

Two Neolignan Glucosides and Antihistamine Release Activities from *Bidens parviflora* WILLD.

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Two new neolignan glucosides, bidenlignasides A (1) and B (2), were isolated from the whole plant of *Bidens parviflora* WILLD. The structures of the two new compounds 1 and 2 established to be 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-[5E-(3-hydroxypropenyl)-3-methoxy-2-O-β-D-glucosyl]phenyl] propan-1-one and 3-{3-[1,3-dihydroxy-3-(4-hydroxy-3-methoxyphenyl)propyl]-4-hydroxy-5-methoxyphenyl}-allyl-O-β-D-glucoside, respectively, on the basis of spectral and chemical evidence. Compounds 1 and 2 were found to inhibit histamine release from peritoneal exudate mast cells induced by antigen-antibody reaction.

Key words *Bidens parviflora*; bidenlignaside A; bidenlignaside B; antiallergic agent; histamine release inhibitor

In our bioactivity screening of *Compositae* plants, the extract of *Bidens parviflora* WILLD. has been shown to have high activity against histamine release from rat mast cells induced by compound 48/80. In previously studies, sterols,¹⁾ flavonones,²⁾ flavonoids,³⁾ polyacetylene glucosides,⁴⁾ chalcones,^{5,6)} aurones,⁷⁾ and flavonol glycosides⁸⁾ were isolated from the *Bidens* family. For the purpose of finding biological active compounds, the chemical constituents of this plant have been extensively studied. During our previous studies, it was reported that five polyacetylene glucosides⁹⁾ and three sucrose coumaroylestere were thought to contribute to the inhibition of histamine release.¹¹⁾ In this paper, we report the isolation and structural elucidation of two new neolignan glycosides, bidenlignasides A (1) and B (2). The extraction and separation were carried out as described in Experimental.

Bidenlignaside A (1) was obtained as a colorless powder (MeOH); $[\alpha]_D^{25} -16.5^\circ$. Its molecular formula $C_{26}H_{32}O_{12}$ was deduced from the FAB-MS, m/z 537 (M+H)⁺; high-resolution positive-ion FAB-MS Calcd for $C_{26}H_{33}O_{12}$ ([M+H]⁺): 537.19712; Found: 537.19659. The IR spectrum of 1 showed absorption bands at 3395, 2932, 1662, 1587, 1462, and 1423 cm^{-1} ascribable to hydroxyl, carboxyl, aromatic ring, and ether functions. In the UV spectrum of 1, absorption maxima were observed at 248 (log ϵ 3.95) and 267 (log ϵ 4.04) nm, which suggest a phenol structure. The 1D ¹H-NMR spectrum of 1 and 2D ¹H-¹H COSY spectra showed the presence of a 1,3,4-trisubstituted benzene ring (ring A) [δ 7.68 (1H, d, $J=2.1$ Hz, H-2), δ 6.79 (1H, d, $J=8.3$ Hz, H-5),

and δ 7.69 (1H, dd, $J=8.3, 2.1$ Hz, H-6)], a 1,3',4',5'-tetra-substituted benzene ring (ring B) [δ 6.98 (1H, d, $J=1.9$ Hz, H-2), δ 6.72 (1H, d, $J=1.9$ Hz, H-6)], an oxygen-bearing methine [δ 5.52 (1H, dd, $J=9.2, 3.5$ Hz, H-9)] and a methylene [δ 4.36 (1H, dd, $J=10.4, 9.2$ Hz, H-8a), δ 3.74 (1H, dd, $J=10.4, 3.5$ Hz, H-8b)], and a hydroxy propylene group [δ 6.47 (1H, d, $J=15.9$ Hz, H-7), δ 6.25 (1H, dt, $J=15.9, 5.5$ Hz, H-8) and δ 4.17 (2H, dd, $J=5.5, 1.5$ Hz, H-9)], in addition to two methoxyl groups [δ 3.90 (3H, s, C-3), δ 3.88 (3H, s, C-3)]. ¹H- and ¹³C-NMR spectra (Table 1) indicated the presence of a β-D-glucosyl moiety at δ 4.92 (1H, d, $J=7.6$ Hz, glc-H-1), δ_C 105.9 (glc-C-1), 78.5 (glc-C-5), 78.0 (glc-C-3), 76.1 (glc-C-2), 70.9 (glc-C-4), and 62.3 (glc-C-6). Acid hydrolysis of 1 with 5% sulfuric acid (H₂SO₄) furnished D-glucose, which was identified by gas-liquid chromatography (GLC) analysis of the trimethylsilyl (TMS) thiazolidine derivative.¹¹⁾ In its ¹³C-NMR spectrum, except for the signals due to the glucose, the remaining carbon signals consisted of a carbonyl group (δ_C 199.3, C-7), an oxygen-bearing methine (δ_C 64.5, C-9), a methylene (δ_C 49.3, C-8), a hydroxy propylene group (δ_C 130.8, C-7, δ_C 130.6, C-8, δ_C 63.6, C-9), and two aromatic rings. The HMBC correlations between the δ_C 143.6 (C-4) and δ_H 4.92 (glc-H-1) suggested that the glucopyranosyl moiety was attached at the C-4 of ring B. HMBC correlations were observed between H-2 and H-6/C-7 and between H-2 and H-6/C-7. These results indicate that 1 has two phenylpropanoid units. Significant correlations were also observed between H-8/C-1 and C-5 and between H-9/C-

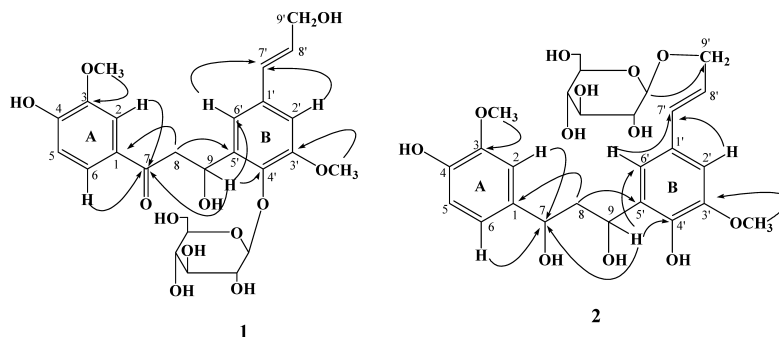


Fig. 1. HMBC Correlations of 1 and 2

7 and C-4, C-6. The position of the methoxyl group was determined to be at C-3 and C-3 based on NOESY (3-OCH₃/H-2, 3-OCH₃/H-2) and HMBC experiments (3-OCH₃/C-3, 3-OCH₃/C-3). The structure of **1** was elucidated to be 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-[5*E*-(3-hydroxyprop-1-enyl)-3-methoxy-2-*O*- β -D-glucosyl]-phenyl]-propan-1-one, which was designated bidenlignaside A.

Bidenlignaside B (**2**) was also obtained as a colorless powder (MeOH); [α]_D²⁵ -18.2°. Its molecular formula C₂₆H₃₄O₁₂ was deduced from the FAB-MS, *m/z* 537 (M-H)⁺; high-resolution positive-ion FAB-MS Calcd for C₂₆H₃₃O₁₂ ([M-H]⁺): 537.19712; Found: 537.19700. The IR spectrum of **2** showed absorption bands at 3412, 2931, 1715, 1604, 1512, 1482, 1422, and 1369 cm⁻¹ ascribable to hydroxyl, aromatic ring and ether functions. In the UV spectrum of **2**, absorption maxima were observed at 246 (log ϵ 4.45) and 268 (log ϵ 3.02) nm, which suggest a phenol structure. The 1D NMR spectrum of **2** and 2D NMR spectra also showed the presence of a 1,3,4-trisubstituted benzene ring (ring A) and a 1,3,4,5-tetrasubstituted benzene ring (ring B). ¹H- and ¹³C-NMR spectra (Table 1) indicated the presence of a β -D-glucosyl moiety. Acid hydrolysis of **2** with 5% sulfuric acid (H₂SO₄) furnished D-gluconic acid, which was identified by GLC analysis of the TMS thiazolidine derivative.¹¹⁾ In its ¹³C-NMR spectrum, except for the signals due to glucose, the remaining carbon signals consisted of two oxygen-bearing methines (δ _C 74.2, C-7, δ _C 62.3, C-9), a methylene (δ _C 46.3, C-8), a hydroxy propylene group [δ _C 134.4, (C-7),

125.2, (C-8), 70.9, (C-9)], and two aromatic rings. The HMBC correlations between δ _C 7.09 (C-9) and δ _H 4.35 (glc-H-1) suggested that the glucospyranosyl moiety was attached at the C-9 of the hydroxy propylene group in ring B. Correlations were observed between H-2 and H-6/C-7 and between H-2 and H-6/C-7. These results indicate that **2** has two phenylpropanoid units. The most significant HMBC correlations were observed between H-8/C-1 and C-5 and between H-9/C-7 and C-4, C-6. The position of the methoxyl group was determined to be at C-3 and C-3 based on NOESY (-OCH₃/H-2, -OCH₃/H-2) and HMBC correlations (-OCH₃/C-3, -OCH₃/C-3). The structure of **2** was elucidated to be 3-[3-[1,3-dihydroxy-3-(4-hydroxy-3-methoxyphenyl)-propyl]-4-hydroxy-5-methoxyphenyl]allyl-*O*- β -D-glucoside and we designated it bidenlignaside B.

It has been noted that the stereochemistry at C-9 for **1**, and at C-7 and C-9 for **2**, has not been confirmed. We examined the inhibitory activity of bidenlignaside A (**1**) and bidenlignaside B (**2**) on histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction. The inhibitory effects of **1** (IC₅₀, 25.1 μ g) and **2** (IC₅₀, 37.2 μ g) had the same activity as the potent antiinflammatory drug, indomethacin (IC₅₀, 24.3 μ M). These results suggest that bidenlignaside A and bidenlignaside B have prospects in the further development of antiallergic drugs and therapy of related diseases.

Experimental

General Experimental Procedures All the melting points were determined on a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV spectrum was obtained on a Hitachi 200-10 spectrophotometer, and the IR spectrum was recorded on a JASCO IR A-2 spectrophotometer. The ¹H- and ¹³C-NMR spectra were recorded on JEOL GL-500 and JEOL FX-100 spectrometers, respectively, using TMS as an internal standard. The MS were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out with silica gel (Wako gel C-300, Wako Pure Chemical Industry Ltd.) and Sephadex LH-20 (20–100 mm, Pharmacia Fine Chemical Co., Ltd.). Thin-layer chromatography (TLC) was performed on Sigma-Aldrich TLC plates (250 μ m thickness), with compounds visualized by spraying with 5% (v/v) H₂SO₄ in ethanol solution.

Plant Materials The whole plant of *B. parviflora* WILLD. was collected from Liaoning province (China) and was identified by Prof. Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, China). A voucher specimen is deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and Isolation The air-dried whole plant (5.5 kg) was extracted twice with 60% ethanol under reflux. Evaporation of the solvent under reduced pressure from the combined extract gave the 60% EtOH extract (674.2 g). The extract was dissolved and suspended in water (2.0 l) and partitioned with hexane (3 \times 2 l), ethyl acetate (3 \times 2 l) and *n*-butanol (3 \times 2 l), respectively, and then solvents were evaporated to yield the hexane extract (136 g), ethyl acetate extract (126 g), butanol extract (176 g), and aqueous extract (376 g). The butanol extract was subjected to silica gel column chromatography (SiO₂, 400 g; eluted with CHCl₃ and MeOH in increasing polarity to obtain nine fractions. Fr. 7 was subjected to Sephadex LH-20 column chromatography eluted with 50% MeOH to obtain fr. 7–8 and was purified by preparative HPLC (Sunshu Park, Pegasil ODS, 35% MeOH) to give 14 mg of compound **1** and 12 mg of compound **2**.

Bidenlignaside A (**1**): Colorless powder (MeOH); [α]_D²⁵ -16.5° (*c*=0.14). UV (methanol) λ _{max} (log ϵ): 222 (4.34), 248 (3.95), 267 (4.04). IR ν _{max} cm⁻¹: 3395, 2932, 1662, 1587, 1462, 1423, 1273, 1232. FAB-MS, *m/z* 537 (M+H)⁺, 163 (100); high-resolution positive-ion FAB-MS Calcd for C₂₆H₃₃O₁₂ (M+H)⁺: 537.19712; Found: 537.19659. ¹H- and ¹³C-NMR spectral data (in MeOH-*d*₄; 500 MHz and 125 MHz; see Table 1).

Bidenlignaside B (**2**): Colorless powder (MeOH); [α]_D²⁵ -18.2° (*c*=0.31). UV (methanol) λ _{max} (log ϵ): 207 (4.39), 246 (4.45), 268 (2.09). IR ν _{max} cm⁻¹: 3412, 2931, 1715, 1604, 1512, 1482, 1422, 1369. FAB-MS, *m/z* 537 (M+H)⁺, 163 (100); high-resolution negative-ion FAB-MS Calcd for C₂₆H₃₄O₁₂ (M+H)⁻: 537.19712; Found: 537.19700. ¹H- and ¹³C-NMR spec-

Table 1. ¹³C- and ¹H-NMR Data of Compounds **1** and **2**

Aglycone	1		2	
	δ _C	δ _H	δ _C	δ _H
1	130.2 s		134.1 s	
2	112.8 d	7.68 d (2.1)	111.9 d	7.01 d (2.1)
3	148.8 s		148.7 s	
4	153.2 s		147.1 s	
5	115.9 d	6.79 d (8.3)	115.7 d	6.72 d (8.3)
6	124.9 d	7.69 dd (8.3, 2.1)	121.1 d	6.82 dd (8.3, 2.1)
7	199.3 s		74.2 d	4.83 dd (5.8, 3.2)
8	49.3 t	4.36 dd (10.4, 9.2) 3.74 dd (10.4, 3.5)	46.3 t	3.80 dt (9.6, 5.8) 3.67 dt (9.6, 3.2)
9	64.5 d	5.52 dd (9.2, 3.5)	62.3 d	4.37 dd (5.8, 3.2)
3-OCH ₃	56.6 q	3.90 s	56.6 q	3.76 s
1'	136.1 s		132.8 s	
2'	118.8 d	6.98 d (1.9)	120.9 d	6.87 d (1.9)
3'	133.6 s		139.2 s	
4'	143.6 s		149.2 s	
5'	154.0 s		151.9 s	
6'	110.4 d	6.72 d (1.9)	111.5 d	7.01 d (1.9)
7'	130.8 d	6.47 d (15.9)	134.4 d	6.58 d (15.9)
8'	130.6 d	6.25 dt (15.9, 5.5)	125.2 d	6.23 dt (15.9, 6.4)
9'	63.6 t	4.17 dd (5.5, 1.5)	70.9 t	4.49 dd (12.5, 6.4) 3.88 dd (12.5, 6.4)
3'-OCH ₃	56.5 q	3.88 s	56.4 q	3.79 s
Glucose				
1	105.9 d	4.92 d (7.6)	103.3 d	4.35 d (7.6)
2	76.1 d	3.22 m	75.2 d	3.20 m
3	78.0 d	3.49 dm	78.0 d	3.36 m
4	70.9 d	3.49 m	71.7 d	3.28 m
5	78.5 d	3.26 m	78.2 d	3.26 m
6	62.3 t	3.85 dd 3.78 dd	62.7 t	3.84 dd 3.76 dd

Assigned by the ¹H-¹H COSY, HMQC, and HMBC spectra. 125 MHz to ¹³C-NMR, 500 MHz to ¹H-NMR, TMS as int. standard (ppm, in MeOH-*d*₄).

tral data (in MeOH- d_4 ; 500 MHz and 125 MHz; see Table 1).

Acid Hydrolysis of 1 and 2 A solution of 1 and 2 (2 mg each) in 5% aqueous H₂SO₄-1,4-dioxane (0.5 ml, 1:1, v/v) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH⁻ form) and the residue was removed by filtration. After solvent was removed from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H₂O and MeOH. The H₂O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose. GLC conditions: Supelco STM-1, 30 m×0.25 mm (i.d.) capillary column; injector temperature, 230 °C, detector 230 °C; column temperature, 230 °C; He gas flow rate, 15 ml/min; and t_R , 24.5 min (from 1 and 2).

Histamine Release Assay The isolated compounds were assayed using the HPLC-fluorometry method previously described¹²⁾ with modifications. Male Wistar rats (Japan SLC, Shizuoka) weighing 180–200 g were exsanguinated and injected intraperitoneally with 10 ml of tyrode solution. The abdominal region was gently massaged for 3 min and then the peritoneal exudates were collected. The peritoneal cavity fluid containing mast cells was suspended in PBS, then layered on BSA ($d=1.068$) in a test tube at room temperature for 20 min. After centrifugation at 300×*g* and 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 3 ml of PBS (pH 7.0) and suspended in the same medium. Cell viability was determined using trypan blue. Mast cells (1×10^6 cells/ml) were preincubated with test samples (10 μ l) at 37 °C for 10 min, followed by addition of histamine releasers, either compound 48/80 (5 μ g/ml) or anti-DNP-IgE (5 μ g/ml) and phosphatidyl serine (100 μ g/ml). The mixtures were incubated again for 10 min, the quantity of histamine released was expressed in peak height, and the percentage inhibition was calculated.

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