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Chinese Journal of Natural Medicines 2017, **15**(4): 0288–0291
Chinese Journal of Natural Medicines

Megastigmane glucosides isolated from *Dichrocephala benthamii*

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Available online 20 Apr., 2017

[ABSTRACT] The present study was designed to investigate the chemical constituents of the whole herb of *Dichrocephala benthamii*. A new megastigmane glucoside (compound **1**), together with its four known analogues (compounds **2**–**5**), was obtained. Their structures were elucidated on the basis of spectroscopic analyses (UV, IR, MS, and 1D and 2D NMR). The absolute configuration of compound **1** was assigned on the basis of CD method and chemical evidence. In addition, their cytotoxicity against human hepatoma cells (HepG-2) was evaluated by the MTT method. Compound **5** showed weak activity against HepG-2, while the other compounds did not show remarkable inhibitory effects.

KEY WORDS] Asteraceae; *Dichrocephala benthamii*; Megastigmane glucoside; Cytotoxicity

CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2017)04-0288-04

Introduction

Dichrocephala benthamii C. B. Clarke, an annual herbaceous plant belonging to the Asteraceae family, is distributed in China and India [1]. It is used as a folk medicine among the Dai nationality of China for the treatment of various disorders, such as indigestion, common cold, fever in children, pneumonia, and hepatitis [2]. Previous studies of this medicinal plant have yielded diverse secondary metabolites, including flavonoids, anthraquinones, alkaloids, terpenoids, and sinapyl alcohol derivatives [3-7]. In our ongoing mining of new and bioactive compounds from this medicinal plant, a new megastigmane glucoside, together with its four known analogues, was isolated and characterized (Fig. **1**). This paper presents the isolation and structural elucidation of these five compounds as well as the determination of their cytotoxicity against human hepatoma cell line HepG-2.

[Received on] 07-May-2016

[Research funding] This work was supported financially by the Chinese National S&T Special Project on Major New Drug Innovation (No. 2013ZX09508104) and Innovative Research Team in Institute of Medicinal Plant Development (IMPLD)

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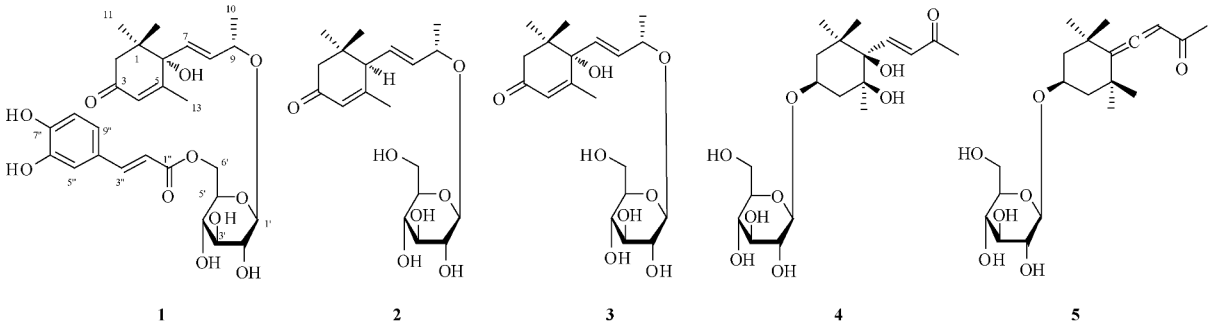
These authors have no conflict of interest to declare.

Results and Discussion

Structure elucidation

Compound **1** was obtained as an amorphous powder with a molecular formula of $C_{28}H_{36}O_{11}$, as determined by HR-ESI-MS analysis (*m/z* 547.218 4 [M − H]$^-$, Calcd. 547.217 9). Analysis of the ^{1}H and ^{13}C NMR spectra showed that compound **1** might contain a phenylpropanoid group, a megastigmane fragment, and a monosaccharide moiety. The ^{1}H NMR spectrum showed diagnostically ABX type-aromatic proton signals [δ 7.01 (1H, d, *J* = 2.0 Hz), 6.92 (1H, dd, *J* = 8.0, 2.0 Hz), and 6.75 (1H, d, *J* = 8.0 Hz)], two *trans*-olefinic proton signals [δ 7.54 (1H, d, *J* = 19.2 Hz) and 6.24 (1H, d, *J* = 19.2 Hz)], and the ^{13}C NMR spectrum exhibited one carbonyl carbon signal (δ 169.1), two sp^2 carbon signals (δ 147.4, 114.9), and six aromatic carbon signals (δ 149.8, 146.9, 127.6, 123.1, 116.6, and 115.3). Interpretation of the ^{1}H-^{1}H COSY NMR data led to the identification of two isolated proton spin-systems corresponding to C-2″–C-3″ and C-8″–C-9″ units. The HMBC correlations from H-3″ to C-5″ and C-9″ and from H-2″ to C-1″ finally established a (*E*)-caffeoyl group. The ^{1}H-^{1}H COSY spectrum revealed a four-carbon spin system from C-7–C-8–C-9–C-10 in the megastigmane fragment. The HMBC correlations from CH_3-11 and CH_3-12 to C-1, C-2, and C-6 indicated that the two methyls at δ 0.99 (H-11) and 0.93 (H-12) were connected with the carbon at δ 42.5 (C-1). The correlations from CH_3-13 to C-4, C-5, and C-6 showed that the methyl at δ 1.90 (H-13) connected



1 **2** **3** **4** **5**

Fig. 1 Structures of compounds 1−5

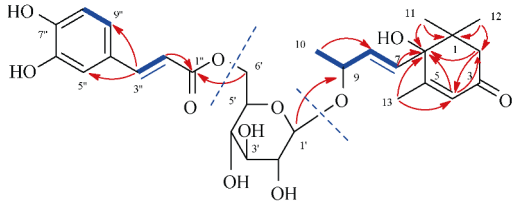


Fig. 2 Selected ^{1}H-^{1}H COSY (bold) and HMBC (arrow) correlations of compound 1

to C-5. Furthermore, the correlations of CH_2-2 with C-3 and C-4, together with H-4 with C-2, C-3, and C-5, and H-7 with C-1, C-5, and C-6, established the megastigmane fragment (Fig. 2). These main correlations from CH_2-6′ to C-1″ and H-1′ to C-9 finally established the connection of the (*E*)-caffeoyl, the megastigmane and the glucose moiety. Considering the molecular weight and chemical shift values of C-6, C-6″, and C-7″, these three carbons must be connected with a free hydroxyl group, respectively. Thus the planar structure of compound **1** was determined.

The monosaccharide in compound **1** was determined to be D-glucose on the basis of acid hydrolysis with TLC experiment, compared with the authentic sample. In the ^{1}H NMR spectrum, the coupling constant (7–8 Hz) suggested the glucose to be *β*-configuration. The absolute configuration of C-6 was established by using circular dichroism (CD) spectrum. The positive Cotton effect at 242 nm and the negative Cotton effect at 316 nm indicated the absolute configuration at C-6 to be *S* configuration [9]. It was reported that the chemical shifts of C-9 and C-10 were indicative for 9*R* (δ_9 77.3−79.1, δ_{10} 21.2−21.8), and 9*S* (δ_9 74.7−76.3, δ_{10} 22.3−22.6) configurations in $\Delta^{7,8}$-type of 9-hydroxy megastigmane 9-*O*-*β*-D-glucopyranosides [10-11]. Thus, the absolute configuration at C-9 was tentatively assigned as *S* on the basis of the diagnostic chemical shifts of C-9 (δ 75.0) and C-10 (δ 22.3) in the ^{13}C NMR data (Table 1). Based on the above spectral analyses, the structure of dichrocephoside A (compound **1**) was determined to be 6*S*, 9*S*-ionone 9-*O*-(6-*O*-(*E*)-caffeoyl)-*β*-D-glucopyranoside.

The four known compounds were identified as (6*R*, 7*E*, 9*S*)-9-hydroxy-megastigman-4, 7-dien-3-one-9-*O*-*β*-D-glucopyranoside (**2**) [12], corchoionoside C (**3**) [12], pisumionoside (**4**) [13], and staphylionoside D (**5**) [14] (Fig. 1), respectively, based on the NMR experiments.

Table 1 ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound 1 in CD_3OD

Position	δ_C (mult)	δ_H (mult) (*J* in Hz)
Megastigmane moiety		
1	42.5	
2a	50.8	2.12 (1H, d, 17.0)
2b		2.49 (1H, d, 17.0)
3	201.3	
4	127.2	5.85 (1H, brs)
5	167.1	
6	80.0	
7	133.9	5.86 (1H, d, 15.5)
8	133.7	5.70 (1H, dd, 15.5, 7.5)
9	75.0	4.43 (1H, m)
10	22.3	1.26 (3H, d, 6.5)
11	23.5	0.99 (3H, s)
12	24.8	0.93 (3H, s)
13	19.7	1.90 (3H, d, 1.0)
Glucose moiety		
1′	101.4	4.26 (1H, d, 8.0)
2′	74.9	3.21 (1H, t, 8.0)
3′	78.3	3.28 (1H, m)
4′	71.9	3.28 (1H, m)
5′	75.6	3.36 (1H, m)
6′a	64.8	4.28 (1H, dd, 13.5, 6.0)
6′b		4.44 (1H, dd, 13.5, 2.0)
(*E*)-caffeoyl moiety		
1″	169.1	
2″	114.9	6.24 (1H, d, 19.2)
3″	147.4	7.54 (1H, d, 19.2)
4″	127.6	
5″	115.3	7.01 (1H, d, 2.0)
6″	149.8	
7″	146.9	
8″	116.6	6.75 (1H, d, 8.0)
9″	123.1	6.92 (1H, dd, 8.0, 2.0)