Hastatusides A and B: Two New Phenolic Glucosides from *Rumex hastatus*

by **Lan-Sheng Zhang**[a])[b]), **Zhen Li**[a])[c]), **Ren-Qiang Mei**[a]), **Guang-Ming Liu***[b]), **Chun-Lin Long**[d]), **Yue-Hu Wang**[d]), and **Yong-Xian Cheng***[a])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China (phone: +86-871-5223048; fax: +86-871-5223048; e-mail: yxcheng@mail.kib.ac.cn)

[b]) College of Pharmacy, Dali University, Dali 671000, Yunnan, P. R. China (phone: +86-872-2257390; fax: +86-872-2257401; e-mail: lgm888999@yahoo.com.cn)

[c]) College of Marine Life Sciences, Ocean University of China, Qingdao 266003, Shandong, P. R. China

[d]) Department of Ethnobotany, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China

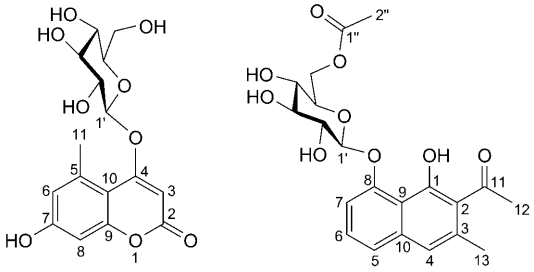
Two new compounds, hastatusides A and B (**1** and **2**, resp.), together with five known compounds, resveratrol, rumexoside, torachrysone-8-yl β-D-glucopyranoside, rutin, nepodin, and orientaloside were isolated from the roots of *Rumex hastatus*. Their structures were determined by spectroscopic methods, including 1D- and 2D-NMR spectroscopy.

Introduction. – In continuation of our search for new bioactive compounds in *Rumex nepalensis*, we have investigated the chemical constituents of the roots of *R. hastatus* D. DON. The plant belongs to the family Polygonaceae, and is widely distributed in the Yunnan, Sichuan, and Tibet Provinces of China. It has been used in ancient and current traditional Chinese medicine for the treatment of cough, headache, and fever [1]. Up to now, several anthraquinones have been isolated from the plant [2]. On the other hand, information on the other types of chemical constituents of this plant is still scarce.

Here, we report on the constituents of the 95% EtOH extract of the roots of *R. hastatus*, from which eight non-anthraquinone compounds were isolated. Compounds **1** and **2** (*Fig. 1*), named hastatusides A and B, respectively, are new natural products, and the six known compounds were isolated for the first time from this plant.

Results and Discussion. – Compound **1** was obtained as colorless crystals. The molecular formula was determined as $C_{16}H_{18}O_9$ by HR-ESI-MS (negative-ion mode; m/z 353.0864 ($[M-H]^-$; calc. 353.0872)). The IR spectrum of **1** showed the presence of a CO group (1685 cm^{-1}). Analysis of the ^1H- and ^{13}C-NMR data (*Table*) indicated the presence of a coumarin derivative. The diagnostic UV absorptions at 262 and 313 nm were also indicative for a coumarin skeleton. HMBC and HMQC experiments allowed the assignment of all H- and C-atoms.

The ^1H-NMR spectrum of **1** indicated a sugar moiety with an anomeric H-atom (δ(H) 5.77, *d*, $J=7.6$) with a β-configuration together with three olefinic H-atoms (δ(H) 6.92, *s*; 6.87, *s*; 6.28, *s*), and a Me group (δ(H) 2.82, *s*). The ^{13}C-NMR spectrum



1 **2**

Fig. 1. *Structures of* **1** *and* **2** *isolated from* R. hastatus

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 400 and 100 MHz resp.; δ in ppm, J in Hz.

Position	**1** (in (D_5)pyridine)		**2** (in (D_6)DMSO)	
	δ(C)	δ(H)	δ(C)	δ(H)
1			150.3 (*s*)	
2	163.0 (*s*)		125.4 (*s*)	
3	90.7 (*d*)	6.28 (*s*)	133.0 (*s*)	
4	168.2 (*s*)		119.6 (*d*)	7.22 (*s*)
5	139.6 (*s*)		122.5 (*d*)	7.47 (*d*, $J = 8.0$)
6	117.2 (*d*)	6.87 (*s*)	127.4 (*d*)	7.40 (*t*, $J = 8.0$)
7	162.2 (*s*)		110.6 (*d*)	7.25 (*d*, $J = 8.0$)
8	101.5 (*d*)	6.92 (*s*)	154.1 (*s*)	
9	157.8 (*s*)		113.2 (*s*)	
10	107.2 (*s*)		135.9 (*s*)	
11	23.9 (*q*)	2.82 (*s*)	204.9 (*s*)	
12			32.1 (*q*)	2.51 (*s*)
13			19.3 (*q*)	2.24 (*s*)
Glc:				
1′	101.3 (*d*)	5.77 (*d*, $J = 7.6$)	102.3 (*d*)	5.10 (*d*, $J = 7.2$)
2′	74.9 (*d*)	4.31–4.35 (*m*)	73.3 (*d*)	3.32–3.42 (overlapped)
3′	79.1 (*d*)	4.07–4.09 (*m*)	76.0 (*d*)	3.32–3.42 (overlapped)
4′	71.3 (*d*)	4.35–4.37 (*m*)	69.9 (*d*)	3.20–3.26 (*m*)
5′	78.7 (*d*)	4.33–4.36 (*m*)	74.2 (*d*)	3.69–3.73 (*m*)
6′	62.6 (*t*)	4.43 (*dd*, $J = 11.6, 3.2$), 4.33–4.36 (overlapped)	63.3 (*t*)	4.36 (*dd*, $J = 12.0, 2.0$), 4.13 (*dd*, $J = 12.0, 6.8$)
1″			170.3 (*s*)	
2″			20.7 (*q*)	2.03 (*s*)

showed the presence of a sugar moiety corresponding to a glucopyranose (δ(C) 101.3, 79.1, 78.7, 74.9, 71.3, and 62.6), together with three CH groups (δ(C) 117.2, 101.5, and 90.7), five olefinic quaternary C-atoms, and a CO group. The ^{1}H- and ^{13}C-NMR data indicated **1** to be a coumarin glucoside. The aglycone was identified as 4,7-dihydroxy-5-methylcoumarin by comparing its spectroscopic data with those of 5-methylcoumarin-4-yl β-D-glucoside and 4,7-dihydroxy-5-methylcoumarin [3]. This conclusion was

further confirmed by the following HMBC correlations (*Fig. 2*): the H-atoms of Me(11) with C(5), C(6), and C(10); H–C(6) with C(7), C(8), and C(10); H–C(8) with C(10); and H–C(3) with C(2) and C(10). The linkage of sugar moiety was deduced from the correlation between H–C(1′) and C(4). Acid hydrolysis afforded D-glucose which was identified by TLC comparison with an authentic sample, and its optical rotation ($[\alpha]_D^{26.0} = +52.60$ ($c = 0.06$, H_2O)). Taken together, the structure of the new compound **1** was deduced as 7-hydroxy-5-methylcoumarin-4-yl β-D-glucopyranoside, and named hastatuside A.

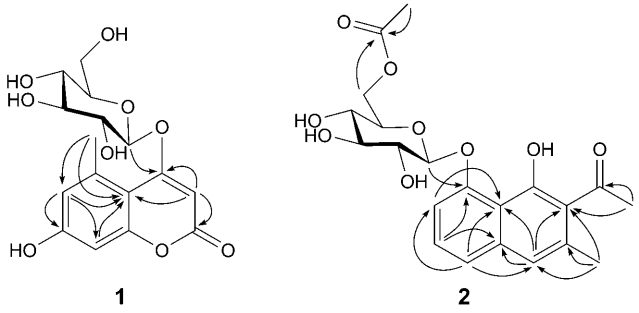


Fig. 2. *Key HMBC correlations for* **1** *and* **2**

Compound **2** was obtained as a pale yellow amorphous powder. Its molecular formula was determined as $C_{21}H_{24}O_9$ by HR-ESI-MS (negative-ion mode; m/z 419.1338 ($[M-H]^-$; calc. 419.1342)). The IR spectrum of **2** showed the presence of OH (3384 cm^{-1}) and CO groups (1737, 1679 cm^{-1}), and a naphthalene ring (1631, 1579 cm^{-1}). Analysis of the ^1H- and ^{13}C-NMR data (*Table*) indicated the presence of a naphthalenol. The ^1H-NMR spectrum of **2** indicated a sugar moiety with an anomeric H-atom (δ(H) 5.10, *d*, $J = 7.2$) with a β-configuration.

The ^1H-NMR spectrum of **2** showed signals of one benzene ring bearing a H-atom (δ(H) 7.22, *s*), and another benzene ring bearing three H-atoms (δ(H) 7.47, *d*, $J = 8.0$; δ(H) 7.40, *t*, $J = 8.0$; δ(H) 7.25, *d*, $J = 8.0$). Furthermore, the NMR spectra displayed signals of an AcO (δ(C) 204.9, 32.1) and a Me group (δ(C) 19.3; δ(H) 2.24 (*s*)). The aglycone was identified as nepodin by comparing their spectroscopic data [4]. This conclusion was confirmed by the following HMBC correlations (*Fig. 2*): the H-atoms of Me(12) with C(2) and C(11); the H-atoms of Me(13) with C(2), C(3), and C(4); H–C(4) with C(2), C(9), C(10), and Me(13); H–C(5) with C(4), C(7), C(9), and C(10); H–C(6) with C(8) and C(10); and between H–C(7) and C(5), C(8), and C(9). Besides, the HMBC correlation between H–C(1′) and C(8) indicated that the sugar was linked at C(8) of the naphthalene. The NMR data of **2** were very similar to those of torachryson-8-yl β-D-glucopyranoside, which has been already isolated from *Rumex* species [5]. However, the ^{13}C-NMR spectrum indicated that compound **2** contained, in addition, an AcO group (δ(C) 170.3, C(1″), and 20.7, C(2″)). The position of attachment was deduced from the HMBC correlation between the H-atoms of C(6′) and C(1″). Similarly, acid hydrolysis gave D-glucose. Thus, the structure of compound **2** was deduced as nepodin-8-yl β-D-(6′-*O*-acetyl)glucopyranoside, and named hastatuside B.

The known compounds were identified as resveratrol [6], rumexoside [7], torachryson-8-yl β-D-glucopyranoside [5], rutin [8], nepodin [4], and orientaloside [7]. These compounds were all isolated for the first time from *R. hastatus.*

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Experimental Part

General. All solvents were distilled before use. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm, *Qingdao Marine Chemical Inc.*, P. R. China), *RP-18* (40–63 μm, *Daiso Co.*, Japan), *Sephadex LH-20* (*Amersham Biosciences*, Sweden), and *MCI* gel *CHP 20P* (75–150 μm, *Mitsubishikasei*, Japan). TLC: SiO_2 GF_{254} (10–40 μm, *Qingdao Marine Chemical Factory*, P. R. China). Fractions were monitored by TLC and spots were visualized by heating the SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. M.p.: *XRC-1* micro-melting point apparatus, uncorrected. Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrometer; λ_{max} in nm. IR Spectra: *Bruker Tensor 27 FT-IR* spectrophotometer; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AM-400* spectrometer; chemical shift δ in ppm rel. to Me_4Si as an internal reference, and coupling constant J in Hz. $^1H,^1H$-COSY, HMQC, and HMBC Spectra: *DRX-500* spectrometer. FAB-MS: *VG Auto Spec-3000* mass spectrometer; in m/z, with glycerol as matrix. HR-ESI-MS: *API QSTAR Pulsar 1* spectrometer.

Plant Material. The roots of *R. hastatus* were collected at suburban area of Kunming in Yunnan Province, P. R. China, in July 2007, and identified by Prof. *Hua Peng*, Kunming Institute of Botany. The voucher specimen (CHYX0184) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming, P. R. China.

Extraction and Isolation. The air-dried and powdered roots of *R. hastatus* (8.0 kg) were extracted three times with 95% EtOH (3 × 20 l, 3 d each) at r.t. The extracts were combined and evaporated to dryness under reduced pressure to afford a crude extract (574 g), which was suspended in H_2O (1000 ml), followed by successive partition with petroleum ether (PE; 3 × 1500 ml), AcOEt (3 × 1500 ml), and BuOH (3 × 1000 ml), resp. The BuOH extract (120 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 1:0 → 0:1) to afford five fractions (*Frs. 1–5*). *Fr. 1* (23 g) was further chromatographed by CC (SiO_2; $CHCl_3$/MeOH 9:1): *Frs. 1.1–1.3*. *Fr. 1.2* (6.1 g) was subjected to CC (*MCI* gel *CHP 20P*; MeOH/H_2O 60:40 to 100:0): *Frs. 1.2.1–1.2.4*. *Fr. 1.2.1* (2.3 g) was purified by CC (*Sephadex LH-20*; MeOH): **1** (16 mg) and rumexoside (17 mg). *Fr. 3* (30 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 8:2): *Frs. 3.1–3.4*. *Fr. 3.1* (8.2 g) was further separated by CC (*RP-18*; MeOH/H_2O 50:50 to 100:0): *Frs. 3.1.1–3.1.4*. *Fr. 3.1.3* (1.7 g) was purified by CC (*Sephadex LH-20*; MeOH): torachryson-8-yl β-D-glucopyranoside (31 mg). *Fr. 2* (21 g) was subjected by CC (SiO_2; $CHCl_3$/MeOH 6:4): *Frs. 2.1–2.4*. *Fr. 2.1* (5 g) was separated by CC (*RP-18*; MeOH/H_2O 50:50 to 90:10): *Frs. 2.1.1–2.1.4*. *Fr. 2.1.4* (1.2 g) was purified by CC (*Sephadex LH-20*; MeOH/H_2O 1:1): **2** (13 mg) and nepodin (28 mg). *Fr. 4* (19 g) was separated by CC (*RP-18*; MeOH/H_2O 50:50 to 90:10): *Frs. 4.1–4.4*. *Fr. 4.4* (2.1 g) was further purified by CC (*RP-18*; MeOH/H_2O 60:40 to 90:10): rutin (14 mg). *Fr. 5* (18 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 3:7): *Frs. 5.1–5.4*. *Fr. 5.2* (3.4 g) was subjected to CC (*MCI* gel *CHP 20P*; MeOH/H_2O 50:50 to 90:10): *Frs. 5.2.1–5.2.4*. *Fr. 5.2.3* (2.1 g) was further purified by CC (*RP-18*; MeOH/H_2O 60:40 to 90:10): **3** (30 mg) and orientaloside (22 mg).

Hastatuside A (= *7-Hydroxy-5-methyl-2-oxo-2*H-*chromen-4-yl β-*D-*Glucopyranoside*; **1**). Colorless crystals (in MeOH). M.p. 227–228°. $[\alpha]_D^{26.0} = +35.2$ ($c = 0.10$, MeOH). UV (MeOH): 207 (4.47), 220 (4.20), 262 (3.27), 313 (4.10). IR (KBr): 3375, 2915, 2884, 1685, 1604, 1245, 1084. ^1H- and ^{13}C-NMR: *Table.* FAB-MS (neg.): 353 ($[M-H]^-$). HR-ESI-MS (neg.): 353.0864 ($[M-H]^-$, $C_{16}H_{17}O_9^-$; calc. 353.0872).

Hastatuside B (= *7-Acetyl-8-hydroxy-6-methylnaphthalen-1-yl 6-*O-*Acetyl-β-*D-*glucopyranoside*; **2**). Pale yellow amorphous powder. M.p. 157–159°. $[\alpha]_D^{26.0} = -73.5$ ($c = 0.07$, MeOH). UV (MeOH): 240 (4.22), 263 (4.15). IR (KBr): 3384, 2959, 1737, 1679, 1631, 1579, 1355, 1250, 1080. ^1H- and ^{13}C-NMR:

Table. FAB-MS (neg.): 419 ($[M-H]^-$). HR-ESI-MS (neg.): 419.1338 ($[M-H]^-$, $C_{21}H_{23}O_9^-$; calc. 419.1342).

Acid Hydrolysis of **1** *and* **2**. A soln. of **1** (6 mg) or **2** (5 mg) in 2M HCl (6 ml) was heated in a H_2O bath at 70° for 6 h. After cooling, the mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. TLC Comparison (SiO_2, $CHCl_3$/MeOH 6:4) with authentic samples revealed the presence of glucose in the H_2O layer (R_f 0.29). Furthermore, the D-form of glucose was established by optical rotation for **1** ($[\alpha]_D^{26.0} = +52.6$ ($c = 0.06$, H_2O)) and for **2** ($[\alpha]_D^{24.0} = +36.4$ ($c = 0.11$, H_2O)).

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