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A NEW AURONE GLUCOSIDE AND A NEW CHALCONE GLUCOSIDE FROM *BIDENS BIPINNATA* LINNE

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Abstract—A new aurone glucoside, bidenoside A, (*Z*)-6-*O*-(3, 6-di-*O*-acetyl-β-D-glucopyranosyl)-6, 7, 3', 4'-tetrahydroxyaurone (**1**) and a new chalcone glucoside, bidenoside B, 2', 4', 6'-trimethoxy-4-*O*-β-D-glucopyranosyl-dihydrochalcone (**4**), together with five known constituents have been isolated from the aerial parts of *Bidens bipinnata*. These structures have been elucidated on the basis of spectroscopic methods.

Bidens bipinnata, a weed of the Asterceae family, is widely distributed in China. It has been used as a folk medicine for various diseases, such as inflammations, rheumatism, sore throat, hypertension and diabetes.[1] In this paper we report the isolation and structural elucidation of a new aurone glucoside, (*Z*)-6-*O*-(3, 6-di-*O*-acetyl-β-D-glucopyranosyl)-6, 7, 3', 4'-tetrahydroxyaurone (**1**), two known auron glucosides, (*Z*)-6-*O*-β-D-glucopyranosyl-6, 7, 3', 4'-tetrahydroxyaurone (**2**)[2] and (*Z*)-6-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-6, 7, 3', 4'-tetrahydroxyaurone (**3**),[2] a new chalcone glucoside, 2', 4', 6'-trimethoxy-4-*O*-β-D-glucopyranosyldihydrochalcone (**4**), together with three known flavonoid glycosides, quercetin 3-*O*-β-D-glucopyranoside (**5**),[3] quercetin 3-*O*-α-L-rhamnoside (**6**)[4] and iso-okanin 7-*O*-β-D-glucopyranoside (**7**)[5] from this plant.

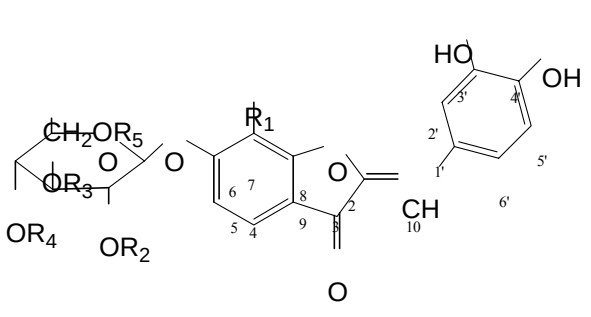
The EtOAc soluble part obtained from EtOH extract was separated on silica gel and octadecyl silica gel (ODS) column chromatography, successively, followed by HPLC to isolate compounds (**1**, **4**).

The *n*-butanol fraction was separated on silica gel and octadecyl silica gel (ODS) column chromatography, successively, followed by HPLC to isolate compounds (**2**, **3**, **5**, **6**, **7**).

Bidenoside A (**1**), an orange powder, showed positive to the Molish reaction and give an $[M+H]^+$ ion peak at *m/z* 533 in positive ion FAB-MS. The molecular formula was determined as $C_{25}H_{24}O_{13}$ on the basis of HR-FAB-MS (M+1; Calcd for 533.1294; Found: 533.1271). The ^{1}H- and ^{13}C-NMR spectra showed a presence of glucopyranosyl unit. The anomeric proton signal at 5.01 ppm (d, *J*= 8.0Hz) in the ^{1}H-NMR spectrum indicated β-glucose. The ^{1}H-NMR signals at δ 7.46 (d, *J*= 1.8 Hz) , 6.76 (d, *J*= 8.3 Hz) and 7.26 (dd, *J*= 8.3, 1.8 Hz) indicated the presence of 2, 5, 6 related aromatic protons (aurone with a 3', 4'-disubstituted B-ring) and at δ 7.13 (d, *J*= 8.5 Hz) and 6.97 (d, *J*= 8.5 Hz) showed ortho related protons in the A-ring. All data closely related to those of the co-occurring aurone glucoside (**2**, **3**). On comparison of its ^{1}H- and ^{13}C-NMR spectral data with those of **3**, **1** was very similar to **3** except for the sugar moiety (Table 1). The two singlet signals of **1** at δ 1.97 and 2.05 in the ^{1}H-NMR were consistent with two acetyl groups attached to the sugar moiety. Therefore, compound **1** has another acetyl group more than **3**. On comparing with **3**, C-2", C-3" and C-4" of **1** appeared with up- and/or down- field shifts, −1.8 ppm, +0.5 ppm and −1.9 ppm, respectively. These data indicated that another acetyl group was linked to the C-3' '-hydroxyl position of the glucose moiety. All carbon and proton signals were assigned by the aids of ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY.

Thus **1** was determined as (*Z*)-6-*O*-(3, 6-di-*O*-acetyl-β-D-glucopyranosyl)-6, 7, 3 ', 4'-tetrahydroxyaurone.

Bidenoside B (**4**), a light yellow powder, showed positive to the Molish reaction and gave an $[M+H]^+$ ion peak at *m/z* 479 in postive ion FAB-MS. The molecular formula was determined as $C_{24}H_{30}O_{10}$ on the basis of HR-EI-MS (Calcd for 478.1838; Found: 478.1824). The ^{1}H-NMR signals of **4** at δ 6.98 (2H, d, *J*= 8.7 Hz) and δ 7.08 (2H, d, *J*= 8.7 Hz) indicated a chalcone with a 4-substituted B-ring and two same meta-H atoms at δ 6.19 (2H, s) in the A-ring. The H-α (δ 2.99 2H, t, *J*= 7.3 Hz) and H-β (δ 2.85 2H, t, *J*= 7.3 Hz) of this compound indicated that **4** was a dihydrochalcone. The coupling constant of the doublet for H-1" of **4** in the ^{1}H-NMR spectrum (*J*= 7.5 Hz) indicated that it has one β-D-glucose.



1 R_1=OH, R_2=R_4=H, R_3=R_5=Ac
3 R_1=R_2=R_3=R_4=H, R_5=Ac

Chart 1 The structures of **1** and **3**

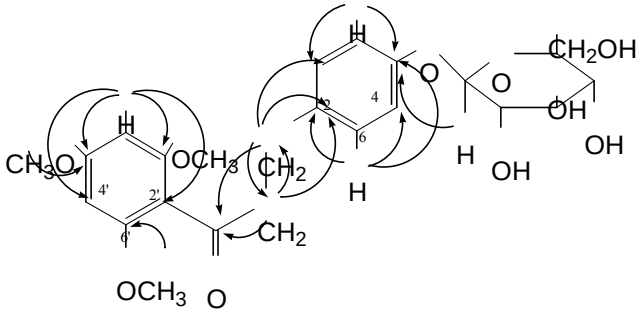


Figure 1 Partial Coherence of **4** in HMBC

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1** and **3** [a)]

	1		**3**	
Aglycone				
2	147.3		147.6	
3	185.2		185.5	
4	115.2	7.13 d *J*=8.5	115.7	7.24 d *J*=8.5
5	113.5	6.97 d *J*=8.5	113.8	7.07 d *J*=8.5
6	153.2		153.6	
7	134.8		134.7	
8	156.2		156.4	
9	119.2		119.3	
10	115.5	6.67 s	115.7	6.76 s
1'	125.3		125.5	
2'	119.2	7.46 d *J*=1.8	119.4	7.55 d *J*=1.8
3'	146.5		146.8	
4'	149.5		149.7	
5'	116.6	6.76 d *J*=8.3	116.8	6.86 d *J*=8.2
6'	126.6	7.26 dd *J*=8.3, 1.8	126.8	7.35 dd *J*=8.2, 1.8
Glucose				
1"	102.7	5.01 d *J*=8.0	103.2	4.99 d *J*=7.6
2"	**73.0**	3.64 dd *J*=9.3, 8.0	74.8	
3"	**78.0**	5.00 dd *J*=9.6, 9.3	77.5	
4"	**69.7**	3.48 dd *J*=9.6, 9.6	71.6	
5"	75.4	3.70-3.73 m	75.8	
6"	64.3	4.18 dd *J*=11.9, 5.3 4.34 br.d *J*=11.9	64.6	4.26 dd *J*=11.9, 6.7 4.43 dd *J*=11.9, 2.3
Acetyl				
	172.2		172.6	
	172.1			
	20.8	2.05 s	20.7	2.06 s
	21.1	1.97 s		

a) Chemical shifts are in δ -values from TMS and are followed by multiplicities and *J*-values (in Hz), 25℃, in CD_3OD (500 MHz)

The singals at δ 3.81 and 3.71 (× 2) were consistent with three methoxyl groups attached to the A-ring. The ^{1}H-^{13}C heteronuclear multiple bond coherence (HMBC) (between Glc-1"-H and 4-C) spectra revealed the glucose was connected at the 4-OH of **2** (Figure 1). The three methoxyl groups were connected at 2',

4', 6' on A-ring of **2**. The ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra were utilized to assign all carbon and proton signals. Thus, **2** was established as 2', 4', 6'-trimethoxy-4-*O*-β-D-glucopyranosyldihydrochalcone.

EXPERIMENTAL

General procedures All melting points were determined on a Yanagimoto melting point apparatus and were uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-LA 500, BM 400, and JNM-EX 270 spectrometer. FAB-MS spectra were measured on a JEOL JMS-DX 302 mass spectrometer. Optical rotations were determined in MeOH on a JASCO DIP-140 polarimeter. Preparative HPLC was performed on a Hitachi (L-6000 pump) instrument with a Waters 5 C_{18}-AR-Ⅱ column (10×250 mm) and Waters 5 SL (10×250 mm) using RI ERC-7520 and UV SSC-5200.

Plant Material The aerial part of *Bidens bipinnata* was collected at the wild field of Heilongjiang, in China and was identified by Prof. Gui-Jun Zhang and a voucher specimen has been deposited at the Chinese Medicine Museum of Heilongjiang University of Traditional Chinese Medicine, Harbin, China.

Extraction and Isolation The air-dried aerial part (4 kg) of *Bidens bipinnata* was extracted with hot EtOH (10L) twice for 2 h. at 60-70 ℃ and the combined extract was concentrated *in vacuo* to a syrup, followed by suspension in water. The suspension was extracted with *n*-hexane, ethyl acetate and then *n*-butanol, successively. The EtOAc extract (20 g) was chromatographed on silica gel and eluted with *n*-hexane-EtOAc (4 : 2 to 4 : 6, gradient elute), to give 12 fractions (Fr. 1-12). Fraction No.9 (1.5 g) was subjected to silica gel column, eluted with *n*-hexane-EtOAc (4 : 6 to 2 : 8), to give 4 fractions. Fraction No.3 (0.6 g) was subjected to reversed-phase (ODS) column chromatography (eluting with 50 % aqueous MeOH), followed by preparative HPLC on silica gel column (EtOAc-Me_2CO-H_2O 6:0.5:0.1) to afford **1** (6.3 mg) and **4** (7.6 mg). The butanol extract (50 g) was chromatographed on silica gel and eluted successively with EtOAc-MeOH gradient elute, to give 10 fractions (Fr. 1-10). Fraction No.4 (2.5 g) was subjected to reversed-phase (ODS) column chromatography (eluting with 40 % aqueous MeOH) followed by preparative HPLC (40% aqueous MeOH) to afford **2** (5 mg), **3** (8 mg), **5** (16 mg), **6** (6 mg) and **7** (9 mg).

Bidenoside A (**1**): an orange amorphous powder (MeOH), mp 165-167 ℃, $[\alpha]_D^{25}$-52.5° (*c* 0.21, MeOH).UV λ^{MeOH}max nm(log ε) : 408 (4.12), 336 (4.04), 271 (3.91). FAB-MS（pos）*m/z*: 533 $[M+1]^+$. HR-FAB-MS: $C_{25}H_{25}O_{13}$ (M+1; Calcd for 533.1294; Found: 533.1271). ^{1}H and ^{13}C-NMR: given in Table 1.

Bidenoside B (**4**): a light yellow powder (MeOH), mp 196-198℃, $[\alpha]_D^{25}$ -130.2° (*c* 0.21, MeOH). UV λ^{MeOH}max nm(log ε) : 285 (4.12), 230 (4.31). FAB-MS（pos）*m/z*： 479 $[M+1]^+$. HR-EI-MS: $C_{24}H_{30}O_{10}$

(Calcd for 478.1838; Found: 478.1824) ^{1}H-NMR (CD_3OD）: 2.99 (2H, t, *J*= 7.3 Hz, H-α), 2.85 (2H, t, *J*= 7.3 Hz, H-β), 6.98 (2H, d, *J*= 8.7 Hz, H-2, 6), 7.08 (2H, d, *J*= 8.7 Hz, H-3, 5), 6.19 (2H, brs, H-3' , 5'), 3.71 (6H, s, H-2', 6' OCH_3), 3.81 (3H, s, H-4' OCH_3), 4.83 (1H, d, *J*= 7.5 Hz, H-1"), 3.68 (1H, dd, *J*= 12.0, 5.2 Hz, H-6"a), 3.87 (1H, dd, *J*= 12.0, 2.0 Hz, H-6" b). ^{13}C-NMR (CD_3OD): 136.1 (C-1), 130.3 (C-2), 117.7 (C-3), 157.4 (C-4), 117.7 (C-5), 130.3 (C-6), 206.3 (C=O), 47.5 (α-C), 30.3 (β-C), 114.0 (C-1'), 159.8 (C-2', 6'), 91.8 (C-3', 5'), 164.4 (C-4'), Glucose 102.6 (C-1"), 75.0 (C-2"), 78.1 (C-3"), 71.4 (C-4"), 78.0 (C-5"), 62.6 (C-6").

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