Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

The constituents of *Urtica cannabina* used in Uighur medicine

Halisha Aishan, Masaki Baba, Noriaki Iwasaki, HaiXue Kuang & Toru Okuyama

To cite this article: Halisha Aishan, Masaki Baba, Noriaki Iwasaki, HaiXue Kuang & Toru Okuyama (2010) The constituents of *Urtica cannabina* used in Uighur medicine, Pharmaceutical Biology, 48:5, 577-583, DOI: 10.3109/13880200903214215

To link to this article: https://doi.org/10.3109/13880200903214215

Published online: 28 Apr 2010.

Pharmaceutical Biology, 2010; 48(5): 577–583

informa
healthcare

RESEARCH ARTICLE

The constituents of *Urtica cannabina* used in Uighur medicine

Halisha Aishan[1, 2], Masaki Baba[2], Noriaki Iwasaki[2], HaiXue Kuang[3], and Toru Okuyama[2]

[1]*Xinjiang Medical University, Urumqi, China,* [2]*Department of Natural Medicine and Phytochemistry, Meiji Pharmaceutical University, Tokyo, Japan, and* [3]*Department of Integrating Traditional Chinese With Western Medicine Heilongjiang University of Traditional Chinese Medicine, Harbin, China*

Abstract

Urtica cannabina L. (Urticaceae) is a perennial herb that grows in Xinjiang Uighur Autonomous Region (northwest China). Two megastigmanes (**1, 2**) and five flavonoid glycosides (**3-7**) were isolated from its fruit. Compound **1** was determined by spectroscopic analysis to be (+)-blumenol A, and its absolute stereochemistry was determined in detail using chemical conversion and a modification of Mosher's method. Other compounds were identified as (+)-dehydrovomifoliol (**2**), isovitexin (**3**), isoquercitrin (**4**), astragalin (**5**), afzelin (**6**), and quercitrin (**7**) using spectroscopic (NMR, HMBC, MS) and physical methods (melting point and optical rotation). Compounds **1-7** were isolated from this plant for the first time, while this is the first report of megastigmanes in the Urticaceae family. The chemotaxonomic significance of the isolation of these megastigmanes and flavonoid glycosides from *Urtica* species is discussed.

Keywords: *Flavonoid glycoside; megastigmane (+)-blumenol A; Urtica cannabina*

Introduction

"The genus Urtica (Urticaceae) contains about 50 taxa distributed from temperate to subtropical regions, e. g. Europe, North Africa, Northwest China, Japan and North America. In China, there are 23 taxa (16 species, 6 subspecies, and 1 variety). In China there are 23 taxa (16 species, 6 subspecies, and 1 variety), including seven species that are native to the Xinjiang Uighur Autonomous Region (northwest China) (Wang et al., 2002) (*Urtica cannabina* L., *U. angustfolia* Fisch., *U. laetevirens* Maxi., *U. dioica* L., *U. kunlunshanica* Y.Yang., *U. thunbergiana* Sieb. et Zucc., and *U. urens* L.).

The leaves of *Urtica* are nutritious and rich in micronutrients; however, they need to be steamed or otherwise cooked before ingestion to destroy their stinging hairs, which contain histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotrienes, 5-hydroxytryptamine, and other irritants (Wagner et al., 1994; Emmelin & Feldberg, 1949). Contact with the hairs leads to a mildly painful sting, development of an erythematous macule, and itching or numbness for a period lasting from minutes to days.

Plants of the genus *Urtica* are commonly used in the traditional medicine of Turkey (Baser et al., 1986), various countries in Europe (Cai, 2001), and Japan (Namba, 1980) for the treatment of rheumatism, internal disease, skin conditions such as rashes, bleeding due to wounds, and benign prostatic hyperplasia (BPH) (Iida et al., 1994).

The hydrophilic components of *Urtica*, including polysaccharides and lectins, appear to be important medicine, and particularly inhibit prostatic hyperplasia (Lichius et al., 1999). The importance of *Urtica* root lignans, such as (-)-3,4-divanillyltetrahydrofuran, to improve benign prostatic hyperplasia (BPH) and other androgen- and estrogen-sensitive conditions, may be due to interference with the binding of sex hormone binding globulin (SHBG) to testosterone, the testosterone receptor, and/or the SHBG receptor (Schottner et al., 1997; Hryb et al., 1995). The steroidal compounds stigmasterol, stigmast-4-en-3-one, and campesterol

Address for Correspondence: Professor Toru Okuyama, Department of Natural Medicine and Phytochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose-shi, Tokyo 204-8588 Japan. Tel./Fax: +81-42-495-8904; E-mail: okuyama@my-pharm.ac.jp

(Received 29 October 2008; revised 07 April 2009; accepted 08 April 2009)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2010 Informa UK Ltd
DOI: 10.3109/13880200903214215

http://www.informahealthcare.com/phb

inhibit the prostatic sodium/potassium pump, which may contribute to the effects of *Urtica* on BPH (Hirano et al., 1994). However, the small quantity of β-sitosterol contained in *Urtica* root (<0.01% of the total mass) is unlikely to have an effect on BPH, given that 60 mg β-sitosterol daily is the usual amount necessary to reduce symptoms (Berges et al., 1995). *U. dioica* agglutinin (UDA) is a heat- and acid-resistant lectin found in stinging *Urtica*, primarily in the root (Galelli & Truffa, 1993). UDA activates the cell-mediated immunity, not observed for any other known plant lectin (Galelli & Truffa, 1993). UDA appears to prevent formation of a systemic lupus erythematosus-like condition in mice, and has diverse antiviral effects *in vitro* (Musette et al., 1996; Balzarini et al., 1992). It also antagonizes the epidermal growth factor receptor that may inhibit the formation of BPH (Wagner et al., 1995).

In Xinjiang Uighur Autonomous Region (northwest China), *U. cannabina* aerial portion and fruit are medicinal plants used in Uighur traditional medicine (Liu et al., 1999; Yasen et al., 1999). The fruit as a component of a preparation has been used for chronic bronchitis (Yasen et al., 1999). However, there have been few studies of the constituent chemicals of the fruit. Zhang et al. (2005) isolated scutellarein-7-*O*-α-L-rhamnoside and vicenin-2 from the leaves, while Ma et al. (2004) isolated β-sitosterol, scopoletin, caprylic alcohol, daucosterol, *p*-coumaric acid methyl ester, and *p*-coumaric acid from the whole herb. In the present study, we attempted to identify useful components of the fruit of *U. cannabina* to support its development as a Uighur traditional medicinal resource.

Herein, we report the isolation of the megastigmanes **1** and **2** from a methanol (MeOH) extract of *U. cannabina* fruit. To the best of our knowledge, this is the first report on the Urticaceae family. Compounds **3-7** were also isolated from this plant for the first time. In addition, the chemotaxonomic significance of these compounds was evaluated and compared among *Urtica* species.

Materials and methods

General experimental procedures

Melting points (mp) were determined using a Yanaco micro melting point apparatus, and were uncorrected. UV spectra were obtained using a Shimadzu UV 1600 spectrophotometer and optical rotation data were obtained using a Horiba SEPA-300 digital polarimeter. Electron Ionization Mass Spectrometry (EI-MS) and Fast Atom Bombardment Mass Spectrometry (FAB-MS) data were obtained using a JEOL JMS-700 mass spectrometer. Circular dichroism (CD) spectra were obtained in MeOH using a JASCO J-820 spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained using a JEOL JNM-AL 400 spectrometer with tetramethylsilane as an internal standard. TLC was performed using silica gel 60 F_{254} TLC plates (Merck) and RP-18 F_{254S} TLC plates (Merck). Silica gel 60 N (particle size: 100-200 μm, Kanto Chemical) was used for silica gel open column chromatography. Separation was carried out using normal and reverse phase HPLC with an SPD-20A detector (Shimadzu) at 254 nm.

Plant materials

Dried fruits of *U. cannabina* were purchased from Xinjiang Hospital of Uighur Medicine (Urumqi, Xinjiang, China), for use as an herbal medicine in May 2006. A voucher specimen was identified by Kuang Hai Xue (Heilongjiang University of Traditional Chinese Medicine) and deposited at the Department of Natural Medicine and Phytochemistry, Meiji Pharmaceutical University (Tokyo) (Accession No. 06UC001).

Extraction and isolation

Dried fruits of *U. cannabina* (8.5 kg) were extracted with 13 L of MeOH under reflux for 4 h (3 times). The MeOH extract was concentrated under reduced pressure to yield a sample of 416.6 g, of which 410 g was suspended in water and partitioned with ethyl acetate (AcOEt). The AcOEt-soluble portion (10 g) was subjected to column chromatography on silica gel and eluted stepwise using a series of *n*-hexane/AcOEt mixtures (20:1, 10:1, 5:1, 2:1, 1:1, 1:2, and 1:5), AcOEt, and MeOH to yield 10 fractions (A1-A10). Fraction A7 (1034.6 mg) was further separated by chromatography on a Sephadex LH-20 column (φ 40 × 1200 mm, GE Healthcare) using MeOH into 7 subfractions, of which subfraction A7-2 (147.4 mg) was subjected to preparative reverse phase HPLC (COSMOSIL $5C_{18}$-ARII column of φ 10 × 250 mm, at 1 mL/min with UV detection at 254 nm) using MeOH/H_2O (5:4) to yield compound **1** (31.2 mg). Fraction A6 (1072.6 mg) was purified by normal phase HPLC (COSMOSIL 5SL-II column of φ 20 × 250 mm, at 2 mL/min, with UV detection at 254 nm) using *n*-hexane/AcOEt (1:2) to give 4 subfractions, of which subfraction A6-2 (32.3 mg) was purified by reverse phase HPLC on the COSMOSIL $5C_{18}$-ARII column as above using MeOH/H_2O (2:3) to yield compound **2** (5.8 mg). Fraction A10 (2.6 g) was further separated by chromatography on the Sephadex LH-20 column (φ 40 × 1200 mm, GE Healthcare) using MeOH to yield 8 subfractions, of which subfraction A10-7 (100.5 mg) was purified by reverse phase HPLC on the COSMOSIL $5C_{18}$-ARII column as above using MeOH/H_2O (5:4) to yield compounds **3** (2.9 mg) and **4** (7.7 mg). Fraction A9 (2.2 g) was further separated by chromatography

Table 1. ^{1}H-NMR data of compounds **1** and **2**.

Proton	1[a)]	2[b)]
2a	2.41 (1H, d, *J* =17.0 Hz)	2.28 (1H, d, *J* =17.0 Hz)
2b	2.20 (1H, d, *J* =17.0 Hz)	2.60 (1H, d, *J* =17.0 Hz)
4	5.87 (1H, s)	5.93 (1H, s)
7	5.75 (1H, d, *J* =15.6 Hz)	6.99 (1H, d, *J* =15.9 Hz)
8	5.82 (1H, dd, *J* =15.6, 5.1 Hz)	6.43 (1H, d, *J* =15.9 Hz)
9	4.37 (1H, m)	
10	1.26 (3H, d, *J* =6.6 Hz)	2.30 (3H, s)
11	1.05 (3H, s)	1.02 (3H, s)
12	0.98 (3H, s)	1.06 (3H, s)
13	1.86 (3H, d, *J* =1.5 Hz)	1.90 (3H, d, *J* =1.5 Hz)

ppm from TMS, in $CDCl_3$[a)], CD_3OD[b)], 400 Hz, at room temperature.

Table 2. ^{13}C-NMR data of compounds **1** and **2**.

Carbon	1[a)]	2[b)]
1	41.24	42.66
2	49.74	50.52
3	197.83	199.99
4	126.68	127.82
5	162.80	164.34
6	79.01	79.90
7	128.84	148.05
8	135.54	131.49
9	68.02	200.29
10	23.84	27.71
11	23.02	23.58
12	24.15	24.81
13	19.07	19.24

ppm from TMS, in $CDCl_3$[a)], CD_3OD[b)], 100 Hz, at room temperature.

on the Sephadex LH-20 column (φ 40×1200 mm, GE Healthcare) using MeOH to yield 9 corresponding fractions, of which subfraction A9-7 (38 mg) was purified by reverse phase HPLC on the COSMOSIL 5C_{22}-ARII column using MeOH/H_2O (5:4) to yield compounds **5** (6.3 mg) and **6** (5.0 mg). Subfraction A9-8 (9.4 mg) was purified by reverse phase HPLC on the COSMOSIL 5C_{22}-ARII column using MeOH/H_2O (5:4) to yield compound **7** (2.7 mg).

Compound 1

(+)-Blumenol A (**1**): white crystal, mp 108-109°C. $[\alpha]_D^{26}$ +245.2° (*c* = 0.69, MeOH). UV λ_{max} (MeOH) nm (log ε) 237 (4.06). CD (*c* = 1.34×10^{-4} M, MeOH) △ε (nm): +14.89 (241.6), -0.85 (320.2). Positive-mode FAB-MS *m/z* 225 $(M+H)^+$, 247 $(M+Na)^+$. Negative-mode FAB-MS *m/z* 223 $(M-H)^-$. NMR (Tables 1 and 2).

Preparation of (R)- and (S)-MTPA esters 1a and 1b from compound 1

A working solution of (*R*)-MTPA [(*R*)-MTPA (Sigma-Aldrich) 51.5 mg and DCC (Nacalai) 36 mg in 0.5 mL of dry CH_2Cl_2] was prepared. Compound **1** (2.9 mg, 13 μmol) and DMAP (Wako) (16.4 mg) were dissolved in dry CH_2Cl_2 (0.5 mL) and stirred at room temperature for 30 min. The (*R*)-MTPA working solution was then added and the mixture was stirred at room temperature for 1 h. After the addition of 5 mL each of water and CH_2Cl_2, the solution was washed successively with 5% HCl (5 mL), saturated $NaHCO_3$ (5 mL) and brine (5 mL). The organic soluble part was dried with Na_2SO_4 and concentrated *in vacuo* to yield a residue (Kai et al., 2007). **1a** (2.9 mg, 50.8%) was purified on a silica gel (2 mL) column using *n*-hexane/AcOEt. Using a similar procedure, **1b** (2.3 mg, 40%) was prepared from compound **1** (2.9 mg, 13 μmol) with (*S*)-MTPA (49.7 mg), DCC (36 mg) and DMAP (17.3 mg) (Figure 1).

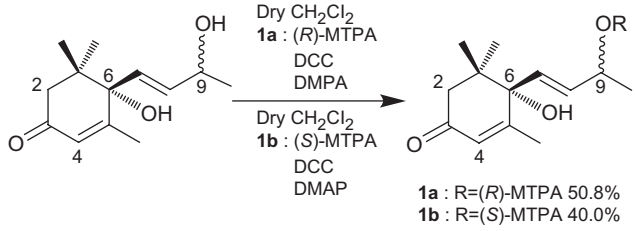

Figure 1. Preparation of (*R*)- and (*S*)-MTPA esters **1a** and **1b** from compound **1**.

9-*O*-(*R*)-MTPA ester (1a)

Colorless oil, $[\alpha]_D^{29}$ +189.3° (*c* = 0.097, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε) 239.5 (4.24). EI-MS *m/z* (rel. int. %) 440 (1) $(M)^+$, 206 (26), 189 (77), 150 (100), ^{1}H-NMR (Table 3).

9-*O*-(*S*)-MTPA ester (1b)

Colorless oil, $[\alpha]_D^{28}$ +159.1° (*c* = 0.067, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε) 239.5 (4.32). EI-MS *m/z* (rel. int. %) 440 (1) $(M)^+$, 206 (25), 189 (77), 150 (100), ^{1}H-NMR (Table 3).

Compound 2

(+)-Dehydrovomifoliol (**2**): yellow oil, $[\alpha]_D^{26}$ +119.9° (*c* = 0.48, MeOH). UV λ_{max} (MeOH) nm (log ε) 239 (4.04). CD (*c* = 1.26×10^{-4} M, MeOH) △ε (nm) +20.44 (243). EI-MS *m/z* (rel. int. %) 222 (1) $(M)^+$, 166 (32), 149 (10), 124 (100), NMR (Tables 1 and 2).

Compound 3

Isovitexin (**3**): yellow amorphous solid, mp 210°-215°C. $[\alpha]_D^{27}$ +40.8° (*c* = 0.21, MeOH). UV λ_{max} (MeOH) nm (log ε) 207 (3.06), 271.0 (2.73), 333.5 (2.74). FAB-MS *m/z* (neg.) 431 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.77 (1H, s, H-3), 6.5 (1H, s, H-8), 7.93 (2H, d, *J* = 8.8, H-2′, 6′), 6.93 (2H, d, *J* = 8.8, H-3′, 5′), 4.59 (1H,

Table 3. ^{1}H-NMR spectral data of compound **1** and derivatives **1a** and **1b**.

				$\Delta(\delta_{1b}-\delta_{1a})$	
	1	1b	1a	ppm	Hz
2a	2.41 (1H, d, *J* =17.0 Hz)	2.31 (1H, d, *J* =17.0 Hz)	2.38 (1H, d, *J* =17.0 Hz)	-0.072	-28.8
2b	2.20 (1H, d, *J* =17.0 Hz)	2.21 (1H, d, *J* =17.0 Hz)	2.24 (1H, d, *J* =17.0 Hz)	-0.034	-13.6
4	5.87 (1H, s)	5.88 (1H, brs)	5.90 (1H, brs)	-0.019	-7.6
7	5.75 (1H, d, *J* =15.6 Hz)	5.75 (1H, d, *J* =15.4 Hz)	5.85 (1H, d, *J* =15.4 Hz)	-0.093	-37.2
8	5.82 (1H, dd, *J* =15.6, 5.1 Hz)	5.80 (1H, d, *J* =15.4 Hz)	5.90 (1H, d, *J* =15.4 Hz)	-0.097	-38.8
9	4.37 (1H, m)	5.64 (1H, m)	5.61 (1H, m)	+0.023	+9.2
10	1.26 (3H, d, *J* =6.6 Hz)	1.44 (3H, d, *J* =6.6 Hz)	1.39 (3H, d, *J* =6.6 Hz)	+0.045	+18.0
11	1.05 (3H, s)	1.04 (3H, s)	1.06 (3H, s)	-0.015	-6.0
12	0.98 (3H, s)	0.92 (3H, s)	0.94 (3H, s)	-0.019	-7.6
13	1.86 (3H, d, *J* =1.5 Hz)	1.82 (3H, d, *J* =1.5 Hz)	1.85 (3H, d, *J* =1.5 Hz)	-0.031	-12.4
Ph		7.50-7.34 (5H, m)	7.50-7.34 (5H, m)		
OCH_3		3.56 (3H, d, *J* =1.2 Hz)	3.51 (3H, d, *J* =1.2 Hz)	+0.052	+20.8

ppm from TMS, in $CDCl_3$, 400 Hz, at room temperature.

Table 4. ^{13}C-NMR data of compounds **3**-**7**.

Carbon	3[a)]	4[b)]	5[b)]	6[a)]	7[a)]
2	163.50	158.73	158.93	156.84	156.85
3	102.51	135.40	135.33	133.90	134.00
4	181.42	179.13	179.23	177.27	177.30
5	160.29	162.73	162.79	160.91	161.03
6	108.71	99.77	99.82	98.57	98.80
7	163.04	165.73	165.67	164.01	164.69
8	93.52	94.60	94.69	93.57	93.64
9	155.91	158.17	158.26	156.16	156.27
10	102.98	105.53	105.67	103.85	103.70
1′	120.83	122.99	122.69	120.26	120.54
2′	128.14	117.39	132.05	130.30	115.32
3′	115.75	145.63	115.94	115.15	145.05
4′	160.86	149.57	161.30	159.63	148.39
5′	115.75	115.82	115.94	115.15	115.47
6′	128.14	122.88	132.05	130.30	120.93
1″	72.98	104.20	104.13	101.59	101.78
2″	70.10	75.65	75.70	70.20	70.51
3″	78.82	78.04	78.04	70.49	70.42
4″	70.50	71.16	71.42	70.99	71.27
5″	81.39	78.29	78.32	69.95	70.07
6″	61.38	62.53	62.71	17.48	17.60

ppm from TMS, in DMSO-d_6, CD_3OD[b)], 100 Hz, at room temperature.

d, *J* = 9.8, H-1″ of Glc), 13.55 (1H, brs, OH-5), ^{13}C-NMR (Table 4).

Compound 4

Isoquercitrin (**4**): yellow powder, mp 175°-177°C. $[\alpha]_D^{23}$ -5.2° (*c* = 0.10, MeOH). UV λ_{max} (MeOH) nm (log ε) 202.5 (4.59), 257 (4.29), 356.5 (4.16). FAB-MS *m/z* (neg.) 463 $(M-H)^-$. ^{1}H-NMR (400 MHz, CD_3OD) δ 6.2 (1H, d, *J* = 2.2, H-6), 6.39 (1H, d, *J* = 2, H-8), 7.71 (1H, brs, H-2′), 6.87 (1H, d, *J* = 8.2, H-5′), 7.58 (1H, dd, *J* = 8.2, H-6′), 5.25 (1H, d, *J* = 7.6, H-1″ of Glc), ^{13}C-NMR (Table 4).

Compound 5

Astragalin (**5**): yellow powder, mp 174°-178°C. $[\alpha]_D^{27}$ -11.3° (*c* = 0.32, MeOH). UV λ_{max} (MeOH) nm (log ε) 266 (4.26), 350 (4.22). FAB-MS *m/z* (neg.) 447 $(M-H)^-$. ^{1}H-NMR (400 MHz, CD_3OD) δ 6.22 (1H, d, *J* = 2, H-6), 6.41 (1H, d, *J* = 2, H-8), 8.05 (2H, d, *J* = 8.9, H-2′, 6′), 6.89 (2H, d, *J* = 8.9, H-3′, 5′), 5.22 (1H, d, *J* = 7.6, H-1″ of Glc), ^{13}C-NMR (Table 4).

Compound 6

Afzelin (**6**): yellow powder, mp 173°-175°C. $[\alpha]_D^{26}$ -104° (*c* = 0.1, MeOH). UV λ_{max} (MeOH) nm (log ε)

265 (3.98), 342 (3.81). FAB-MS *m/z* (neg.) 431 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.21 (1H, d, *J* = 2, H-6), 6.40 (1H, d, *J* = 2, H-8), 7.75 (2H, d, *J* = 8.8, H-2′, 6′), 6.91 (2H, d, *J* = 8.8, H-3′, 5′), 5.3 (1H, d, *J* = 1.5, H-1″ of Rha), 12.6 (1H, brs, OH-5), ^{13}C-NMR (Table 4).

Compound 7

Quercitrin (**7**): yellow powder, mp 178°-180°C. $[\alpha]_D^{27}$ -96° (*c* = 0.1, MeOH). UV λ_{max} (MeOH) nm (log ε) 206 (4.64), 256.5 (4.19), 351.5 (4.06). FAB-MS *m/z* (neg.) 447 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.18 (1H, d, *J* = 2.2, H-6), 6.37 (1H, d, *J* = 2.2, H-8), 7.26 (1H, d, *J* = 2.2, H-2′), 6.85 (1H, d, *J* = 8.3, H-5′), 7.22 (1H, d, *J* = 8.3, 2.2, H-6′), 5.2 (1H, d, *J* = 1.2, H-1″ of Rha), 12.58 (1H, brs, OH-5), ^{13}C-NMR (Table 4).

Results and discussion

Here we report the first isolation of megastigmanes **1** and **2** and flavonoid glycosides **3-7** (Figure 2) from *U. cannabina* as well as the first isolation of megastigmanes from plants of the Urticaceae family.

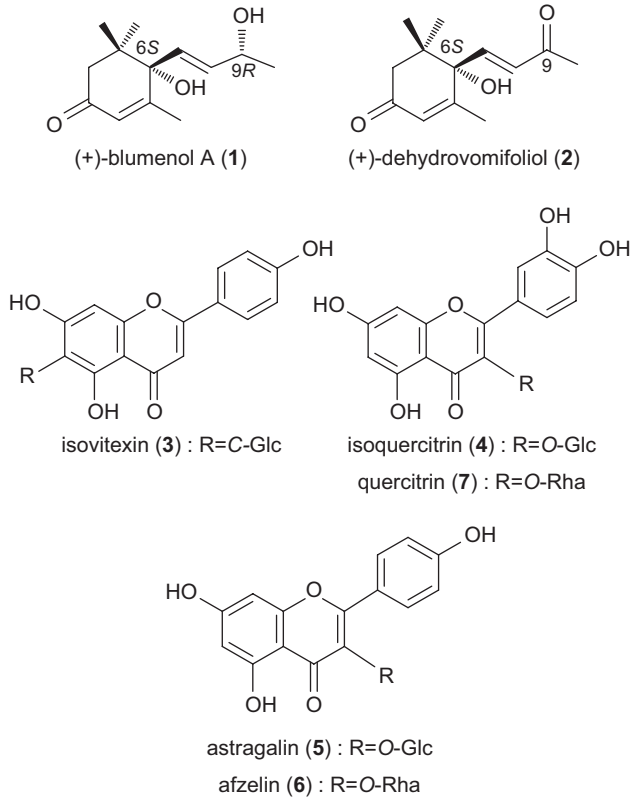

Figure 2. Structures of compounds **1**-**7**.

The absolute configuration of **1** at C-6 was determined by application of the circular dichroism (CD) helicity rule (Weiss et al., 1973) based on the known compound (+)-dehydrovomifoliol (**2**). The CD spectrum of **2** showed a positive Cotton effect (△ε +20.44) at 243 nm (Figure 3), so the 6-position was determined to have the *S* configuration. On the basis of this evidence, because the CD spectrum of **1** exhibited a positive Cotton effect (△ε +14.89) at 241.6 nm (Figure 3), we also concluded that the 6-position of **1** has the *S* configuration. In order to

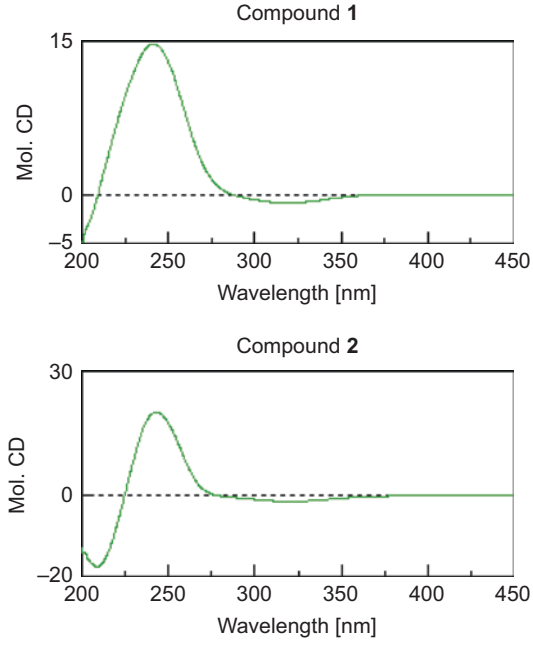

Figure 3. CD curves of compounds **1** and **2**.

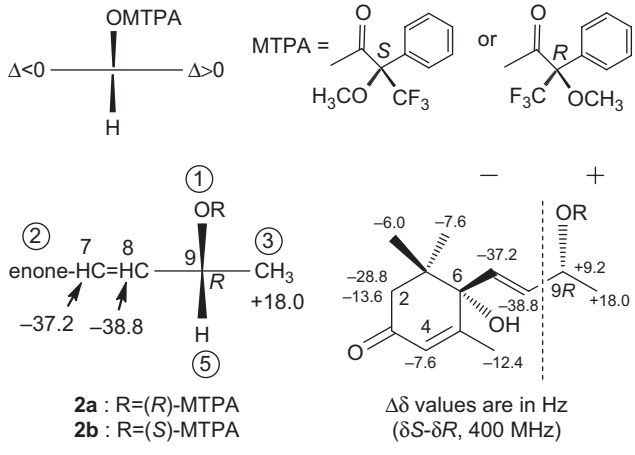

Figure 4. Results of modified Mosher's method of **2a** and **2b**.

clarify the absolute stereostructure at the 9-position of compound **1**, a modified Mosher's method was used. Compound **1** was treated with either (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), *N,N*-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine in dry dichloromethane at room temperature to yield the 9-*O*-(*R*)-MTPA ester **1a** (50.8%), and the 9-*O*-(*S*)-MTPA ester **1b** (40.0%), respectively (Figure 1). As shown in Figure 4, the NMR signals due to protons attached at the 2a, 2b, 4, 7, 8, 11, 12, and 13-positions in **1a** were observed at a lower field than in **1b** (negative △δ), while the signal of the protons at the 10-position in **1a** were observed at a higher field than in **1b** (positive △δ). Consequently, the absolute configuration at the 9-position was elucidated to be *R*. On the basis of this evidence, the structure of **1** was determined to be (+)-blumenol A.

The other five compounds were identified as isovitexin (**3**), isoquercitrin (**4**), astragalin (**5**), afzelin (**6**), and quercitrin (**7**) by comparison of their spectroscopic data (NMR, UV, MS, melting points and optical rotation) with reported values.

(+)-Blumenol A (**1**) has been previously isolated from the leaves of *Podocarpus blumei* Endl. (Podocarpaceae) (Galbraith & Horn, 1972), while (+)-dehydrovomifoliol (**2**) has been isolated from the aerial parts of *Beta vulgaris* L. var. *cicla* L. (Chenopodiaceae) (Kim et al., 2004). However, this is the first report of the isolation of megastigmanes from plants of the Urticaceae family. Afzelin (**6**) and quercitrin (**7**) have been isolated from the fruits of *Ocotea velloziana* Meisn. (Lauraceae) (Garcez et al., 1995) and *Juniperus communis* L. (Cupressaceae) (Hiermann et al., 1996). Isovitexin (**3**) has been isolated from other Urticaceae species, including the leaves of *Phenax angustifolius* Wedd. (Piccinelli et al., 2005). Isoquercitrin (**4**) and astragalin (**5**) have been isolated from flowers of *U. dioica* (Wang et al., 2006), and roots of *U. fissa* (Chaurasia & Wichtl, 1987).

The presence of megastigmanes **1** and **2**, afzelin (**6**) and quercitrin (**7**) has chemotaxonomic significance for Urticaceae family. Isovitexin (**3**) does not appear to have a significant chemotaxonomic presence among *Urtica* species, although it has been reported in *Phenax*, another member of the Urticaceae family. As isovitexin has not previously been reported in *Urtica* species, its presence in *U. cannabina* is also of chemotaxonomic significance.

Naturally occurring flavonoids have been isolated from more than 6000 species, and several have been reported to exhibit anti-allergic, anti-inflammatory, anticancer and antiviral activities. Megastigmanes have been reported to exhibit radical scavenging activity (Matsunami et al., 2006). In future studies, the compounds identified in this plant may be investigated for activity against chronic bronchitis, which this plant used for in Uighur traditional medicine.

Acknowledgement

Authors are grateful to Professor Dr. Uper Hamurat of Xinjiang Medical University in China, and Dr. Hisahiro KAI of Kyushu University of Health and Welfare in Japan. A part of this work was supported by Japan China Medical Association in 2007.

Declaration of interest

The authors report no conflict of interest. The authors are responsible for the content and writing of the paper.

References

Balzarini J, Neyts J, Schols D, Hosoya M, Van DE, Peumans W, De CE (1992): The mannose-specific plant lectins from *Cymbidium hybrid* and *Epipactis helleborine* and the (N-acetylglucosamine)$_n$-specific plant lectin from *Urtica dioica* are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication *in vitro*. *Antiviral Res* 18: 191–207.

Baser KHC, Honda G, Miki W (1986): *Herb Drugs and Herbalists in Turkey*. Tokyo, Toyo Publishing, pp. 146, 161, 188, 193, 222.

Berges RR, Windeler J, Trampisch HJ, Senge T (1995): Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia. Beta-sitosterol study group. *Lancet* 345: 1529–1532.

Cai Q (2001): Rheuma-Hek capsule. *World Phytomedicines* 16: 282.

Chaurasia N, Wichtl M (1987): Flavonol glycosides from *Urtica dioica*. *Planta Med* 53: 432–444.

Emmelin N, Feldberg W (1949): Distribution of acetylcholine and histamine in nettle plants. *New Phytol* 48: 143–148.

Galbraith MN, Horn DHS (1972): Structures of the natural products blumenols A, B, and C. *J Chem Soc Chem Comm* 3: 113–114.

Galelli A, Truffa BP (1993): *Urtica dioica* agglutinin. A superantigenic lectin from stinging nettle rhizome. *J Immunol* 151: 1821–1831.

Garcez WS, Yoshida M, Gottlieb OR (1995): Benzylisoquinoline alkaloids and flavonols from *Ocotea vellosiana*. *Phytochemistry* 39: 815–816.

Hiermann A, Kompek A, Reiner J, Auer H, Schubert-Zsilavecz M (1996): Investigation of flavonoid pattern in fruits of *Juniperus communis*. *Sci Pharm* 63: 437–444.

Hirano T, Homma M, Oka K (1994): Effects of stinging nettle root extracts and their steroidal components on the Na^+, K (+)-ATPase of the benign prostatic hyperplasia. *Planta Med* 60: 30–33.

Hryb DJ, Khan MS, Romas NA, Rosner W (1995): The effect of extracts of the roots of the stinging nettle (*Urtica dioica*) on the interaction of SHBG with its receptor on human prostatic membranes. *Planta Med* 61: 31–32.

Iida M, Asami Y, Tanaka T, Honda G, Tabata M, Sezik E, Yesilada E (1994): Studies on genus *Urtica* in Turkey (1). Identification of *Urtica* in Turkey by internals of axis and petiole. *Nat Med* 48: 237–243.

Kai H, Baba M, Okuyama T (2007): Two new megastigmanes from the leaves of *Cucumis sativus*. *Chem Pharm Bull* 55: 133–136.

Kim I, Chin YW, Lim SW, Kim YC, Kim J (2004): Norisoprenoids and hepatoprotective flavone glycosides from the aerial parts of *Beta vulgaris* var. *cicla*. *Arch Pharm Res* 27: 600–603.

Lichius JJ, Renneberg H, Blaschek W, Aumüller G, Muth C (1999): The inhibiting effects of components of stinging nettle roots on experimentally induced prostatic hyperplasia in mice. *Planta Med* 65: 666–668.

Liu YM, Liu WX, Shawuti YKM, Zou Y (1999): *Pharmacography of Uighur (Part Two)*. Urumuqi, Xinjiang Science Technology Hygiene Publishing House, pp. 510.

Matsunami K, Takamori I, Shinzato T, Aramoto M, Kondo K, Otsuka H, Takeda Y (2006): Radical-scavenging activities of new megastigmane glucosides from *Macaranga tanarius* (L.) Mull.-Arg. *Chem Pharm Bull* 54: 1403–1407.

Ma XM, Guo YJ, Wang LS (2004): Study on chemical contents of *Urtica cannabina* L. *Zhongguo Zhongyao Zazhi* 29: 472.

Musette P, Galelli A, Chabre H (1996): *Urtica dioica* agglutinin, a V beta 8.3-specific superantigen, prevents the development of the systemic lupus erythematosus-like pathology of MRL *lpr/lpr* mice. *Eur J Immunol* 26: 1707–1711.

Namba T (1980): *Coloured Illustrations of Wakan-Yaku*, Vol. I. Osaka, Hoikusha Publishing, pp. 106.

Piccinelli AL, Mahmood N, Mora G, Poveda L, De Simone F, Rastrelli L (2005): Anti-HIV activity of dibenzylbutyrolactone-type lignans from *Phenax* species endemic in Costa Rica. *J Pharm Pharmacol* 57: 1109–1115.

Schottner M, Gansser D, Spiteller G (1997): Lignans from the roots of *Urtica dioica* and their metabolites bind to human sex hormone binding globulin (SHBG). *Planta Med* 63: 529–532.

Wagner H, Willer F, Samtleben R, Boos G (1994): Search for the antiprostatic principle of stinging nettle (*Urtica dioica*) roots. *Phytomedicine* 1: 213–224.

Wagner H, Geiger WN, Boos G, Samtleben R (1995): Studies on the binding of *Urtica dioica* agglutinin (UDA) and other lectins in an *in vitro* epidermal growth factor receptor test. *Phytomedicine* 2: 287–290.

Wang MY, Wei YF, Huang XY (2002): Researches in medicinal plant of *Urtica* L. *J Chin Mater Med* 25: 58–60.

Wang MY, Wei YF, Li XB (2006): Chemical constituents of anti-rheumatism fraction from *Urtica fissa*. *Zhongcaoyao* 37: 1300–1303.

Weiss G, Koreeda M, Nakanishi K (1973): Stereochemistry of theaspirone and the blumenols. *J Chem Soc Chem Comm* 16: 565–566.

Yasen TRS, Tian RW, Cheng LY, Hamulati WPR, Liu YM (1999): *People's Republic of China Medical Department Drugs Standard Uighur Medicine Fascicle*. Urumuqi, Xinjiang Science Technology Hygiene Publishing, pp. 198.

Zhang ML, Li ZP, Jia XM (2005): Study on chemical constituents of *Urtica cannabina* L. *Nat Prod Res Dev* 17: 175–176.