Antiallergic Agents from Natural Sources. 3.[1] Structures and Inhibitory Effects on Nitric Oxide Production and Histamine Release of Five Novel Polyacetylene Glucosides from *Bidens parviflora* WILLD.

Naili WANG,*[,a] Xinsheng YAO,[a] Rie ISHII,[b] and Susumu KITANAKA*[,c]

Department of Natural Products Chemistry, Shenyang Pharmaceutical University,[a] 103 Wenhua Road, Shenhe District Shenyang , Shenyang 110015, China, Saitama Institute of Public Health,[b] 639–1 Kamiokubo, Urawa, Saitama 338–0824, Japan, and College of Pharmacy, Nihon University,[c] 7–7–1 Narashinodai, Funabashi, Chiba 274–8555, Japan.
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Five new polyacetylene glucosides, bidensyneosides A_1, A_2, B, C (1—4), and 3-deoxybidensyneoside B (5), have been isolated from the air-dried whole plant of *Bidens parviflora* WILLD. The structures were identified based on spectroscopic analysis, physicochemical properties, and application of the modified Mosher method to be 3(*R*),8(*E*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-glucopyranoside (1), deca-3(*R*),8(*Z*) 8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-glucopyranoside (2), 3(*R*)-deca-4,6,8-triyne-1,3-diol 1-*O*-β-D-glucopyranoside (3), 3(*R*),8(*E*)-8-decene-4,6-diyne-1,3,10-triol 1-*O*-β-D-glucopyranoside (4), and 8(*E*)-8-decene-4,6-diyne-1,10-diol 1-*O*-β-D-glucopyranoside (5), respectively. These compounds inhibited nitric oxide (NO) production in lipopolysaccharide and interferon-γ activated murine macrophages (RAW264.7) and also inhibited histamine release from rat mast cells stimulated by the antigen–antibody reaction.

Key words *Bidens parviflora* WILLD.; polyacetylene glucoside; Mosher method; mast cell; macrophage; histamine release inhibitor; NO production inhibitor

The plant *Bidens parviflora* WILLD. has been used in traditional Chinese medicine as an antipyretic, antiinflammatory, and antirheumatic treatment.[2,3] Previous studies have isolated sterols,[4] monoterpenes,[5] flavonones,[6] flavonoids,[7] polyacetylene glucosides,[8] chalcones,[9] aurones,[10] and flavonol glycosides[11] from this family. The isolation of four flavones from the leaves of *B. parviflora* has also been reported by others.[12] In screening the bioactivity of *Compositae* plants, we found that a *B. parviflora* WILLD. extract inhibits histamine release from rat mast cells induced by compound 48/80. We are therefore studying the chemical constituents of this plant in an effort to identify the antiallergic agents. In the present report, we describe the isolation and structural determination of five new polyacetylene glucosides (**1**—**5**) from *B. parviflora*. We also report on the inhibition of histamine release and NO production by these compounds.

The molecular formula of bidensyneoside A_1 (**1**), a brown powder, $[\alpha]_D^{23}$ −146.4° (c=0.6, MeOH), was determined to be $C_{16}H_{22}O_7$ (m/z 326.13690 $[M]^+$) by high resolution (HR) EI-MS and EI-MS (m/z 164 $[M-\text{hexose}]^+$). The spectral properties and hydrolysis with β-glucosidase indicated that **1** is a glucoside. In the IR spectrum, absorption bands attributable to acetylene (2231 cm^{-1}, 2138 cm^{-1}), hydroxyl (3394 cm^{-1}), and ethylene (1675 cm^{-1}) groups were observed. The UV spectra of **1** were typical for an ene-diyne chromophore (λ_{max}=220, 241, 253, 268, 284 nm).[13] Extensive analysis of the ^{1}H-NMR spectra together with ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{1}H-detected heteronuclear correlation through multiple quantum coherence (HMQC) spectra indicated the presence of a methyl proton at δ 1.80 (3H, dd, J=7.1, 1.8 Hz, Me-10) coupling with two *E*-configured olefinic protons at δ 6.32 (1H, dq, J=15.9, 7.1Hz, H-9) and δ 5.58 (1H, dd, J=15.9, 1.8 Hz, H-8) indicating a methyallyl moiety, a methine proton at δ 4.64 (1H, t, J=6.7 Hz, H-3), a methylene proton at δ 1.97 (2H, m, H-2), and nonequivalent methylene protons at δ 3.99 (1H, dt, J=10.4, 5.8 Hz, H-1a) and δ 3.72 (1H, dt, J=10.4, 6.4 Hz, H-1b). The correlations of protons at H-8, H-9, and H-10, and H-1, H-2 and H-3 were confirmed by ^{1}H–^{1}H COSY spectral data. The presence of hydroxyl groups at positions 1 and 3 was confirmed by a distortionless enhancement in a polarization transfer (DEPT) experiment, showing one methine group at δ 60.3 and one methylene group at δ 66.9. C-4, C-5, C-6, and C-7 were confirmed to be ethynyl carbons in a ^{13}C-DEPT experiment showing the four quaternary carbons at δ 69.8, 72.5, 78.2, and 83.1. Long-range correlations were observed in the heteronuclear multiple-bond connectivity (HMBC) spectrum between the following: CH_3-10 (δ 1.80) and C-9 (δ 145.1), C-8 (δ 110.6); H-9 (δ 6.32) and C-10 (δ 18.8), C-8 (δ 110.6), C-7 (δ 78.2); H-8 (δ 5.58) and C-10 (δ 18.8), C-9 (δ 145.1), C-7 (δ 78.2), C-6 (δ 72.5); H-3 (δ 4.64) and C-1 (δ 66.9), C-2 (δ 39.1), C-5 (δ 69.8), C-6 (δ 72.5); H-2 (δ 1.97) and C-1 (δ 66.9), C-3 (δ 60.3), C-4 (δ 83.1), C-5 (δ 69.8); and H-1a

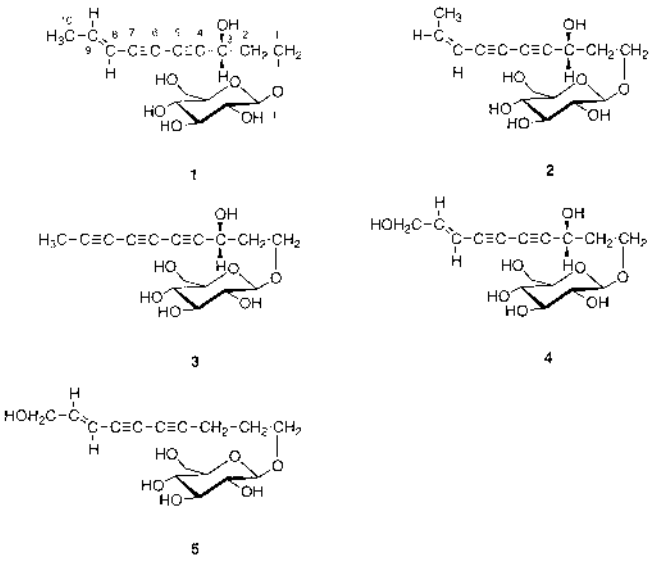

Chart 1

* To whom correspondence should be addressed. e-mail: kitanaka@pha.nihon-u.ac.jp

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**5** (in MeOH-d_4)

Proton	1	2	3	4	5
1	3.99 dt (10.4, 5.8)	4.00 dt (10.8, 5.4)	3.98 dt (10.1, 5.6)	3.99 dt (9.8, 5.8)	3.96 dt (9.8, 5.8)
	3.72 dt (10.4, 6.4)	3.74 dt (10.8, 6.8)	3.72 dt (10.1, 6.8)	3.73 dt (9.8, 6.7)	3.72 dt (9.8, 6.6)
2	1.97 m	1.97 m	1.96 m	1.97 m	1.83 m
3	4.64 t (6.7)	4.67 t (8.2)	4.62 t (7.1)	4.65 t (6.7)	2.47 t (7.3)
8	5.58 dq (15.9, 1.8)	5.53 dq (10.5, 2.1)		5.81 dq (15.9, 1.9)	5.76 dq (15.9, 1.9)
9	6.32 dq (15.9, 7.1)	6.20 dq (10.5, 6.8)		6.39 dq (15.9, 4.9)	6.34 dq (15.9, 4.6)
10	1.80 dd (7.1, 1.8)	1.89 dd (6.8, 2.1)	1.96 s	4.13 dd (4.9, 1.9)	4.13 dd (4.6, 1.9)
Glc-1′	4.26 d (7.7)	4.26 d (7.8)	4.25 d (7.6)	4.26 d (8.0)	4.24 d (8.0)

Chemical shift (δ) in ppm and coupling constant (J) in Hz in parentheses.

Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**5** (in MeOH-d_4)

Position	1	2	3	4	5
1	66.9 t	66.8 t	66.7 t	66.8 t	69.2 t
2	39.1 t	38.9 t	38.9 t	39.0 t	29.8 t
3	60.3 d	60.2 d	60.0 d	60.2 t	16.9 t
4	83.1 s	85.2 s	79.4 s	84.4 s	84.3 s
5	69.8 s	69.5 s	69.7 s	69.6 s	66.2 s
6	72.5 s	76.0 s	64.6 s	74.2 s	74.1 s
7	78.2 s	78.5 s	59.1 s	77.6 s	75.3 s
8	110.6 d	109.7 d	64.9 s	108.6 d	109.2 d
9	145.1 d	144.0 d	78.0 s	148.1 d	147.1 d
10	18.8 q	16.5 q	3.8 q	62.6 t	62.7 t
Glucose moiety					
1′	104.6 d	104.6 d	104.6 d	104.6 d	104.5 d
2′	75.2 d	75.1 d	75.1 d	75.1 d	75.2 d
3′	78.1 d	78.5 d	78.1 d	78.5 d	78.1 d
4′	71.7 d	71.6 d	71.6 d	71.6 d	71.7 d
5′	78.0 d	78.0 d	77.9 d	78.0 d	77.9 d
6′	62.8 t	62.7 t	62.7 t	62.7 t	62.8 t

The multiplicities of carbon signals were determined using the DEPT method, and are indicated as s, d, t, and q.

(δ 3.99), H-1b (δ 3.72) and C-1′ (δ 104.6), C-3 (δ 60.3), C-4 (δ 83.1), C-5 (δ 69.8). Aglycone was identified to be $C_{10}H_{12}O_2$ by EI-MS. In the HMBC spectrum of **1**, the glucose anomeric proton (δ_H 4.26) showed a correlation with a methylene carbon at δ 66.9, indicating that the glucose must be linked at position C-1 of the aglycone. Thus, the planar structure of **1** was established to be 3(*R*),8(*E*)-8-decene-4,6-diyne-1,3-diol 1-*O*-β-D-glucopyranoside.

Bidensyneoside A_2 (**2**) was obtained as a brown powder, $[\alpha]_D^{23}$ −157.5° (c=0.4, MeOH). The HR-EI-MS data indicated that **2** has the same molecular structure as **1** ($C_{16}H_{22}O_7$). The UV, IR, ^{1}H-, and ^{13}C-NMR spectra of **2** were nearly identical to those of **1** (Tables 1, 2), except for a methyl chemical shift observed in the ^{1}H- and ^{13}C-NMR spectra. The methyl proton (δ 1.89) was observed δ 0.09 ppm downfield and the methyl carbon (δ 16.5) was observed 2.3 ppm upfield from that in **1**. The H-8 and H-9 coupling constants in **2** showed J values (10.5 Hz) that suggested a *Z*-configuration for the double-bond protons. The nuclear Overhauser effect (NOE) experiments indicated that irradiation of the 10-methyl proton signal at δ 1.89 caused an NOE increase at H-8, and irradiation of the proton signal at δ 5.53 (H-8) caused an NOE increase at the 10-methyl proton signal. Thus the double-bond configuration was assumed to be *Z*.

Bidensyneoside B (**3**) was isolated as a brown solid, $[\alpha]_D^{23}$ −52.2° (MeOH, c=0.6), and its molecular formula was identified to be $C_{16}H_{20}O_7$ by HR-EI-MS analysis. The UV spectrum of **3** was typical for a triyne chromophore (λ_{max}= 220, 241, 253, 268, 284 nm), but absorption was very weak. In the IR spectrum, absorption bands of acetylene (2221, 2138 cm^{-1}) were stronger than those of **1**. ^{1}H- and ^{13}C-NMR data (Tables 1, 2) for **3** were partially similar to those of **1**, showing three acetylenes and one terminal methyl at δ 1.96 (3H, s, Me-10). Long-range correlations were observed in the HMBC spectrum between the following: CH_3-10 (δ 1.96) and C-9 (δ 78.1), C-8 (δ 64.9), C-7 (δ 59.1), C-6 (δ 64.6); H-3 (δ 4.62) and C-1 (δ 66.7), C-2 (δ 38.9), C-5 (δ 69.6), C-6 (δ 74.2); H-2 (δ 1.96) and C-1 (δ 66.9), C-3 (δ 60.0), C-4 (δ 79.4), C-5 (δ 69.7), C-6 (δ 74.2); and H-1a (δ 3.98), H-1b (δ 3.72) and C-1 (δ 104.6), C-2 (δ 38.9), C-3 (δ 60.0), C-4 (δ 79.4), C-5 (δ 69.7). The planar structure of **3** was determined to be 3(*R*)-deca-4,6,8-triyne-1,3-diol 1-*O*-β-D-glucopyranoside.

Bidensyneoside C (**4**) was obtained as a brown solid, $[\alpha]_D^{23}$ −71.6° (MeOH, c=0.5), and its molecular formula was identified to be $C_{16}H_{22}O_8$ by HR-EI-MS analysis. The UV, IR, ^{1}H-, and ^{13}C-NMR spectra of **4** were nearly identical to those of **1**, with the exception of a terminal hydroxymethyl at δ 4.13 (2H, dd, J=4.9, 1.9 Hz, H-10) in **4** coupling with two *E*-configurated olefinic protons at δ 6.39 (1H, dt, J=15.9, 4.9 Hz, H-9) and 5.81 (1H, dt, J=15.9, 1.9 Hz, H-8) in the ^{1}H-NMR spectrum. The ^{1}H-NMR, ^{1}H–^{1}H COSY, and HMQC spectra indicated the presence of a methine proton at δ 4.65 (1H, t, J=6.7 Hz, H-3), a methylene proton at δ 1.97 (2H, m, H-2), and a methylene proton at δ 3.99 (1H, dt, J=9.8, 5.8 Hz, H-1a) and δ 3.73 (1H, dt, J=9.8, 6.7 Hz, H-1b). Long-range correlations were observed in the HMBC spectrum from $HOCH_2$-10 (δ 4.13) to C-9 (δ 148.1) and C-8 (δ 108.6). The hydroxymethyl position was indicated to combine at C-9. Thus the planar structure of **4** was elucidated as deca-8(*E*)-en-4,6-diyne-1,3,10-triol 1-*O*-β-D-glucopyranoside.

3-Deoxybidensyneoside B (**5**) was obtained as colorless crystals, mp 164 °C and $[\alpha]_D^{23}$ −67.7° (c=0.5, MeOH). The molecular formula of **5** was identified by HR-EI-MS analysis to be $C_{16}H_{20}O_8$, identical to that of **1**. The UV, IR, ^{1}H-, and ^{13}C-NMR spectra of **5** were nearly identical to those of **1**. However, unlike **1**, **5** does not have a terminal methyl, but has a hydroxymethyl at δ 4.13 (2H, dd, J=4.6, 1.9 Hz, H-10) coupling with two *E*-configurated olefinic protons at δ 6.34 (1H, dt, J=15.9, 4.6 Hz, H-9) and 5.76 (1H, dt, J=15.9, 1.9 Hz, H-8) in the ^{1}H-NMR spectrum. The ^{1}H-NMR, ^{1}H–^{1}H COSY, and HMQC spectra indicated the presence of three methylene protons at δ 2.47 (2H, t, J=7.3 Hz, H-3), δ 1.83

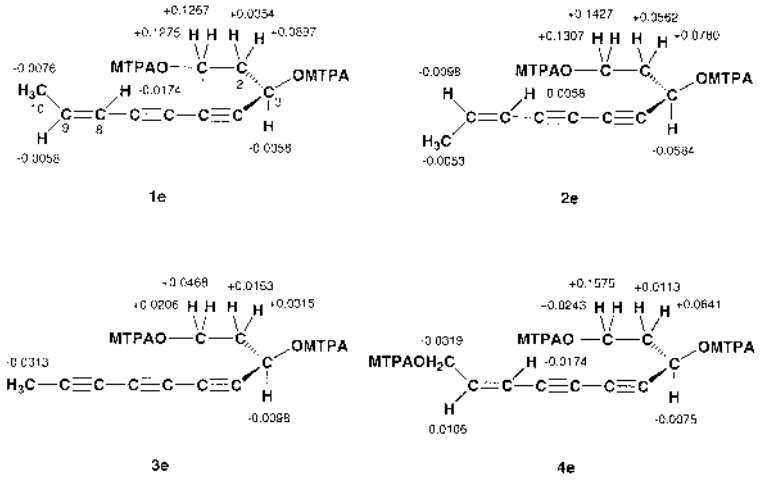

Fig. 1. $\delta=\delta_S-\delta_R$ Values (ppm) Obtained from the MTPA Esters **1e**—**4e** of the Aglycones **1a**—**4a** of Polyacetylene Glucosides **1**—**4** in $CHCl_3$ at 25 °C

Table 3. Inhibitory Effects of Polyacetylene Glucosides on Histamine Release from Mast Cells Induced by the Antigen–Antibody Reaction

Compound	IC_{50} (μM)
1	0.074
2	0.119
3	0.186
4	0.072
5	0.085
Indomethacin	0.625

Table 4. Inhibitory Activity of Polyacetylene Glucosides on NO Production by Macrophages

Compound	Assay I[a] IC_{50} (μM)	Assay II[b] IC_{50} (μM)
1	0.225	0.111
2	>1.00	>1.00
3	0.141	0.081
4	0.193	0.126
5	0.116	0.078

a) Cells were treated with a sample in the presence of 100 ng/ml LPS at 37 °C for 18 h. *b*) Cells were treated with a sample in the presence of 100 ng/ml LPS +10 U/ml IFN-γ at 37 °C for 18 h.

(2H, m, H-2), and δ 3.96 (1H, dt, J=9.8, 5.8 Hz, H-1a) and δ 3.72 (1H, dt, J=9.8, 6.6 Hz, H-1b). Long-range correlations were observed in the HMBC spectrum between the following: $HOCH_2$-10 (δ 4.13) and C-9 (δ 148.1), C-8 (δ 108.6); H-3 (δ 2.47) and C-1 (δ 69.2), C-2 (δ 29.8), C-4 (δ 84.3), C-5 (δ 66.2); H-2 (δ 1.83) and C-1 (δ 69.2), C-3 (δ 16.9), C-4 (δ 84.3), C-5 (δ 66.6); and H-1a (δ 3.96), H-1b (δ 3.72) and C-1′ (δ 104.5), C-2 (δ 29.8), C-3 (δ 16.9), C-4 (δ 84.3). Two hydroxymethyl positions were indicated at C-10 and C-1, and thus the planar structure of **5** was deduced to be (*E*)-8-decene-4,6-diyne-1,10-diol 1-*O*-β-D-glucopyranoside.

The sugar moiety in each compound was identified by thin-layer chromatography (TLC) after hydrolysis with β-glucosidase.[14] In all five compounds, the sugar unit was found to be glucose. This was confirmed by the ^{1}H- and ^{13}C-NMR spectral data. For compounds **1**—**5**, the coupling constant of H-1 was about 8 Hz, which indicated a β-glucose. The anomeric protons of all five compounds showed correlations in the HMBC spectrum with their methylene carbons at about δ 66.9, indicating that the glucose must be linked at the hydroxyl group at C-1 of the aglycone.

The stereochemistry at chiral centers (C-3) within compounds **1**, **2**, **3** and **4** was determined using the modified Mosher method[15] on the *R*-(+)-α-methoxy-α-trifluoromethylphenylacytyl (MTPA) esters (**1b**, **2b**, **3b**, **4b**) and *S*-(−)-MTPA esters (**1c**, **2c**, **3c**, **4c**) in their aglycones. In the ^{1}H-NMR spectra of the *R*-(+)-MTPA esters (**1b**, **2b**, **3b**, **4b**), the protons H-1 and H-2 appeared upfield, suggesting an effect of the MTPA phenyl ring.[15] In contrast, H-8, H-9, and H-10 were downfield from the corresponding *S*-(−)-MTPA derivatives (**1c**, **2c**, **3c**, **4c**). These results are shown in Fig. 1. Thus the absolute configurations at C-3 of **1**, **2**, **3**, and **4** were determined to be *R*.

We examined the inhibitory activity of **1**—**5** on histamine release from rat peritoneal exudate cells induced by the antigen–antibody reaction.[16] As shown in Table 3, the inhibitory effect of compounds **1**—**5** was much higher than that of the potent antiinflammatory agent, indomethacin.

Macrophages play major roles in inflammation and host defense mechanisms against bacterial as well as viral infections.[17] The nitric oxide (NO) radical, together with oxyradicals, is known to be an effector molecule for the antitumor and antimicrobial effects of macrophages. However, excessive production of NO may also lead to severe injury to host cells and tissues during acute and chronic inflammation.[18] We compared the inhibitory effects of **1**—**5** on NO production by the murine macrophage-like cell line RAW264.7 activated by lipopolysaccharide (LPS) and recombinant mouse interferon-γ (IFN-γ) (Table 4). Compounds **1**, **3**, **4**, and **5** were found to have the high activity. Interestingly, the inhibitory effects of polyacetylene derivatives on histamine release by mast cells and NO production by macrophages were observed first.

Experimental

Plant Materials *B. parviflora* WILLD. was collected in Da-Hi-Shan county, Liaoning province, China, in July 1999 and was identified by Professor Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, China). A voucher specimen is deposited in the Department of Natural Products Chemistry of Shenyang Pharmaceutical University.

General Procedures The melting point was determined on an uncorrected Yanagimoto micro-melting-point apparatus. UV spectra were obtained on a Hitachi 200-10 spectrophotometer and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were read on a JEOL GL-500 spectrometer, using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out using silica gel (Wako gel C-300, Wako Pure Chemical Ind., Ltd.) and Sephadex LH-20 (20—100 μm, Pharmacia Fine Chemical Co., Ltd.). TLC was performed on Merck TLC plates (0.25 mm thickness) and compounds visualized by 5% (v/v) H_2SO_4 in ethanol solution.

Extraction and Isolation The air-dried whole plants (5.5 kg) were extracted two times with 60% ethanol. 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 (3×2 l), ethyl acetate (3×2 l) and *n*-butanol (3×2 l), respectively. Evaporation of the solvent yielded a hexane fraction (P1, 67.2 g), ethyl acetate fraction (P2, 43.3 g), 1-butanol fraction (P3, 174.6 g), and the aqueous fraction (P4, 358.3 g). The 1-butanol fraction (166.2 g) was subjected to Sephadex LH-20 column chromatography with MeOH–H_2O (0→100) to give fractions P3-A (11.1 g), P3-B (37.2 g), P3-C (32.6 g), P3-D (15.3 g), P3-E (7.9 g), P3-F (8.9 g), P3-G (5.6 g), P3-H (6.5 g), P3- I (12.1 g), and P3-J (13.1 g). Fraction P3-D (15.3 g) was separated by normal-phase silica gel column chromatography (SiO_2, 400 g, eluted with $CHCl_3$ and MeOH in increasing polarity) to obtain nine fractions [P3-Da (0.17 g), P3-Db (0.89 g), P3-Dc (2.85 g), P3-Dd (0.65 g), P3-De (0.87 g), P3-Df (1.32 g), P3-Dg (1.16 g), P3-Dh (0.35 g), P3-Di (1.1 g)]. Fraction P3-Dc (2.85 g) was then purified by Sephadex LH-20 column chromatography (120 g), eluted with MeOH–H_2O (1 : 1), and further separated by preparative high-performance liquid chromatography (HPLC) (1NW 125 Fluofix, 10 mm i.d.×250 mm) eluted with 18% CH_3CN to give compounds **1** (P3-Dc-5, 110 mg), **2** (P3-Dc-4, 25 mg), **3** (P3-Dc-3, 90 mg), **4** (P3-Dc-2, 27 mg), and **5** (P3-Dc-1, 36 mg).

Bidensyneoside A_1 (1) A brown powder, $[\alpha]_D^{23}$ −146.4° (c=0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3394, 2923, 2231, 1675, 1160, 1074. UV λ_{max}^{MeOH} nm (ε): 284 (2300), 268 (3100), 253 (2300), 241 (2000). HR-EI-MS *m/z*: 326.13690 (Calcd for $C_{16}H_{22}O_7$: 326.13655). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Bidensyneoside A_2 (2) A brown powder, $[\alpha]_D^{23}$ −157.5° (c=0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3382, 2921, 2231, 1635, 1160, 1074. UV λ_{max}^{MeOH} nm (ε): 283 (2300), 267 (3200), 253 (2300), 240 (1600). HR-EI-MS *m/z*: 326.13633 (Calcd for $C_{16}H_{22}O_7$: 326.13655). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Bidensyneoside B (3) A brown powder, $[\alpha]_D^{23}$ −52.2° (c=0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3386, 2924, 2221, 1643, 1161, 1074. UV λ_{max}^{MeOH} nm (ε): 284 (1400), 267 (1600), 253 (1190), 239 (400). HR-EI-MS *m/z*: 324.12116 (Calcd for $C_{16}H_{20}O_7$ $[M]^+$, 324.12090). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Bidensyneoside C (4) A brown powder, $[\alpha]_D^{23}$ −71.6° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3381, 2886, 2231, 1641, 1160, 1074. UV λ_{max}^{MeOH} nm (ε): 283 (25700), 267 (33200), 253 (23400), 241 (12200). HR-EI-MS *m/z*: 342.13145 (Calcd for $C_{16}H_{22}O_8$ $[M]^+$, 342.13145). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Dehydrobidensyneoside B (5) Colorless crystal, mp 164 °C, $[\alpha]_D^{23}$ −67.7° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3330, 2927, 2231, 1627, 1160, 1074. UV λ_{max}^{MeOH} nm (ε): 328 (17500), 283 (24900), 267 (23700), 252 (21900), 239 (20900). HR-EI-MS *m/z*: 326.13658 (Calcd for $C_{16}H_{22}O_7$ $[M]^+$, 326.13655). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Enzymatic Hydrolysis A solution of compounds (**1**, **2**, **3**, **4**, **5**, each *ca.* 13 mg) and β-glucosidase (8 mg) in water (2 ml) was allowed to stand for 20 h at 37 °C. The reaction mixture was then extracted with AcOEt, and the AcOEt layer was evaporated to dryness under reduced pressure. The residue (*ca.* 6 mg) was purified by HPLC [Aquasil SS-2031 column, eluted with hexane–AcOEt (1 : 1)]. The aglycones (**1a**, **2a**, **3a**, **4a**, **5a**, each *ca.* 3 mg) were identified by ^{1}H-NMR ($CDCl_3$) spectral comparisons of the samples. The aqueous layer was evaporated to dryness, and the residue showed the presence of D-glucose on TLC (solvent $CHCl_3$: MeOH : H_2O=60 : 35 : 5, *Rf*=0.32).

Preparation of MTPA Ester The aglycones (**1a**, **2a**, **3a**, **4a**, each *ca.* 1 mg) were dissolved in pyridine (0.2 ml) and added to (+)-α-methoxy-α-trifluoro-methylphenylacetyl chloride or (−)-α-methoxy-α-trifluoro-methylphenylacetyl chloride (10 μl). The mixture was stirred overnight at room temperature and purified by HPLC [Aquasil SS-2031 column, eluted with hexane–AcOEt (1 : 1)] to afford the MTPA esters (**1b**, **2b**, **3b**, **4b**, and **1c**, **2c**, **3c**, **4c**, each *ca.* 0.8 mg).

Assay of Inhibitory Activity on Histamine Release All the isolated compounds were assayed using a modified HPLC-fluorometry method.[16] Male Wistar rats (Japan SLC, Shizuoka) weighing 180—200 g were exsanguinated and injected intraperitoneally with of Tyrode 10 ml 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 phosphate-buffered saline (PBS), then layered on bovine serum albumin (d=1.068) in a test tube at room temperature for 20 min. After centrifugation at 300×g at 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with PBS 3 ml (pH 7.0) and suspended in the same medium. Cell viability was determined using trypan blue dye exclusion. Mast cells (1.0×10^6 cells/ml) were preincubated with test samples (10 μl) at 37 °C for 10 min, followed by addition of histamine releasers (either compound 48/80 5 μg/ml or anti-DNP-IgE 5 μg/ml) and phosphatidyl serine (100 μg/ml). The mixtures were incubated again for 10 min, the quantity of histamine released was expressed in peak height, and % inhibition was calculated.

Assay of Inhibitory Activity on NO Production by Activated Macrophages[19] The RAW264.7 cells were seeded at 1.2×10^6 cells/ml onto a 35-mm Petri dish and then incubated at 37 °C for 2 h. Test compounds were then added to the culture simultaneously with both LPS 100 ng/ml and INF-γ 10 U/ml, and the cells were incubated at 37 °C, usually for 8 h. After incubation, the cells were chilled on ice, scraped from the dish with a cell scraper, collected in a microfuge tube, and then immediately centrifuged at 7000 rpm at 4 °C. A 7000 μl volume of the supernatant was placed in a new microfuge tube and stored at 4 °C before assaying for nitrite (NO_2^-). The cells were washed twice PBS without divalent cations by repeated centrifugation at 7000 rpm. The final cell pellet was extracted with a lysis buffer at 40 °C, comprised of 1% (V/V) Triton X-100, EDTA 0.1 mM and 1% aprotinin (Sigma) in HEPES NaOH buffer 20 mM, pH 7.5, at 4 °C for 30 min. The cell lysate was centrifuged at 10000 rpm at 4 °C for 1 min, and 30 μl of the resultant supernatant was placed in a new microfuge tube. The final cell extract was stored at −80 °C until use. One hundred microliters of the culture supernatant was placed in a well in duplicate 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was also placed in other wells on the same plate. To quantitate NO_2^-, Griess reagent 50 μl, 1% sulfanilamide in 5% H_3PO_4, and 0.1% *N*-1-naphthylethylenediamine dihydrochloride were added to each well. After 10 min, the reaction products were colorimetrically quantitated at 550 nm with subtraction of the background absorbance at 630 nm, using a Corona MTP-120 microplate reader (Corona Electric Co., Ltd.).

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