4)			152.0	
6″	122.9	7.78 (dd, 9.0, 2.4)	131.1	7.96 (d, 8.4)			106.8	7.50 (d, 1.8)
7″	165.1		165.0				164.8	
3″-OMe	55.5	3.79, s					55.9	3.61, s
5″-OMe							56.5	3.97, s
1‴	96.7	5.38 (d, 7.8)	98.5	5.21 (d, 7.8)			101.0	5.33 (d, 7.2)
2‴	69.8	3.58 (td, 7.8, 2.4)	72.9	3.37, m [b)]			73.9	3.30, m [b)]
3‴	71.7	3.98, m [b)]	76.9	3.37,m [b)]			76.7	3.30, m [b)]
4‴	68.2	3.45, m	70.7	3.18, m [b)]			71.1	3.12, m
5‴	71.2	4.26, m	73.5	3.97, m [b)]			73.8	3.49, m
6‴	65.4	4.11, m[b)] 4.37 (br d, 11.4)	65.1	4.06, m 4.41 (br d, 10.2)[b)]			64.9	3.91,m 4.39 (dd, 11.4, 2.4)

[a)] Assignments were confirmed by ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments; chemical shifts (δ) in ppm, *J* values in parentheses were recorded in Hz; [b)] overlapped signals

^{1}H-^{1}H COSY and HMQC spectral analysis, the proton and carbon signals of two sugar moieties were unambiguously assigned. On acid hydrolysis, **1** afforded vanillic acid together with D-glucopyranose and D-allopyranose, which was described in the experimental section. Moreover, the coupling constant of 7.2 Hz for the anomeric proton H-1′ (δ_H5.24) and 7.8 Hz for H-1‴ (δ_H5.38) indicated β-configuration for both sugar residues. Thus, two sugar moieties were identified as β-D-glucopyranose (δ_C98.3, 72.8, 76.9, 70.6, 73.5, 65.1), and β-D-allopyranose (δ_C96.7, 69.8, 71.7, 68.2, 71.2, 65.4), respectively.

The connections between sugar moieties and aromatic units were established mainly based on the HMBC spectrum. In the HMBC spectrum (Figure 2), the anomeric proton signal at δ_H5.24 (H-1′) correlated with the carbon signal at δ_C149.8 (C-4), and the proton signals at δ_H 4.09 and 4.41 (H-6′) correlated with the carbonyl signal at δ_C165.1 (C-7″), which indicated that the β-D-glucopyranose moiety was attached to C-4 and C-7″. Similarly, the β-D-allopyranose moiety was located at C-4″ and C-7 as evidenced by the cross-peaks between the anomeric proton signal at δ_H5.38 (H-1‴) and the carbon signal at δ_C150.1 (C-4″), and between the proton signals at δ_H4.11 and 4.37 (H-6‴) and the carbonyl signal at δ_C165.2 (C-7) in the HMBC spectrum. The positions of substituting groups in the aromatic units were also determined on the basis of HMBC data together with NOESY results (Figure 2). In the HMBC spectrum, correlations between H-2, H-6/C-7; H-2, H-6/C-4; CH_3O-3/C-3; H-2″, H-6″/C-7″; H-2″, H-6″/C-4″; CH_3O-3″/C-3″ suggested the presence of two 1,3,4-trisubstituted benzene rings, and the positions of methoxy groups were at C-3 and C-3″. Such determination of methoxy groups were further supported by the NOESY spectrum, which showed cross-peaks between the signals at δ_H7.41 (1H, d, H-2), 7.42 (1H, d, H-2″) and δ_H3.79 (6H, s, 3-OCH_3 and 3″-OCH_3), respectively. Consequently, **1** was determined as benzoic acid, 4-[[6′-O-[4″-(β-D-allopyranosyloxy)-3″-methoxybenzoyl]-β-D-glucopyranosyl]oxy]-3-methoxy-intramol.1,6‴-ester and named as clemoarmanoside A.

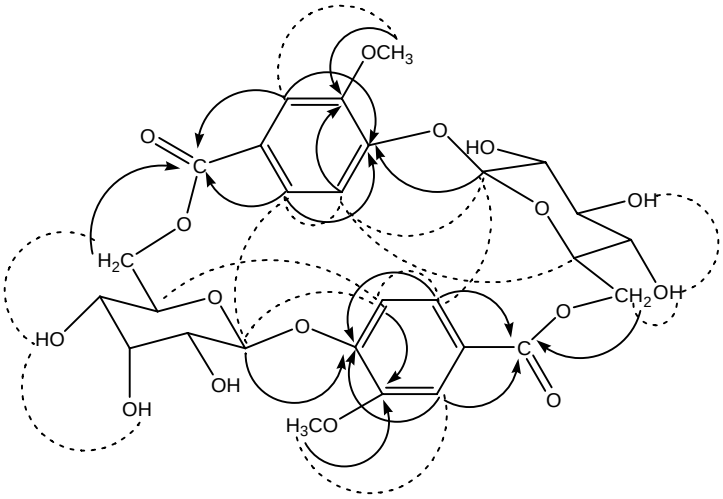

Figure 2. Key HMBC (H C) and NOESY (H H) spectrum of **1**

Clemoarmanoside B (**2**) was isolated as a white amorphous powder. Its molecular formula of $C_{27}H_{30}O_{15}$ with thirteen degrees of unsaturation was determined by HR-ESI-MS (617.1516 $[M+Na]^+$). The UV and

IR spectra of **2** were very similar to those of **1**. ^{1}H- and ^{13}C-NMR data (Table 1) suggested that **2** was a macrocyclic compound like **1**. The ^{1}H-NMR spectrum of **2** revealed the presence of one aromatic ABX system at δ_H7.40 (1H, d, J = 8.4 Hz), 7.41 (1H, d, J = 1.8 Hz) and 7.78 (1H, dd, J = 8.4, 1.8 Hz). Whilst, signals at δ_H7.96 (2H, d, J = 8.4 Hz) and 7.19 (2H, d, J = 8.4 Hz) were attributed to a 1,4 bis-substituted benzene ring under highly symmetrical circumstance. There also existed one methoxy proton signal at δ_H3.79 (3H, s), two anomeric proton signals at 5.25 (1H, d, J = 7.8 Hz) and δ_H5.21 (1H, d, J = 7.8 Hz), and other signals (δ_H4.41-3.18) due to sugars. The presence of two carbonyl groups, one methoxy group, two substituted benzene rings, and two sugar moieties were confirmed by the ^{13}C-NMR data (Table 1). In addition, twelve carbon signals were observed almost in pairs between δ_C98.5 and 65.0 including two anomeric carbon signals at δ_C98.2 and 98.5, indicating that two sugar moieties were the same. The sugar moieties were confirmed to be β-D-glucopyranose by comparing with the known compounds (**3**)[5] and (**4**)[7] and an experiment of acid hydrolysis (The hydrolysate of **2** gave vanillic acid, *p*-salicylic acid and D-glucopyranose).

The connections of two sugar moieties and two aromatic moieties were deduced by the C-H long-range couplings between H-1′ at δ_H5.25 and C-4 at δ_C149.9; H-6′ at δ_H4.10 and 4.39 and C-7″ at δ_C165.0; H-1‴ at δ_H5.21 and C-4″ at δ_C160.4; H-6‴at δ_H4.06 and 4.41 and C-7 at δ_C165.1 in the HMBC spectrum of **2** (Figure 3). Furthermore, the HMBC spectrum exhibited correlations between H-2, H-6/C-7; H-2, H-6/C-4; CH_3O-3/C-3; H-2″, H-6″/C-7″; H-2″, H-6″/C-4″, which confirmed the presence of a 1,3,4-trisubstituted benzene ring and a 1,4 bis-substituted benzene ring. The position of the methoxy group in the aromatic unit was also confirmed by the cross-peaks between δ_H3.79 (3H, s) and 7.41 (1H, d, H-2) in the NOESY spectrum. Thus, all the proton and carbon signals of **2** were fully assigned as shown in Table 1 and Figure 3. Consequently, the structure of **2** was formulated as benzoic acid, 4-[[6′-O-[4″-(β-D-glucopyranosyloxy)benzoyl]-β-D-glucopyranosyl]oxy]-3-methoxy-intramol.1,6‴-ester, and named as clemoarmanoside B.

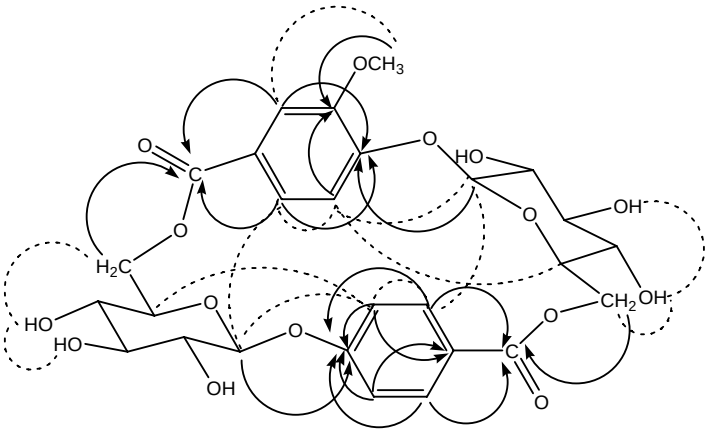

Figure 3. Key HMBC (H C) and NOESY (H H) spectrum of **2**

Berchemolide (**3**)[5] and clemochinenoside B (**4**)[7] were known compounds, and their structures were

determined by comparing the ^{1}H- and ^{13}C-NMR data with those in the literature. Compound (**4**) was first reported by C. Q. Song and R. S. Xu in 1993. Carbon signals at δ_C106.6, 112.3, 106.6, 114.5 were attributed to C-2, 5, 2″, 6″ in the literature.[7] Based on the 2D-NMR spectra (including ^{1}H-^{1}H COSY, HMQC and HMBC) of **4**, we suggested that the carbon signals at δ_C111.9, 114.4, 107.0, 106.8 were assigned to C-2, 5, 2″, 6″, respectively (Table 1).

EXPERIMENTAL

General Experimental Procedures

Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were obtained on a Philips PYE Unican Pu8800 spectrophotometer, and IR spectra on an IMPACT-400 instrument with KBr pellets. NMR spectra were run on a VARIAN INOVA 600 spectrometer using DMSO-d_6 as solvent. EI-MS measurements were performed on a ZabSpec mass spectrometer. ESI-MS data were recorded on a Q-Trap LC/MS/MS with turbo ion spray source. HR-ESI-MS data were obtained on an ACCUTOF CS (GEOL) instrument. Precoated Silica gel GF_{254} plates (Qingdao Haiyang Chem. Co.) were employed for TLC. Spots were visualized under UV light (254 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating. For column chromatography, Silica gel (Qingdao Haiyang Chem. Co.) and Sephadex LH-20 (Pharmacia) were used. The MPLC were performed on a system equipped with a Büchi pump and Büchi columns with the stationary phase Silica gel 60 (15-40 μm, Qingdao Haiyang Chem. Co.). HPLC analyses were performed on a Shimadzu LC-10AT_{vp} series apparatus with a Alltech ELSD 800 detector. β-D-allopyranose was purchased from Sigma-Aldrich Co.

Plant Materials

The stems of *Clematis armandii* Franch. were collected in Yunnan Province, People's Republic of China, in 1997, and authenticated by Prof. Zailin Li (Food and Drug Administration, Yunnan Province of China). A voucher specimen is deposited in the Natural Medicine Research Center of the Institute of Medicinal Plant Development, China.

Extraction and Isolation

The air-dried stems of *C. armandii* (9.5 kg) were ground and extracted three times with 95% EtOH under reflux. The combined extract was concentrated under reduced pressure to yield 450 g of residue, which was suspended in water and extracted successively with petroleum ether (60-90℃), $CHCl_3$, EtOAc, and

n-BuOH. The n-BuOH extract (200 g) was subjected to a macroporous resin (AB-8) column and eluted with water, and then 10%, 30%, 50%, 90% EtOH, respectively. The 50% EtOH fraction (110 g) was chromatographed over a silica gel (1200 g, 200-300 mesh) column eluted with $CHCl_3$-MeOH-H_2O (9:1:0.1-0:1:0.1), and monitored by TLC analysis to yield fifteen combined fractions (I-XV). Fraction IV and V was further purified by a sephadex LH-20 column to yield **1** (10 mg) and **3** (5 mg). The 30% EtOH fraction (20 g) was subjected to a sephadex LH-20 column to yield five combined fractions (I′-V′). Fraction II′ was subjected to MPLC over silica gel eluted with $CHCl_3$-MeOH-H_2O (85:15:1.5-0:1:0.1), to give seven fractions (II′$_1$-II′$_7$). Purification of fraction II′$_1$ using sephadex LH-20 to give **4** (8 mg). Fraction V′ was purified by a sephadex LH-20 column to afford **2** (6 mg).

Acid Hydrolysis and Determination of the Absolute Configuration of Sugars in 1 and 2

Each compound (1 mg) was heated at 105℃ in a sealed ampoule with dimethyl sulfoxide (DMSO) 100 μL and 2 M trifluoroacetic acid (TFA) 70 μL for 2 h. After removing the TFA and DMSO, the reaction mixture was dissolved in water (10 mL) and extracted with EtOAc (2 × 5 mL). The H_2O layer was concentrated and the residue dissolved with MeCN-H_2O (8 : 2), then sugars were detected by HPLC. Chromatographic conditions: column, Asahipak NH2P-50 (4.6 mm × 25 cm); solvent, 80% MeCN in water; flow rate, 1.0 mL/min. Detection conditions: An Alltech ELSD 800 detector (Alltech, U.S.A.) was used. The carrier gas was nitrogen, the drift tube temperature was set at 41℃, and the gas flow-rate was 2.8 L/min. Consequently, D-allose (t_R 10.61 min) and D-glucose (t_R 12.28 min) were detected from **1**, while only D-glucose was detected from **2**. The EtOAc layers were evaporated, and vanillic acid and *p*-salicylic acid were identified by direct comparison with authentic samples by TLC.

Compound (**1**): white crystals from MeOH; mp 288-290℃; $[\alpha]_D^{20}$ +53.7° (c = 0.41, pyridine); UV (MeOH) λ_{max} nm (logε): 219 (4.58), 253 (4.31) and 292 (3.92); IR (KBr) cm^{-1}: 3446, 1718, 1600, 1514, 1458, 1421, 1296, 1279, 1221, 1088, 1045, 908, 760; EI-MS m/z (rel. int.%): 313 (15), 295 (6), 168 (22), 151 (100); ESI-MS m/z: 647 $[M+Na]^+$; HR-ESI-MS m/z: 647.1558 (calcd. for $C_{28}H_{32}O_{16}Na$, $[M+Na]^+$, 647.1588); ^{1}H- and ^{13}C-NMR: see Table 1.

Compound (**2**): white amorphous powder from MeOH; $[\alpha]_D^{20}$ +45.8° (c = 0.24, pyridine); UV (MeOH) λ_{max} nm (logε): 215 (4.44), 251 (4.34) and 292 (3.86); IR (KBr) cm^{-1}: 3440, 1716, 1606, 1512, 1458, 1421, 1296, 1279, 1221, 1080, 1026, 901, 760; EI-MS m/z (rel. int.%): 313 (15), 295 (6), 283 (5), 168 (50), 151 (100), 121 (48); ESI-MS m/z: 617 $[M+Na]^+$; HR-ESI-MS m/z: 617.1516 (calcd. for $C_{27}H_{30}O_{15}Na$, $[M+Na]^+$, 617.1482); ^{1}H- and ^{13}C-NMR: see Table 1.

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REFERENCES

1. "Xin Bian Zhong Yao Zhi," Vol. 3, Chemical Industry Press, Beijing, 2002, pp. 686-693.
2. Y. F. Chen, J. Liu, R. S. Davidson, and O. W. Howarth, *Tetrahedron*, 1993, **49**, 5169.
3. W. W. Huang, D. Y. Kong, and P. M. Yang, *Chin. J. Nat. Med.*, 2003, **1**, 199.
4. W. W. Huang, D. Y. Kong, and P. M. Yang, *Chin. Tradit. Herb. Drugs.*, 2004, **35**, 621.
5. N. Sakurai, M. Kobayashi, A. Shigihara, and T. Inoue, *Chem. Pharm. Bull.*, 1992, **40**, 851.
6. C. Q. Song and R. S. Xu, *Chin. Chem. Lett.*, 1992, **3**, 119.
7. C. Q. Song and R. S. Xu, *Chin. Chem. Lett.*, 1993, **4**, 505.