New Chemical Constituents of Roots of *Urtica triangularis* HAND-MASS

Xing-Guo YAN,[a] Jing-Ming JIA,[b] Ling TANG,[a] Li-Ying SHI,[a] Yong-Qi WANG,[a] and Bao-Min FENG*,[a,c]

[a] *College of Bioengineering, Dalian University, Dalian Development Zone;* [c] *Liaoning Key Lab of Bioorganic Chemistry, Dalian University, Dalian Development Zone; Dalian 116622, China: and* [b] *School of Traditional Chinese Medicines, Shenyang Pharmaceutical University; Shenyang 110016, China.*

Received March 12, 2008; accepted July 25, 2008; published online July 29, 2008

Studies on the chemical constituents of roots of *Urtica triangularis* HAND-MASS have led to the isolation of four new compounds. The structures, including the absolute configurations, of these constituents have been elucidated through spectral studies including ^{1}H-NMR, ^{13}C-NMR, 2D-NMR experiments (heteronuclear single-quantum coherence, heteronuclear multiple bonding connectivity and nuclear Overhauser effect spectroscopy), high resolution mass spectroscopy (HR-MS) and circular dichroism as (−)-4-methoxy-8′-acetyl olivil, (−)-4-methoxy-8′-acetyl olivil-4-*O*-α-arabinopyronosyl-(1→6)-β-glucopyranoside, (−)-olivil-9-*O*-β-glucopyranoside and cyclo-olivil-9-*O*-β-glucopyranoside.

Key words Urticaceae; *Urtica triangularis* HAND-MASS; lignan

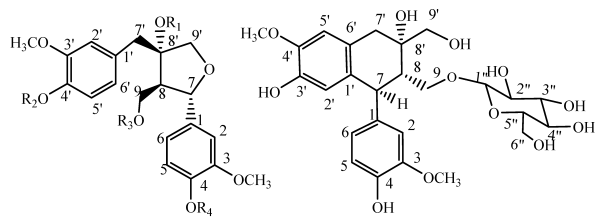
Urtica triangularis HAND-MASS (Urticaceae), a perennial herb indigenous to China, is distributed widely in the southwest of China, especially in Sichuan, Hunan and Gansu provinces.[1,2] It is mainly used as a folk medicine for treating rheumatism.[2] The chemical constituents of *Urtica triangularis* HAND-MASS have never been reported yet. This paper deals with the isolation and structural identification of a new lignan and three new lignan glycosides from the roots of this plant.

Results and Discussion

The EtOH extract of the dried roots of *Urtica triangularis* HAND-MASS was concentrated and partitioned with ether, EtOAc and *n*-BuOH successively. The EtOAc fraction was separated by a silica gel column chromatography to yield one new lignan (**1**) and the *n*-BuOH fraction was purifed by polyporous resin, silica gel and HPLC to yield three new lignan glycosides (**2**—**4**). They were identified as (−)-4-methoxy-8′-acetyl olivil (**1**), (−)-4-methoxy-8′-acetyl olivil 4-*O*-α-arabinopyronsyl-(1→6)-β-glucopyranoside (**2**), (−)-olivil-9-*O*-β-glucopyranoside (**3**) and cyclo-olivil-9-*O*-β-glucopyranoside (**4**) respectively, based on the analyses of NMR, HR-MS, circular dichroism (CD) and other physicochemical properties. The structures of compounds **1**—**4** were shown in Fig. 1.

Compound **1** was obtained as white needles, mp 101—102 °C (CH_3COCH_3), $[\alpha]_D^{20}$ −36.1 (MeOH). Its HR-EI-MS showed $[M]^+$ at *m*/*z* 432.1707, corresponding to the molecular formula $C_{23}H_{28}O_8$ (Calcd 432.1784). The ^{1}H-NMR spectrum of **1** showed a phenolic hydroxyl proton signal at δ 7.40 (1H, s), six aromatic hydrogen signals at δ 7.01 (1H, d, *J*=1.8 Hz), 6.89 (1H, d, *J*=8.3 Hz), 6.93 (1H, dd, *J*=8.5, 2.1 Hz), 6.73 (1H, d, *J*=1.8 Hz), 6.77 (1H, d, *J*=8.1 Hz) and 6.62 (1H, dd, *J*=8.2, 2.1 Hz) which belonged to two 1,2,4-subsitituted phenyl groups, three methylene signals at δ 3.96 (2H, m, $-CH_2$-9′), 3.93 (2H, m, $-CH_2$-9), 3.78 (1H, d, *J*=14.3 Hz, H-7′a) and 3.08 (1H, d, *J*=14.3 Hz, H-7′b), two methines at δ 4.97 (1H, d, *J*=6.4 Hz, H-7) and 2.69 (1H, m, H-8), three methoxyls substituted to benzene at δ 3.85 (3H, s, $-OCH_3$), 3.79 (3H, s, $-OCH_3$), 3.786 (3H, s, $-OCH_3$), one methyl at δ 1.88 (3H, s) and an alcoholic hydroxyl at δ 4.07 (1H, t, *J*=4.5 Hz). Twenty three signals were found in ^{13}C-NMR including one carbonyl carbon (171.6), six tertary aromatic carbons (150.3, 149.7, 148.1, 146.3, 136.7, 129.2) and six aromatic methines (123.0, 119.2, 115.3, 113.9, 112.7, 111.0), one aliphatic tertary carbon (92.8), two aliphatic methines (84.3, 58.5), three aliphatic methylenes (78.3, 60.1, 35.8) and four methyls (56.3, 56.2, 56.1, 22.3). The above data were very similar to those of olivil[3] but one additional methoxy group and an acetyl group. The methoxy group was deduced to be attached to C-4 as demonstrated by the heteronuclear multiple bonding connectivity (HMBC) correlations between –OH (δ_H 7.40) and C-3′ (δ_C 148.1), C-4′ (δ_C 146.3) and C-5′ (δ_C 115.3). The HMBC correlation between –OH (δ_H 4.07) and C-9 (δ_C 60.1) indicated that the acetyl group was linked to C-8′. The relative configurations between C-7 and C-8, C-8 and C-8′ were determined to be *trans* and *cis*, respectively by the correlations between H-7 and 9-CH_2, 9-CH_2 and 7′-CH_2 in nuclear Overhauser effect spectroscopy (NOESY). The CD spectrum of **1** further showed two negative cotton effects [Δε −2.02 (282.7 nm), Δε −8.58 (232.8 nm)] similar to those of (−)-olivil glycoside,[4] suggesting C-7, 8 and 8′ have *S*, *R* and *S* configurations. The above analyses led to the structure of **1**, (−)-4-methoxyl-8′-acetyl oilvil.

Compound **2** was obtained as amorphous powder, $[\alpha]_D^{20}$ −30.8 (MeOH). Its molecular formula was deduced as $C_{34}H_{46}O_{17}$ (Calcd 725.2735) by HR-ESI-MS $[M-1]^-$ (*m*/*z* 725.2866). The ^{1}H-NMR (Table 1) displayed features similar to those of compound **1** excpept the presence of the proton signals of sugar moiety at δ 3.20—4.90. The ^{13}C-NMR spec-



1 R_1 = CH_3CO R_2 = H R_3 = H R_4= CH_3
2 R_1 = CH_3CO R_2 = Ara(1-6)-Gle–
R_3 = H R_4= CH_3
3 R_1 = H R_2 =H R_3 = Glc R_4= H

4

Fig. 1. Structures of Compounds **1**—**4**

* To whom correspondence should be addressed. e-mail: fbmdlu@163.com

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** (CD_3COCD_3) and **2** (CD_3OD) (δ in ppm, J in Hz)

No.	δ_H of **1**	δ_H of **2**	δ_C of **1**	δ_C of **2**
1			136.7	136.6
2	7.01, 1H, d, J=1.8	7.0, 1H, d, J=1.7	111.0	111.2
3			150.3	150.6
4			149.7	150.5
5	6.89, 1H, d, J=8.3	6.91, 1H, d, J=8.3	112.7	113
6	6.93, 1H, dd, J=8.5, 2.1	6.91, H, dd, J=8.3, 1.7	119.2	119.8
7	4.97, 1H, d, J=6.4	4.96, 1H, d, J=6.05	84.3	85.1
8	2.69, 1H, m	2.70, 1H, m	58.5	58.5
9	3.93, 2H, m	3.90, 2H, m	60.1	60.1
1′			129.2	133
2′	6.73, 1H, d, J=1.8	6.75, 1H, d, J=1.95	113.9	115.3
3′			148.1	150
4′			146.3	146.9
5′	6.77, 1H, d, J=8.1	7.15, 1H, d, J=8	115.3	118.2
6′	6.62, 1H, dd, J=8.2, 2.1	6.73, 1H, dd, J=8.1, 1.95	123.0	123.6
7′	3.08, 1H, d, J=14.3	3.06, 1H, d, J=14.3	35.8	36.3
	3.78, 1H, d, J=14.3	3.78, 1H, d, J=14.3		
8′			92.8	92.7
9′	3.96, 2H, m	4.02, 1H, d, J=10.1	78.3	78.3
		3.96, 1H, d, J=9.95		
–OMe-3′	3.85, 3H, s	3.84, 3H, s	56.3	56.8
–OMe	3.790, 3.786 each 3H, s	3.814, 3.810 each 3H, s	56.2	56.6
			(or 56.1)	(or 56.5)
–CO–			171.6	173.1
$-CH_3$	1.88, 3H, s	1.88, 3H, s	22.3	22.4
–OH-9	4.07, 1H, t, J=4.5			
–OH-4′	7.40, 1H, s			
Glc-1		4.86, 1H, d, J=7.35		102.8
2		3.50, 1H, m		74.9
3		3.48, 1H, m		77.8
4		3.39, 1H, m		71.6
5		3.62, 1H, m		77.2
6		3.75, 1H, m		69.4
		4.11, 1H, dd, J=11.5, 2.1		
Ara-1		4.29, 1H, d, J=6.7		105.1
2		3.57, 1H, dd, J=8.7, 6.8		72.4
3		3.49, 1H, m		74.1
4		3.74, 1H, m		69.5
5		3.83, 1H, m		66.6
		3.45, 1H, dd, J=5.8, 4.2		

Table 2. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Data of **3** and **4** (in CD_3OD) (δ in ppm, J in Hz)

No.	δ_H of **3**	δ_H of **4**	δ_C of **3**	δ_C of **4**
1			129.0	133.5
2	6.93, 1H, d, J=1.6	6.80, 1H, d, J=1.9	114.1	114.4
3			147.7	149.1
4			145.9	148.1
5	6.72, 1H, d, J=8.0	6.77, 1H, d, J=8.0	114.4	117.3
6	6.74, 1H, dd, J=8.1, 1.7	6.67, 1H, dd, J=7.6, 2.0	122.7	123.4
7	4.74, 1H, d, J=8.2	4.12, 1H, d, J=11.8	83.9	44.9
8	2.47, 1H, td, J=7.7, 5.2	2.23, 1H, ddd, J=11.9, 3.8, 2.2	58.8	46.7
9	3.74, 1H, dd, J=10.3, 7.4	3.47, 1H, dd, J=10.6, 3.8	67.0	69.2
	4.12, 1H, dd, J=10.3, 5.1	4.14, 1H, dd, J=10.6, 2.2		
1′			133.4	138.2
2′	7.12, 1H, d, J=1.8	6.19, 1H, s	110.2	113.0
3′			147.2	147.5
4′			144.8	145.3
5′	6.75, 1H, d, J=8.1	6.63, 1H, s	114.4	116.2
6′	6.89, 1H, d, J=8.2, 1.9		119.4	126.6
7′	2.92, d, J=14.1	2.62, d, J=16.3	39.3	40.0
	3.06, d, J=14.1	3.27, d, J=16.5		
8′			81.0	74.5
9′	3.62, 1H, d, J=9.2	3.55, d, J=11.3	76.3	68.9
	3.86, 1H, m	3.84, d, J=11.3		
1″	4.30, 1H, d, J=7.8	4.08, 1H, d, J=7.8	103.5	105.3
2″	3.22, 1H, dd, J=9.2, 7.8	3.17, 1H, m	73.8	75.1
3″	3.38, 1H, m	3.29, 1H, m	76.7	78.1
4″	3.29, 1H, m	3.25, 1H, m	70.3	71.5
5″	3.28, 1H, m	3.20, 1H, m	76.7	78.0
6″	3.88, 1H, m	3.85, 1H, m	61.4	62.6
	3.67, 1H, dd, J=11.9, 5.4	3.65, 1H, dd, J=12.0, 5.6		
–OMe	3.85, 3.86, each 3H, s	3.80, 3.81, each 3H, s	55.1	56.4
				56.5

trum and heteronuclear single-quantum coherence (HSQC) experiment also showed the presence of six carbon signals assignable to a glucopyranosyl and five carbon signals assignable to an arabinose (Table 1).[5] The coupling constant of the anomeric proton (4.86, 1H, d, J=7.35 Hz) indicated β configuration of the C-1 of glucopyranosyl. The α orientation of the C-1 of arabinosyl was deduced from the coupling constant of the anomeric proton (4.29, 1H, d, J=6.7 Hz). The sugar unit of **2** was shown to be attached to C-4′ of the aglycone as demonstrated by the HMBC correlations between H-1 (δ_H 4.86) of the glucopyranosyl and C-4′ (δ_C 146.9) of the aglycone. The arabinosyl was shown to be attached to C-6 of the glucopyranosyl as demonstrated by the HMBC correlation between H-1 (δ_H 4.29) of the arabinosyl and C-6 (δ_C 69.4) of the glucopyranosyl. Like compound **1** the relative stereochemistry between C-7 and C-8, C-8 and C-8′ was also determined by NOESY and the CD spectrum of **2** showed two negative cotton effects [$\Delta\varepsilon$ −2.34 (278.2 nm), $\Delta\varepsilon$ −9.58 (229.7 nm)]. Therefore compound **2** was elucidated as (−)-4-methoxy-8′-acetylolivil 4-*O*-α-arabinopyronsyl-(1→6)-β-glucopyranoside (Fig. 1).

Compound **3** was obtained as amorphous powder, $[\alpha]_D^{20}$ −10.3 (MeOH). Its HR-ESI-MS showed $[M-1]^-$ at m/z 537.2049, so its molecular formula was deduced to be $C_{26}H_{34}O_{12}$ (Calcd 537.2050). On the basis of ^{1}H- and ^{13}C-NMR data of compound **3** (Table 2) and further HSQC and HMBC spectroscopic investigations, the skeleton of **3** was elucidated as olivil.[6] Seven proton signals at δ 3.20—4.32 in ^{1}H-NMR and 6 carbon signals at δ 103.5, 73.8, 76.7, 70.3, 76.7, 61.4 in ^{13}C-NMR suggested the presence a glucopyranosyl.[6] The coupling constant of the anomeric proton (4.30, 1H, d, J=7.8 Hz) indicated a β configuration. The glycopyranosyl was shown to be located at C-9 of the aglycone proved by the HMBC correlation between H-1″ (δ_H 4.30) and C-9. On the basis of NOESY and CD analyses it was suggested that C-7, 8 and 8′ had *S*, *R* and *S* configurations.[4] Thus, the structure of compound **3** was determined as (−)-olivil 9-*O*-β-glucopyranoside (Fig. 1).

Compound **4** was obtained as an amorphous powder, $[\alpha]_D^{20}$ +26.1 (MeOH). It had a molecular formula $C_{26}H_{34}O_{12}$ (Calcd 537.2050) by the analysis of HR-ESI-MS $[M-1]^-$ (m/z 537.2047). By comparison of the NMR data (Table 2) with literature[6] and further HSQC, and HMBC spectroscopic investigations (Fig. 2), the skeleton of **4** was elucidated as cyclo-olivil. Relative configuration between C-7 and C-8 was establishes as *trans* by the coupling constant value ($J_{7,8}$=11.8 Hz). Relative stereochemistry between C-8 and C-8′ was also determined to be *trans* on the basis of NOE correlation between H-8 and H-9′. Like compound **3**, the proton signals at δ 3.20—4.32 in ^{1}H-NMR and 6 carbon signals at δ 105.3, 75.1, 78.1, 71.5, 78.0, 62.6 in ^{13}C-NMR was also from

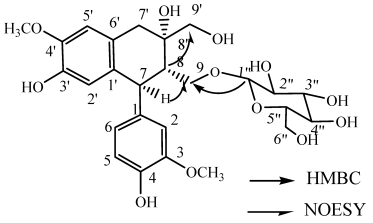


Fig. 2. The Key HMBC and NOESY Correlations of Compound **4**

a glucopyranosyl.[7] A β configuration of the anomeric carbon was deduced on the basis of the coupling constant of H-1 (4.08, 1H, d, J=7.8 Hz) of the glucopyranosyl. The sugar moiety was linked with C-9 based on the HMBC correlation between H-1″ (δ_H 4.08) and C-9. Therefore, the structure of **4** was determined as cyclo-olivil-9-*O*-β-glucopyranoside (Fig. 1) and the absolute configuration was not determined.

Experimental

General Experimental Procedures. General Column chromatography (CC): silica gel (200—300 mesh, Qingdao Marine Chemical Group, Co; C_{18} reverse-phase silica gel (ODS), 250 mesh, Merck); TLC: pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Group, Qingdao). UV Spectra: Hitachi U-2010. NMR spectra: Bruker-ARX-500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz), TMS as internal standard, δ in ppm, J in Hz. The HR-EI-MS spectra were recorded on a ZabSpec magnetic mass spectrometer. The HR-ESI-MS spectra were recorded on an Nano LC-Q-TOF2 mass spectrometer. HPLC: Waters LC 515. The circular dichrosim: JASCO-J810, Japan.

Roots of *U. triangularis* Hand-Mass were collected in August 2004, in Sichuan province, China. A voucher specimen, identified by Prof. Chen Chen (Liaoning Normal University), was deposited with registration No. 04008 in the College of Bioengineering of Dalian University.

The dried roots powder of *U. triangularis* Hand-Mass (10 kg) was extracted with EtOH (95%) under reflux and then filtered by gauze. The EtOH extract was concentrated by evaporation to afford the residue which was extracted with petroleum ether, EtOAC and *n*-BuOH successively suspended in H_2O. The EtOAc extract was separated by repeated column chromatography to yield compound **1** (40 mg). The *n*-BuOH soluble fraction was evaporated to afford the residue, which was separated into several fractions by polyporous resin (D101), eluting with EtOH : H_2O (30 : 70, 50 : 50, 100 : 0). The fraction eluted with EtOH : H_2O (30 : 70) was separated into several fractions by column chromatography on silica gel, eluted with $CHCl_3$ and by mixtures containing increasing amount of MeOH. The fraction eluted with $CHCl_3$: MeOH (5 : 1) was further separated by HPLC (ODS column, 8 μM, 250×10 mm, flow rate 3.0 ml/min, UV 254 nm) eluted with MeOH : H_2O (25 : 75) to afford **2** (65 mg), **3** (92 mg) and **4** (110 mg).

Compound (**1**): White needles; $[\alpha]_D^{20}$ −36.1 (c=0.4, MeOH); CD (c=1.0 g/l) $\Delta\varepsilon$ (nm) −2.02 (282.7), −8.58 (232.8); mp 101—102 °C (CH_3COCH_3); ^{1}H-NMR (CD_3COCD_3, 500 MHz) and ^{13}C-NMR (125 MHz), see Table 1; HR-EI-MS *m*/*z*: 432.1707 (Calcd for $C_{23}H_{28}O_8$, 432.1784).

Compound (**2**): Amorphous powder; $[\alpha]_D^{20}$ −30.8 (c=0.1, MeOH); CD (c=1.0 g/l) $\Delta\varepsilon$ (nm) −2.34 (278.2), −9.58 (229.7); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz), see Table 1; HR-ESI-MS $[M-1]^-$ at *m*/*z*: 725.2866 (Calcd for $C_{34}H_{46}O_{17}$, 725.2735).

Compound (**3**): Amorphous powder; $[\alpha]_D^{20}$ −10.3 (c=0.1, MeOH); CD (c=0.5 g/l) $\Delta\varepsilon$ (nm) −1.79 (288.2), −10.23 (232.7); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz), see Table 2; HR-ESI-MS $[M-1]^-$ at *m*/*z*: 537.2049 (Calcd for $C_{26}H_{34}O_{12}$, 537.2050).

Compound (**4**): Amorphous powder; $[\alpha]_D^{20}$ +26.1 (c=0.2, MeOH); CD (c=1.5 g/l) $\Delta\varepsilon$ (nm) 10.88 (216.3), 6.62 (238.4), 5.43 (276.2), −24.09 (292.1); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz), see Table 2; HR-ESI-MS $[M-1]^-$ at *m*/*z*: 537.2047 (Calcd for $C_{26}H_{34}O_{12}$, 537.2050).

Acknowledgement This project was financially supported by the National Natural Science Foundation of China (No. 30572317) and research project of the Educational Department of Liaoning Province (2004F114).

References

1) Wang M. Y., Wei Y. F., Xing F., Shi Y., *J. Chin. Med. Mater.*, **12**, 857—859 (2001).
2) Wang M. Y., Wei Y. F., Shi Y., Yao Y., *Lishizhen Med. Mater. Med. Res.*, **12**, 676—677 (2001).
3) Kadowaki E., Yoshida Y., Nitoda T., Baba N., Nakajima S., *Biosci. Biotechnol. Biochem.*, **67**, 415—419 (2003).
4) Machida K., Takano M., Kakuda R., *Chem. Pharm. Bull.*, **50**, 669—671 (2002).
5) Shi B. J., Li Q., Zhang Q. X., Wang Y., *Acta Pharm. Sin.*, **42**, 862—866 (2007).
6) Wang H. B., Yu D. Q., Liang X. T., *J. Nat. Prod.*, **52**, 342—345 (1989).
7) Feng W. S., Hao Z. Y., Zheng X. K., Kuang H. X., *Acta Pharm. Sin.*, **42**, 625—630 (2007).