Chemical Constituents from the Roots and Rhizomes of *Clematis hexapetala* Pall.

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Z. Naturforsch. **2007**, *62b,* 854 – 858; received November 1, 2006

A new phenolic glycoside, 2,6-dimethoxy-4-(3-hydroxy-propen-1-yl)phenyl-4-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**1**) and a new D-ribono-γ-lactone derivative, 3-*O*-β-D-glucopyranosyl-2-hydroxymethyl-D-ribono-γ-lactone (**3**), together with thirteen known compounds have been isolated from the roots and rhizomes of *Clematis hexapetala* Pall. The characterization of these compounds was achieved by various chromatographic and spectroscopic methods.

Key words: Clematis hexapetala, Chemical Constituents, Structure Elucidation

Introduction

Clematis hexapetala Pall. (Family *Ranunculaceae*) is widely distributed in the North of China. The roots and rhizomes of the plant are known as "*Weilingxian*" in traditional Chinese medicine and used as an analgesic, diuretic and anti-inflammatory agents. Literature studies revealed that no phytochemical work has been done so far on the plant. Chemical investigations resulted in the isolation and elucidation of a new phenolic glycoside (**1**) and a new D-ribono-γ-lactone derivative (**3**), together with thirteen known compounds, syringin (**2**) [1], 2-hydroxymethyl-D-ribono-γ-lactone (**4**) [2, 3], (+)-pinoresinol-4-*O*-β-D-glucopyranoside (**5**) [4, 5], (+)-lariciresinol-4-*O*-β-D-glucopyranoside (**6**) [5], (+)-lariciresinol-4′-*O*-β-D-glucopyranoside (**7**) [6 – 8], 5,6,7,8,4′-pentamethoxyflavone (tangeretin) (**8**) [9], isoliquiritigenin (**9**) [10], 3,7,4′-trihydroxyflavone (**10**) [11], luteolin (**11**) [12], puerarin (**12**) [13], magnoline (**13**) [14], protocatechuic acid (**14**) [15], and *n*-dotriacontane acid (**15**) [16] (Fig. 1). Herein, we report the isolation and elucidation of the new compounds.

Results and Discussion

Compound **1** was obtained as pale yellow resin, with the molecular formula $C_{23}H_{34}O_{13}$, as deduced from the $[M+Na]^+$ peak at *m/z* = 541.1920 in the HRFABMS and confirmed by ^{13}C NMR spectroscopic data. Acid hydrolysis of **1** afforded 3,5-dimethoxy-4-hydroxyl-phenyl propenol, L-Rha and D-Glc. The ^1H NMR spectrum of **1** presented the characteristic pattern of a 3,5-dimethoxy-4-hydroxyl-phenyl propenol moiety [δ = 6.74 (2H, s), 6.54 (d, J = 15.9 Hz, 1H), 6.32 (dt, J = 5.4, 15.9 Hz, 1H), 4.21 (dd, J = 1.5, 5.4 Hz, 2H) and 3.88 (6H, s, OCH_3)], and two anomeric protons at δ = 4.77 (d, J = 7.5 Hz, 1H) and δ = 4.66 (1H, br). The three-proton doublet at δ = 1.18 (d, J = 6.3 Hz, 3H) indicated the presence of a deoxyhexopyranosyl unit in **1**. In the ^{13}C NMR spectrum, the anomeric C atoms were present at δ = 105.2 and 102.2. The coupling constant (J = 7.5 Hz) of the anomeric H atom suggested that the glucopyranosyl unit existed in β orientation. All the protons and carbon atoms were assigned by ^1H–^1H COSY, HSQC and HMBC experiments. In the HMBC spectrum of **1**, the anomeric proton of the glucopyranosyl unit at δ = 4.77 (d, J = 7.5 Hz, 1H) showed a long-range correlation with C-4 of the aglycone at δ = 135.7, indicating that the glucopyranosyl unit is attached to C-4 of the aglycone. The anomeric proton of the rhamnopyranosyl unit at δ = 4.66 (1H, br) indicated a cross peak with the C-6 of the glucopyranosyl at δ = 68.0, suggesting that the rhamnopyranosyl unit is linked to C-6 of the glucopyranosyl group. Conclusively, the structure of **1** was identified as 2,6-dimethoxy-4-(3-hydroxy-propen-1-yl)phenyl-4-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (Table 1).

0932–0776 / 07 / 0600–0854 $ 06.00

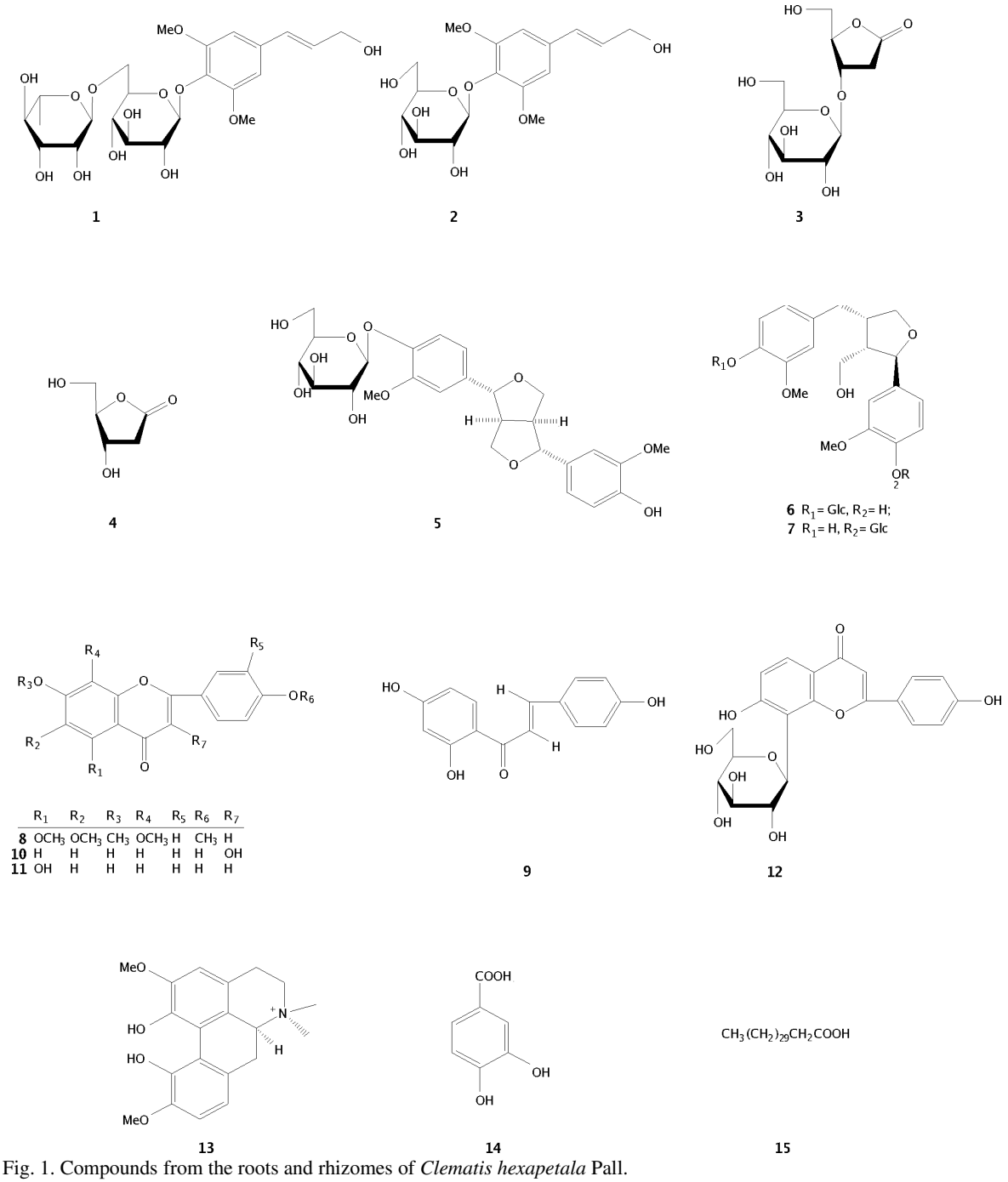

Fig. 1. Compounds from the roots and rhizomes of *Clematis hexapetala* Pall.

Compound **3** was obtained as an amorphous white powder, with a molecular formula $C_{11}H_{18}O_9$ deduced from the *quasi* molecular ion peak at *m/z* = 317.0853 ([M+Na]$^+$) in the HRFABMS. In the ^{13}C NMR spectrum, except for the signals due to a glucopyranosyl unit, the signals due to a 2-hydroxymethyl-ribono-γ-lactone moiety were observed (δ = 176.0, 36.4, 75.6, 85.9 and 61.0) [2]. In addition, the ^{1}H NMR spectrum

NO.	1		3	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	129.9	–	176.0
H(a)-C(2)	6.74 (s)	105.3	2.55 (br.d, J = 18.0)	36.4
H(b)-C(2)	–	–	2.87 (overlapped)	–
H-C(3)	–	154.5	4.56 (m)	75.6
H-C(4)	–	135.7	3.61 (m)	85.9
H(a)-C(5)	–	154.5	3.47 (m)	61.0
H(b)-C(5)	–	–	3.69 (m)	–
H-C(6)	6.74 (s)	105.3	–	–
H-C(7)	6.54 (d, J = 15.9)	135.3	–	–
H-C(8)	6.32 (dt, J = 5.4, 15.9)	131.4	–	–
H-C(9)	6.32 (dd, J = 1.5, 5.4)	63.6	–	–
MeO-C(3)	3.88 (s)	57.0	–	–
MeO-C(5)	3.88 (s)	57.0	–	–
Glc				
H-C(1′)	4.77 (d, J = 7.5)	105.2	4.32 (d, J = 7.8)	102.2
H-C(2′)	3.86 (m)	75.6	3.40 (m)	73.2
H-C(3′)	3.47 (m)	77.9	3.63 (m)	77.0
H-C(4′)	3.45 (m)	71.7	3.23 (m)	69.9
H-C(5′)	3.48 (m)	77.3	3.24 (m)	76.7
H-C(6′a)	3.63 (dd, J = 2.4, 12.0)	68.0	3.71 (dd, J = 1.8, 11.7)	61.0
H-C(6′b)	3.38 (m)		3.47 (dd, J = 6.3, 11.7)	
Rha				
H-C(1″)	4.66 (br.s)	102.2		
H-C(2″)	3.67 (dd, J = 1.8, 3.3)	72.1		
H-C(3″)	3.59 (m)	72.3		
H-C(4″)	3.39 (m)	74.0		
H-C(5″)	3.86 (overlapped)	69.7		
H-C (6″)	1.18 (d, J = 6.3)	18.0		

Table 1. ^{1}H and ^{13}C NMR data of **1** (in CD_3OD) and **3** (in [D_6]DMSO); δ in ppm, J in Hz.

The coupling constants of the sugar H atoms were not assigned due to overlapping.

showed an anomeric proton at δ = 4.32 (d, J = 7.8 Hz, 1H) for the β-glucopyranosyl unit. The anomeric carbon atom was present at δ = 102.2 in the ^{13}C NMR spectrum. In the HMBC spectrum, the correlation between the anomeric proton (δ = 4.32) of the glucopyranosyl unit and C-3 (δ = 75.6) of the aglycone suggested that the glucopyranosyl is attached to C-3 of the aglycone. In the NOESY spectrum, the absence of a NOE correlation between H-3 and H-4 suggested that H-3 and H-4 existed in a *trans* formation. Enzymatic hydrolysis of **3** gave the known compound 2-hydroxymethyl-D-ribono-γ-lactone (**4**) which was identified by comparison of its NMR data and optical rotation with those reported [2, 3]. Acid hydrolysis of **3** afforded D-glucose. Thus, the structure of **3** was identified as 3-*O*-β-D-glucopyranosyl-2-hydroxymethyl-D-ribono-γ-lactone.

Experimental Section

General

Optical rotations were determined using a Perkin Elmer 243B digital polarimeter. EIMS and ESIMS spectra were performed on a Trans-MS and a QSTAR mass spectrometer. HRFABMS were measured on an Autospec-Ultima ETOF spectrometer in positive ion mode. NMR spectra were recorded on an Inova-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) in CD_3OD and [D_6]DMSO using TMS as internal standard reference. Chemical shifts δ are in ppm and coupling constants J are in Hz. Prep-HPLC was performed on a ODS column (Alltech 250 × 10 mm i. d., 5 μm) with a Waters 2487 dual λ absorbance detector. GC analysis was carried out on an Agilent 6890N gas chromatogragh using an HP-5 capillary column (28 × 0.32 mm, i. d.); detector, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2 at 1.0 mL min^{-1}. Column chromatography was performed using silica gel (100 – 200 mesh, 200 – 300 mesh, QingDao Marine Chemical Factory) as an absorbent, or Sephadex LH-20 gel (Pharmacia) and D101 porous polymer resin (Tianjin Chem. Ind. Co. Ltd.). TLC were performed on silica gel 60 F_{254} Merck plates.

Plant material

The roots and rhizomes of *Clematis hexapetala* Pall. were collected in September 2004 from Hailaer in the Inner Mongolian Autonomous Region of China. The identification of the plant was performed by Prof. Peng-Fei Tu, Peking University. A voucher specimen was deposited in the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine (No: 200409011).

Isolation

Air-dried roots and rhizomes (19 kg) were extracted 3 times at 60 °C with 95 % EtOH and 50 % EtOH and filtered. The filtered extract with 50 % EtOH was concentrated at 60 °C in a vacuum evaporator to afford residues (3.5 kg). The residue was suspended in water and extracted successively with EtOAc and *n*-BuOH to afford fraction A (105 g) and B (513 g), respectively.

Fraction A (100 g) was chromatographed on a silica gel column and eluted with $CHCl_3$/MeOH in a gradient to give ten fractions (Fr. 1–10). Fr. 2 was subjected to silica gel to give a crystalline product, which was washed with $CHCl_3$ to yield **15** (18.0 mg). Fr. 3 was subjected to silica gel and purified by prep-HPLC (MeOH/0.05 % TFA H_2O = 55 : 45, 2.5 mL min^{-1}, 254, 280 nm) to yield **8** (5.2 mg) and **9** (6.8 mg). Fr. 4 was chromatographed on a Sephadex LH-20 column and eluted with $CHCl_3$/MeOH (3 : 1 v/v) to give **4** (7.2 mg). Fr. 5 was subjected to silica gel and purified by Sephadex LH-20 column chromatography to yield **5** (8.8 mg). Fr. 6 was subjected to silica gel to give Fr. 6.1 and Fr. 6.2. Fr. 6.1 was chromatographed on a Sephadex LH-20 column and purified by prep-HPLC (MeOH/0.05 % TFA H_2O = 40 : 60, 2.5 mL min^{-1}, 254, 280 nm) to obtain **10** (5.0 mg) and **11** (4.0 mg). Fr. 6.2 was purified by prep-HPLC (MeOH/H_2O = 25 : 75, 2.5 mL min^{-1}, 254, 280 nm) to obtain **14** (4.2 mg). Fr. 7 was chromatographed on a silica gel column and purified by prep-HPLC (MeOH/H_2O = 22 : 78, 2.5 mL min^{-1}, 254, 280 nm) to obtain **6** (12.0 mg) and **7** (14.0 mg). Fr. 8 was chromatographed on a silica gel and Sephadex LH-20 column, and purified by prep-HPLC (MeOH/0.05 % TFA H_2O = 36 : 64, 2.5 mL min^{-1}, 254, 280 nm) to obtain **12** (6.0 mg). Fr. 10 was filtered to give white precipitates, which were washed with MeOH repeatedly to obtain **3** (1200.7 mg).

Fraction B (256 g) was subjected to D101 porous polymer resin and eluted with H_2O, 10, 30, 50 and 70 % MeOH successively. The fraction eluted with 10 % MeOH (9.04 g) was subjected to Sephadex LH-20 column chromatography and eluted with MeOH–H_2O (1 : 9) to afford Fr. 1–3. Fr. 2 was subjected to Sephadex LH-20 column chromatography and eluted with MeOH–H_2O (1 : 9) repeatedly to furnish **2** (7.8 mg). Fr. 3 was subjected to silica gel column chromatography and purified by prep-HPLC (MeOH/H_2O = 32 : 68, 280 nm, 2.5 mL min^{-1}) to afford **1** (9.5 mg). The fraction eluted with 30 % MeOH (30.0 g) was subjected to a strong-acid cation exchange resin column, and an ODS column, and purified by HPLC (CH_3CN/0.1 % NEt_3 = 2 : 98, 2.5 mL min^{-1}; 254, 280 nm) to afford **13** (12.5 mg).

*2,6-Dimethoxy-4-(3-hydroxy-propen-1-yl)phenyl-4O-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside (**1**)*

Pale yellow resin. – $[\alpha]_D^{20} = -30.0°$ (c = 0.5, CD_3OD). – ^1H and ^{13}C NMR data see Table 1. – HRFABMS: m/z = 541.1920 (calcd. 541.1897 for $C_{23}H_{34}O_{13}Na$, $[M+Na]^+$). – EIMS: m/z = 210 (loss of Glc and Rha).

*3-O-β-*D*-Glucopyranosyl-2-hydroxymethyl-*D*-ribono-γ-lactone (**3**)*

Amorphous white powder. – $[\alpha]_D^{20} = -20.4°$ (c = 0.5, [D_6]DMSO). – ^1H and ^{13}C NMR data see Table 1. – HRFABMS: m/z = 317.0853 (calcd. 317.0849 for $C_{11}H_{18}O_9Na$, $[M+Na]^+$).

*Acid hydrolysis of **1***

Compound **1** (2.0 mg) was hydrolyzed with 2 N aq. CF_3COOH (10 mL) at 110 °C for 8 h in a sealed tube. After this period, the reaction mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 × 10 mL). From the combined EtOAc extract the solvent was evaporated under reduced pressure and analyzed by TLC. 4-Hydroxy-3,5-dimethoxyphenylpropenol was detected. The aq. layer was evaporated with MeOH repeatedly under vacuum to remove the solvents completely. The residue was dissolved in anhydrous pyridine (0.100 mL) and mixed with a pyridine solution of L-cysteine methyl ester hydrochloride (0.100 mL). After warming to 60 °C for 1 h, hexamethyldisilazane (0.100 mL) and trimethylsilyl chloride (0.040 mL) were added, the warming to 60 °C was continued for another 30 min, then the mixture was filtered through a 0.45 μm membrane to remove the precipitation and analyzed by GC. L-Rha (5.410 min) and D-Glc (12.866 min) were detected from **1**.

*Enzymatic hydrolysis of **3***

Preparation of the aglycone of **3** by acid hydrolysis (2 N aq. CF_3COOH) failed. Thus **3** (10.0 mg) was incubated with β-glucosidase (25.0 mg) in phosphate buffer (4 mL, pH 5–6) for 48 h at 37 °C. The mixture was extracted with EtOAc (3 × 10 mL) and the combined EtOAc extract was evaporated under reduced pressure. Then, the residue was subjected to silica gel column chromatography ($CHCl_3$/MeOH = 6 : 1) affording **4** (3.6 mg).

Acknowledgements

The authors are thankful to Ministry of Science and Technology of PRC for their financial support (2004AA2Z3730), and to the Institute of Material Medica, Chinese Academy of Medical Science and Peking Union Medical for HRFABMS measurements.

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