Two New Secolignans from the Roots of *Urtica fissa* E. PRITZ

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Two new secolignans, {(3*S*,4*S*)-4-[bis(4-hydroxy-3-methoxyphenyl)methyl]-2-oxotetrahydrofuran-3-yl}methyl β-D-glucopyranoside (**1**) and its stereoisomer {(3*S*,4*R*)-4-[bis(4-hydroxy-3-methoxyphenyl)-methyl]-2-oxotetrahydrofuran-3-yl}methyl β-D-glucopyranoside (**2**), have been isolated from the roots of *Urtica fissa* E. PRITZ. Their structures were determined based on spectroscopic methods (HR-MS, 1D- and 2D-NMR, HMBC, HSQC, and NOESY).

Introduction. – *Urtica* species (Urticaceae) were widely used as common folk medicines and ethnic drugs in Han nationality and other minorities in China for treating many diseases such as rheumatism, inflammation of bruises and sprains, skin pruritus, postnatal convulsion, infantile convulsion, urticaria, *etc.* [1]. Nowadays, it is reported that *Urtica* plants had the effects of anti-fungal, anti-HIV, anti-proliferaty, and anti-BPH (benign prostatic hyperplasia) activities [2–5]. *Urtica fissa* E. PRITZ, a member of the *Urtica* species, is distributed widely in the southwest of China, especially in Sichuan, Guizhou, and Gansu provinces [6]. During our previous study, the BPH rats induced by testosterone propionate were taken as the animal model to screen the 20% EtOH extracts of the *Urtica* plants. *Urtica fissa* E. PRITZ was found to lower the prostatic weight of the model animals, decrease the density of lecithin corpuscle and increase the acid phosphatase level. The following chemical study led to the isolation and identification of two new secolignans (**1** and **2**) described in this work. This kind of lignans has only been found in *Piperomia* species of Piperaceae family till now [7–10].

Results and Discussion. – Compound **1** was obtained as an amorphous powder, with $[\alpha]_D^{20} = -18.8$ ($c = 0.48$, MeOH). The structure of **1** was established by analysis of HR-EI-MS, ^{1}H- and ^{13}C-NMR (*Table*), HMBC, and NOESY spectra.

The HR-EI-MS of compound **1** showed a molecular-ion peak at *m*/*z* 536.1898 corresponding to the molecular formula $C_{26}H_{32}O_{12}$. The ^{1}H-NMR data of **1** showed six signals for aromatic H-atoms at δ(H) 6.69 (*d*, $J = 8.2$, 1 H), 6.77 (*d*, $J = 8.1$, 1 H), 6.81 (*dd*, $J = 8.1, 1.9$, 1 H), 6.92 (*d*, $J = 2.0$, 1 H), 6.93 (*dd*, $J = 8.2, 1.9$, 1 H), and 6.99 (*d*, $J = 1.8$, 1 H), which indicated the presence of two 1,2,4-trisubstituted phenyl groups, two MeO signals at δ(H) 3.82, 3.85 (both *s*, 3 H), two CH_2 signals at δ(H) 4.24 (*dd*, $J = 11.0$,

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**

	1 (CD_3OD)		**2** (CD_3OD)	
	δ(C)	δ(H)	δ(C)	δ(H)
2	181.7		180.7	
3	44.9	2.74 (*dt*, *J* = 9.1, 2.4)	48.0	2.56 (*dt*, *J* = 7.3, 3.4)
4	42.9	3.67–3.73 (*m*)	41.7	3.63 (*dq*, *J* = 11.8, 7.5)
5	74.6	4.24 (*dd*, *J* = 11.0, 8.4, H_β), 3.90 (*t*, *J* = 8.2, H_α)	72.9	4.34 (*dd*, *J* = 8.9, 8.3, H_β), 3.89 (*dd*, *J* = 9.0, 7.0, H_α)
6	56.5	3.83–3.87 (*m*)	56.5	3.77 (*d*, *J* = 11.8)
7	68.9	4.19 (*dd*, *J* = 9.9, 2.5), 3.51 (*dd*, *J* = 9.9, 2.5)	68.6	3.74 (*dd*, *J* = 10.3, 2.4), 3.35 (*dd*, *J* = 10.3, 3.9)
1′	136.5		136.1	
2′	112.5	6.99 (*d*, *J* = 1.8)	113.0	6.93 (*d*, *J* = 2.0)
3′	149.1		149.1	
4′	146.2		146.5	
5′	116.3	6.69 (*d*, *J* = 8.2)	116.4	6.74 (*d*, *J* = 8.2)
6′	120.7	6.93 (*dd*, *J* = 8.2, 1.9)	121.7	6.87 (*dd*, *J* = 8.2, 2.0)
1″	136.5		135.8	
2″	112.7	6.92 (*d*, *J* = 2.0)	113.0	6.91 (*d*, *J* = 2.0)
3″	149.3		149.2	
4″	146.3		146.5	
5″	116.5	6.77 (*d*, *J* = 8.1)	116.4	6.76 (*d*, *J* = 8.1)
6″	121.2	6.81 (*dd*, *J* = 8.1, 1.9)	121.2	6.84 (*dd*, *J* = 8.1, 2.0)
1‴	104.8	4.30 (*d*, *J* = 7.8)	104.7	4.11 (*d*, *J* = 7.8)
2‴	75.1	3.22–3.26 (*m*)	75.1	3.14–3.17 (*m*)
3‴	78.1	3.22–3.26 (*m*)	78.0	3.19–3.22 (*m*)
4‴	71.6	3.28 (*t*, *J* = 8.7)	71.5	3.28–3.32 (*m*)
5‴	78.6	3.42 (*t*, *J* = 8.9)	78.0	3.28–3.32 (*m*)
6‴	62.8	3.82–3.85 (*m*), 3.64 (*dd*, *J* = 12.0, 5.7)	62.7	3.87 (*dd*, *J* = 11.9, 2.4), 3.71 (*dd*, *J* = 11.9, 5.3)
3′,3″-MeO	56.5, 56.6	3.82, 3.85 (2*s*)	56.6, 56.7	3.85, 3.87 (2*s*)

8.4, 1 H), δ(H) 3.90 (*t*, *J* = 8.2, 1 H), δ(H) 4.19 (*dd*, *J* = 9.9, 2.5, 1 H), δ(H) 3.51 (*dd*, *J* = 9.9, 2.5, 1 H), and three CH signals at δ(H) 2.74 (*dt*, *J* = 9.1, 2.4, 1 H), δ(H) 3.67–3.73 (*m*, 1 H), δ(H) 3.83–3.87 (*m*, 1 H). The ^{13}C-NMR spectrum, together with HSQC, showed twelve signals for aromatic C-atoms at δ(C) 112.5, 112.7, 116.3, 116.5, 120.7, 121.2, 136.5, 136.5, 146.2, 146.3, 149.1, and 149.3, two CH_2 signals at δ(C) 74.6, 68.9, three CH signals at δ(C) 44.9, 42.9, 56.5, one CO signal at δ(C) 181.7, and two MeO signals at δ(C) 56.5 and 56.6.

Additionally, the ^{1}H- and ^{13}C-NMR spectra showed signals for a sugar moiety at δ(H) 4.30 (*d*, *J* = 7.8, 1 H), 3.22–3.26 (*m*, 2 H), 3.28 (*t*, *J* = 8.7, 1 H), 3.42 (*t*, *J* = 8.9, 1 H), 3.82–3.85 (*m*, 1 H), and 3.64 (*dd*, *J* = 12.0, 5.7, 1 H), and at δ(C) 104.8, 75.1, 78.1, 71.6, 78.6, and 62.8. Based on the HSQC, HMBC, and NOESY data, this sugar moiety was elucidated as a β-glucopyranosyl moiety. The absolute configuration of the sugar moiety is very likely to be D, but clear-cut experimental evidence was absent.

The HMBC correlations between two MeO groups at δ(H) (3.82, 3.85) and C(3′) and C(3″), respectively, between H–C(2′), H–C(5′), and H–C(6′) and C(4′), between H–C(5″), H–C(6″), and H–C(2″) and C(4″) suggested that the two benzene rings

were 3′(3″)-MeO- and 4′(4″)-OH-substituted. The presence of a bis(4-hydroxy-3-methoxyphenyl)methyl group was proved by HMBC cross-peaks between C(6) and the aromatic H-atoms H–C(1′), H–C(1″), H–C(2′), H–C(2″), H–C(6′), and H–C(6″). A γ-butyrolactone ring was deduced from the HMBC cross peaks between H–C(3), H–C(4), H–C(5) and C(2). The bis(4-hydroxy-3-methoxyphenyl)methyl group was suggested to be located at C(4) of the γ-butyrolactone ring by HMBC correlations between H–C(4) and H–C(5).

The glycopyranosyl unit was shown to be attached to a CH_2 group ($CH_2(7)$), demonstrated by the HMBC between H–C(1‴) of the glucopyranosyl moiety and $CH_2(7)$. The HMBC between $CH_2(7)$ and C(2), C(3), and C(4) suggested the structure of **1** as {(3*S*,4*S*)-4-[bis(4-hydroxy-3-methoxyphenyl)methyl]-2-oxotetrahydrofuran-3-yl}methyl β-D-glucopyranoside. The NOESY correlation between H–C(3) and H–C(4) indicated the *cis* configuration at C(3) and C(4) (*Fig. 1*).

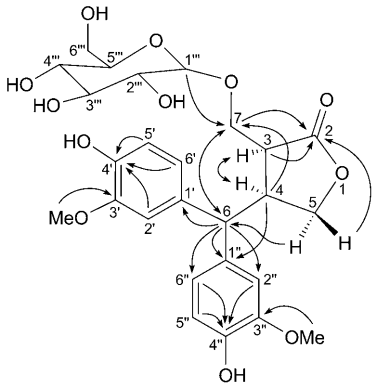

Fig. 1. *Structure and key HMBC* (→) *and NOESY* (↔) *correlations of* **1**

Compound **2** was obtained as an amorphous powder, with $[\alpha]_D^{20} = -39.1$ ($c = 1.15$, MeOH). The structure of **2** was established by analysis of HR-ESI-MS, ^{1}H- and ^{13}C-NMR (*Table*), HMBC, and NOESY.

The HR-ESI-MS of compound **2** gave a molecular-ion peak at *m*/*z* 536.1904, in accordance with the molecular formula $C_{26}H_{32}O_{12}$. The ^{1}H- and ^{13}C-NMR spectra of compound **2** were very similar to those of compound **1** (*Table*), except the *J* values between H–C(3) and H–C(4) ($J(3,4) = 9.1$ in **1**, but $J(3,4) = 7.3$ in **2**). So **2** was proposed to be a stereoisomer of **1**. It was further determined that **2** had *trans* configuration at C(3) and C(4) by NOESY correlation between H–C(3) and H–C(6), and between H–C(4) and H–C(7) (*Fig. 2*). The correlations of the ^{1}H signals to ^{13}C signals were determined by a HSQC spectrum as shown in the *Table*. Thus, the structure of **2** was determined as {(3*S*,4*R*)-4-[bis(4-hydroxy-3-methoxyphenyl)methyl]-2-oxotetrahydrofuran-3-yl}methyl β-D-glucopyranoside.

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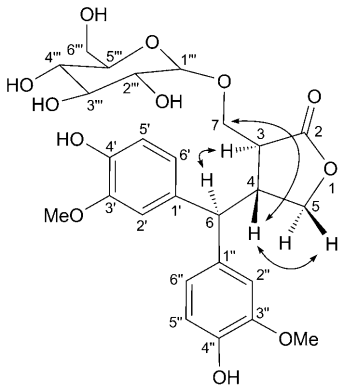

Fig. 2. *Structure and key NOESY* (↔) *correlations of* **2**

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical Group, Co.*), *C18 reverse-phase* (*RP*) silica gel (250 mesh, *Merck*), or *Sephadex LH-20* (*Pharmacia*). TLC: pre-coated SiO_2 GF_{254} plates (*Qingdao Marine Chemical Group, Qingdao*). HPLC: *Waters LC 515*. UV Spectra: *Hitachi U-2010*. NMR Spectra: *Bruker-ARX-500* spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz); TMS as internal standard; δ in ppm, J in Hz. HR-EI-MS: *ZabSpec* magnetic mass spectrometer. HR-ESI-MS: *Nano LC-Q-TOF2* mass spectrometer.

The roots of *Urtica fissa* E. PRITZ were collected in August 2004, in Sichuan province, P. R. China. A voucher specimen, identified by Prof. *Chen Chen* (Liaoning Normal University), was deposited with registration No. 04026 in the College of Bioengineering of Dalian University.

The root powder of *Urtica fissa* E. PRITZ (8 kg) was extracted with EtOH (95%) under reflux and then filtered by gauze. The EtOH extract was concentrated by evaporation and the residue suspended in H_2O and extracted with petroleum ether (PE) and AcOEt successively. The H_2O layer was evaporated and the residue was separated into several fractions by polyporous resin (*D101*), eluting with EtOH/H_2O (30:70, 50:50, and 100:0). The fraction eluted with EtOH/H_2O 50:50 was separated into several fractions by CC over SiO_2, eluting with $CHCl_3$/MeOH 30:1, 15:1, 10:1, 5:1, 2:1, and 0:1. The fraction eluted with $CHCl_3$/MeOH (5:1) was separated by HPLC (*ODS* column, 8 μM, 250 × 10 mm, flow rate 3.0 ml/min, UV 254 nm), eluting with MeOH/H_2O (40:60) to afford **1** (8 mg) and **2** (6 mg).

*{(3*S*,4*S*)-4-[Bis(4-hydroxy-3-methoxyphenyl)methyl]-2-oxotetrahydrofuran-3-yl}methyl β-*D*-Glucopyranoside* (**1**). $[\alpha]_D^{20} = -18.8$ ($c = 0.48$, MeOH). NMR Data: see *Table*. HR-EI-MS: 536.1898 (calc. 536.1894).

*{(3*S*,4*R*)-4-[Bis(4-hydroxy-3-methoxyphenyl)methyl]-2-oxotetrahydrofuran-3-yl}methyl β-*D*–Glucopyranoside* (**2**). $[\alpha]_D^{20} = -39.1$ ($c = 1.15$, MeOH). NMR Data: see *Table*. HR-ESI-MS: 536.1904 (calc. 536.1894).

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