New Iridoid Glucosides from the Aerial Parts of *Verbena brasiliensis*

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Received March 9, 2006; accepted June 20, 2006

Two new iridoid glucosides, verbenabraside A (1) and verbenabraside B (2), were isolated from the aerial parts of *Verbena brasiliensis* VELL., along with six known iridoid glucosides, gelsemiol 3-*O*-*β*-D-glucoside (3), verbraside (4), 9-hydroxysemperoside (5), griselinoside (6), aralidioside (7), and 6*α*-hydroxyforsythide dimethyl ester (8), three known phenylethanoid glycosides, 2-phenylethyl *O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside (9), acteoside (10), and leucosceptoside A (11), two known lignan glucosides, dihydroxymethyl-*bis*(3,5-dimethoxy-4-hydroxyphenyl) tetrahydrofuran-9 (or 9′)-*O*-*β*-glucopyranoside (12) and (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside (13), a known methyl salicylate glucoside, methyl 2-*O*-*β*-D-glucopyranosylbenzoate (14), and two known sterols, *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (15) and *β*-sitosterol (16). Their chemical structures were determined on the basis of spectroscopic data. Compound 1 exhibited stronger scavenging effect on the stable free radical 1,1-diphenyl-2-picrylhydrazyl than that of *α*-tocopherol.

Key words *Verbena brasiliensis*; iridoid glucoside; phenylethanoid glycoside; lignan glucoside; sterol; radical-scavenging effect

Verbena brasiliensis VELL. is native to South America and is now grown wild in Japan. The presence of two iridoid glucosides, brasoside and verbraside, in this plant have been reported.[1,2] In the course of our studies on Verbenaceae plants and natural antioxidants, we examined the constituents of the MeOH extract of the aerial parts of *V. brasiliensis*. The present paper describes the isolation and structural elucidation of two new iridoid glucosides (**1**, **2**), along with six known iridoid glucosides (**3**—**8**), three known phenylethanoid glycosides (**9**—**11**), two known lignan glucosides (**12**, **13**), a known methyl salicylate glucoside (**14**), and two known sterols (**15**, **16**), as well as the radical-scavenging effects of **1** and **2**.

The MeOH extract of the aerial parts of *V. brasiliensis* was partitioned between hexane and aqueous MeOH. The aqueous MeOH layer was successively subjected to Diaion HP20 and silica gel column chromatography as well as HPLC on ODS to afford 15 compounds (**1**—**15**). Chromatography of the hexane layer over silica gel and Diaion HP20 furnished **16**.

Compounds **3**—**16** were identified as gelsemiol 3-*O*-*β*-D-glucoside (**3**),[3] verbraside (**4**),[2] 9-hydroxysemperoside (**5**),[3] griselinoside (**6**),[4] aralidioside (**7**),[4] 6*α*-hydroxyforsythide dimethyl ester (**8**),[5] 2-phenylethyl *O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside (**9**),[6] acteoside (**10**),[7] leucosceptoside A (**11**),[8] dihydroxymethyl-*bis*(3,5-dimethoxy-4-hydroxyphenyl) tetrahydrofuran-9 (or 9′)-*O*-*β*-glucopyranoside (**12**),[9] (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside (**13**),[10] methyl 2-*O*-*β*-D-glucopyranosylbenzoate (**14**),[11] *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**15**),[12] and *β*-sitosterol (**16**),[12] respectively, based on their physical and spectral data, although detailed ^{1}H-NMR spectral data and ^{13}C-NMR spectral data in CD_3OD of **3**—**8** have not been reported in the literature. The structures of compounds **1**—**8** are shown in Fig. 1.

Compound **1**, called verbenabraside A, was obtained as an amorphous powder and exhibited an $[M+Na]^+$ ion peak at *m*/*z* 547 in the positive FAB-MS and $[M-H]^-$ ion peak at *m*/*z* 523 in the negative FAB-MS. The molecular formula of **1** was determined to be $C_{25}H_{32}O_{12}$ using high-resolution (HR) negative FAB-MS. The ^{1}H- and ^{13}C-NMR signals of **1** were similar to those of **3**, with additional signals due to one *trans*-caffeoyl group. These ^{1}H- and ^{13}C-NMR signals (Tables 1, 2, respectively) were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra, and the data of the aglycone moiety of **1** were superimposable on those of **3**. Acidic hydrolysis of **1** afforded D-glucose which was confirmed by optical rotation using chiral detection in HPLC analysis, and the coupling constant of the anomeric proton signal [δ 4.36 (d, J=7.5 Hz)] indicated the mode of glycosidic linkage of the glucosyl group to be *β*. Furthermore, the signal due to H-4 of the glucosyl group was subjected to acylation shift by *ca*. 1.49 ppm in comparison with that of **3** in the ^{1}H-NMR spectrum, and a key correlation was observed between H-4 of the glucosyl group and the carboxyl carbon of the caffeoyl group in the HMBC spectrum. Based on these data, **1** was defined as gelsemiol 3-*O*-(4′-*trans*-caffeoyl)-*β*-D-

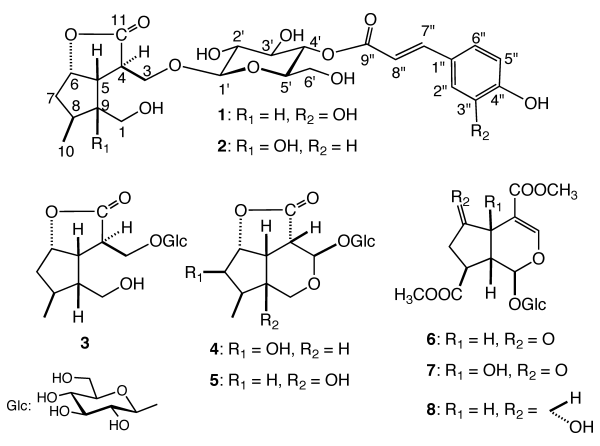

Fig. 1. Structures of **1**—**8**

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Table 1. ^{1}H-NMR Data for **1**—**8** (in CD_3OD 500 MHz)

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1a	3.80 dd (2.0, 11.0)	3.68 d (11.5)	3.79 dd (3.0, 11.0)	4.44 dd (4.0, 12.5)	4.06 d (12.0)	5.15 d (8.0)	5.99 d (2.0)	5.23 d (9.0)
1b	*ca.* 3.57	3.61 d (11.5)	3.58 dd (8.5, 11.0)	3.49 d (12.5)	3.46 d (12.0)			
3a	4.18 dd (3.5, 9.5)	4.22 dd (3.5, 10.0)	4.15 dd (3.5, 10.0)	5.35 s	5.35 s	7.50 s	7.75 s	7.63 s
3b	3.91 dd (4.5, 9.5)	3.97 dd (5.0, 10.0)	*ca.* 3.86					
4	3.02 m	3.03 ddd (3.5, 5.0, 7.0)	3.00 ddd (3.5, 3.5, 5.5)	3.02 d (11.5)	3.24 d (11.5)			
5	3.24 m	3.13 dd (6.5, 7.0)	3.22 ddd (5.5, 7.0, 7.0)	*ca.* 3.31	2.98 dd (6.5, 11.5)	*ca.* 3.38		2.87 ddd (1.0, 3.5, 9.0)
6	5.01 br dd (6.5, 6.5)	5.03 br dd (6.5, 7.0)	5.00 dd (6.5, 7.0)	4.73 d (6.5)	5.04 dd (4.5, 6.5)			4.51 dd (3.5, 3.5)
7a	2.09 br dd (5.5, 14.0)	*ca.* 1.93	2.08 dd (5.5, 14.5)	3.97 d (3.0)	2.00 dd (5.5, 12.5)	2.69 dd (4.5, 18.5)	*a)*	2.13 dd (9.0, 14.0)
7b	1.50 m	*ca.* 1.93	1.50 ddd (6.5, 11.5, 14.5)		1.84 ddd (4.5, 12.5, 12.5)	2.62 dd (10.0, 18.5)	*a)*	1.94 ddd (3.5, 10.0, 14.0)
8	*ca.* 1.75	2.01 m	*ca.* 1.76	1.87 m	1.93 m	*ca.* 3.38	2.90 d (10.0)	3.11 ddd (3.5, 9.0, 10.0)
9	*ca.* 1.77		*ca.* 1.76	1.80 ddd (4.0, 10.0, 12.5)		2.77 dd (2.5, 8.0)	2.75 dd (2.0, 10.0)	2.50 ddd (3.5, 9.0, 9.0)
10	1.02 d (5.5)	0.95 d (6.5)	1.02 d (5.5)	1.03 d (6.5)	0.97 d (6.5)			
10-OCH_3						3.76 s	3.75 s	3.69 s
11-OCH_3						3.73 s	3.68 s	3.72 s
1′	4.36 d (7.5)	4.38 d (8.0)	4.29 d (8.0)	4.51 d (8.0)	4.52 d (8.0)	4.67 d (8.0)	4.62 d (8.0)	4.68 d (8.0)
2′	*ca.* 3.31	3.31 dd (8.0, 9.5)	3.18 dd (8.0, 9.5)	3.23 dd (8.0, 8.5)	3.24 dd (8.0, 9.5)	3.27 dd (8.0, 9.5)	3.20 dd (8.0, 9.0)	3.21 dd (8.0, 9.5)
3′	*ca.* 3.58	3.65 dd (9.5, 9.5)	*ca.* 3.34	3.39 dd (8.5, 8.5)	3.40 dd (9.5, 9.5)	*ca.* 3.38	3.38 dd (9.0, 9.0)	3.38 dd (9.5, 9.5)
4′	*ca.* 4.83	4.85 dd (9.5, 9.5)	*ca.* 3.34	*ca.* 3.34	*ca.* 3.32	*ca.* 3.31	*ca.* 3.31	*ca.* 3.30
5′	*ca.* 3.54	*ca.* 3.53	*ca.* 3.27	*ca.* 3.31	*ca.* 3.31	*ca.* 3.31	*ca.* 3.31	*ca.* 3.30
6a′	*ca.* 3.63	*ca.* 3.66	*ca.* 3.86	3.84 dd (2.0, 12.0)	3.85 dd (2.0, 12.0)	3.88 dd (1.5, 12.0)	3.89 dd (2.0, 12.0)	3.86 d (12.0)
6b′	*ca.* 3.54	*ca.* 3.56	3.67 dd (3.5, 11.5)	3.69 dd (5.0, 12.0)	3.70 dd (5.0, 12.0)	3.64 dd (5.5, 12.0)	*ca.* 3.68	3.68 dd (3.5, 12.0)
2″	7.06 s	7.47 d (8.5)						
3″		6.82 d (8.5)						
5″	6.79 d (8.0)	6.82 d (8.5)						
6″	6.96 d (8.0)	7.47 d (8.5)						
7″	7.60 d (16.0)	7.67 d (16.0)						
8″	6.30 d (16.0)	6.36 d (16.0)						

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses). *a)* Signal not recorded due to D_2O exchange. Assignments are based on ^{1}H–^{1}H COSY, HMQC, and HMBC spectra.

Table 2. ^{13}C-NMR Data for **1**—**8** (in CD_3OD, 125 MHz)

C	1	2	3	4	5	6	7	8
1	61.5	65.3	61.4	57.5	61.2	97.3	95.5	100.4
3	70.8	70.3	70.6	98.6	98.5	154.1	156.8	155.8
4	43.7	44.9	43.7	42.0	44.8	105.1	106.9	106.7
5	46.1	54.2	46.0	35.6	48.0	40.2	74.3	44.2
6	85.4	84.6	85.3	88.3	84.7	213.3	209.5	74.4
7	42.8	39.8	42.8	77.8	39.3	37.8	*a)*	39.1
8	34.1	37.3	34.0	38.1*b)*	37.3	40.1	36.9	45.6
9	52.6	84.3	52.5	38.6*b)*	74.4	41.3	48.5	43.1
10	17.7	11.9	17.7	11.0	10.4	175.7	174.5	177.8
11	180.9	180.5	180.9	177.7	177.2	168.5	167.1	169.4
10-OCH_3						53.0	53.1	52.6
11-OCH_3						52.0	51.9	51.8
1′	104.6	104.5	104.5	103.9	104.0	101.1	100.8	100.8
2′	75.2	75.2	75.0	75.3	75.3	74.6	74.3	74.8
3′	75.8	75.8	78.0	78.0	78.0	78.0	77.6	77.9
4′	72.6	72.6	71.7	71.4	71.4	71.6	71.5	71.6
5′	76.3	76.3	78.1	78.3	78.3	78.4	78.4	78.4
6′	62.6	62.5	62.8	62.6	62.7	62.7	62.6	62.9
1″	127.8	127.2						
2″	115.4	131.3						
3″	146.9	117.0						
4″	149.8	161.4						
5″	116.7	117.0						
6″	123.1	131.3						
7″	147.7	147.3						
8″	114.9	114.9						
9″	168.7	168.6						

δ in ppm from TMS. *a*) Signal not recorded due to D_2O exchange. *b*) Assignments may be interchangeable. Assignments are based on ^{1}H–^{1}H COSY, HMQC, and HMBC spectra.

glucopyranoside.

Compound **2**, called verbenabraside B, was obtained as an amorphous powder. The HR negative FAB-MS of **2** indicated the same molecular formula as that of **1**. The ^{1}H- and ^{13}C-NMR signals of **2** were similar to those of **1**, apart from the appearance of signals due to a 4-substituted phenyl group and one oxygenated quaternary carbon, and the lack of signals due to a 3,4-disubstituted phenyl group and one methine group. In the same manner as for **1**, these ^{1}H- and ^{13}C-NMR signals were examined in detail, and the ^{13}C-NMR data of the aglycone moiety of **2** were very similar to those of 9α-hydroxygelsemiol,[13] except for the signals due to C-3 and C-4, which were considered to be subjected to glycosylation shift,[14,15] while the data on the sugar moiety were almost the same as those of **1**. In the HMBC spectrum, a key cross-peak was observed between C-3 of the aglycone moiety and anomeric proton. The relative stereochemistry in the aglycone moiety was examined from its nuclear Overhauser and exchange spectroscopy (NOESY) spectrum, and key NOEs were observed, as illustrated in Fig. 2. Consequently, the structure of **2** was concluded to be 9α-hydroxygelsemiol 3-*O*-(4′-*trans*-*p*-coumaroyl)-β-D-glucopyranoside.

The scavenging effects of **1** and **2** on the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were examined, and the activity was compared with that of the standard antioxidants α-tocopherol and L-cysteine, each at a 0.02 mM concentration. Compound **1** exhibited more potent activity than that of α-tocopherol; on the other hand, the activity of **2** was weaker than that of L-cysteine. Therefore the catechyl group of **1** was considered to be important for the activity.

As far as we know, **1** and **2** are new iridoid glucosides, and **3** and **5**—**16** are the first examples of isolation from the aerial parts of *Verbena brasiliensis*.

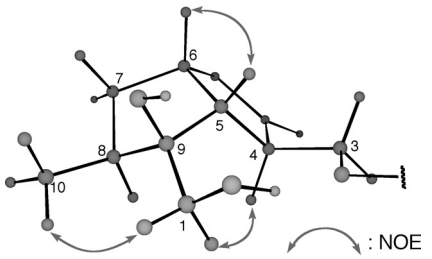

Fig. 2. Selected NOE Correlations Observed in NOESY Spectrum of **2** (in CD_3OD, 500 MHz)

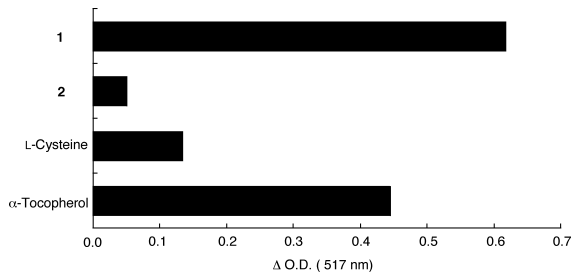

Fig. 3. DPPH Radical-Scavenging Effects of **1**, **2**, L-Cysteine, and α-Tocopherol

The final concentration of each sample tested was 0.02 mM. ΔO.D., O.D. of control at 517 nm (1.1135)—O.D. of sample. DPPH, 0.1 mM.

Experimental

All the instruments and the materials used were the same as cited in the previous report[16] unless otherwise specified.

Plant Material The aerial parts of *V. brasiliensis* were collected in Kumamoto prefecture, Japan, in August 2001, and identified by Professor

Toshihiro Nohara, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University.

Extraction and Isolation The cut fresh aerial parts of *V. brasiliensis* (3704 g) were extracted with MeOH (3900 ml) for 5 d at room temperature, and the solvent was removed under reduced pressure to give a syrup (307 g). The MeOH extract was partitioned between hexane (2900 ml) and aqueous MeOH [MeOH (2500 ml) and H_2O (100 ml)]. Each layer was dried under reduced pressure to furnish the hexane-soluble fraction (26.5 g) and aqueous MeOH-soluble fraction (280.0 g). The hexane-soluble fraction was successively subjected to silica gel column chromatography [hexane–acetone (20 : 1, 5 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1)] and Diaion HP20 column chromatography (80% MeOH, 90% MeOH, MeOH, acetone) to give fr. 1 (286 mg). Fraction 1 was recrystallized from hexane–acetone to afford **16** (22 mg). A part (52.3 g) of the aqueous MeOH-soluble fraction was chromatographed over Diaion HP20 (30% MeOH, 60% MeOH, 90% MeOH, MeOH, acetone) to give fr. 2—6. Chromatography of fr. 3 (11.7 g) over silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] gave fr. 7—19. Fraction 8 (61 mg), fr. 10 (213 mg), fr. 11 (213 mg), fr. 13 (128 mg), and fr. 14 (202 mg) were each subjected to HPLC on COSMOSIL 5C18 AR-II (Nacalai Tesque, Inc., 250 mm×20 mm i.d., 40% MeOH) to afford **14** (15 mg) from fr. 8, **6** (23 mg), **2** (40 mg), and **11** (26 mg) from fr. 10, **1** (20 mg) from fr. 11, **3** (4 mg) from fr. 13, and **12** (11 mg), **13** (28 mg), and **9** (17 mg) from fr. 14. A part (413 mg) of fr. 15 (4717 mg) was subjected to HPLC under the same conditions as for fr. 8 to afford **10** (221 mg). Fraction 5 (2290 mg) was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to give **15** (101 mg). A part (225.0 g) of the aqueous MeOH-soluble fraction was subjected to Diaion HP20 column chromatography (30% MeOH, 60% MeOH, acetone) to give fr. 20—22. Chromatography of fr. 21 (85.4 g) over silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5)] gave fr. 23—26. Fraction 24 (7.00 g) and fr. 25 (7.73 g) were each subjected to HPLC (30% MeOH) under the similar conditions as fr. 8 to afford **8** (165 mg) and **7** (348 mg) from fr. 24, and **4** (54 mg) and **5** (170 mg) from fr. 25.

Verbenabraside A (**1**): An amorphous powder, $[\alpha]_D^{13}$ −86.1° (c=2.1, MeOH). Positive FAB-MS m/z: 525 $[M+H]^+$, 547 $[M+Na]^+$. Negative FAB-MS m/z: 523 $[M-H]^-$. HR negative FAB-MS m/z: 523.1937 (Calcd for $C_{25}H_{31}O_{12}$: 523.1815). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Verbenabraside B (**2**): An amorphous powder. $[\alpha]_D^{13}$ −39.0° (c=4.4, MeOH). Positive FAB-MS m/z: 547 $[M+Na]^+$. Negative FAB-MS m/z: 523 $[M-H]^-$. HR negative FAB-MS m/z: 523.1859 (Calcd for $C_{25}H_{31}O_{12}$: 523.1815). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Acidic Hydrolysis of 1 and 2 Compounds **1** (2 mg) and **2** (2 mg) in 2 N HCl (1 ml) were each heated at 95 °C for 1 h. The reaction mixture was neutralized with 4 N NaOH and then evaporated under reduced pressure to give a residue. The residue was extracted with MeOH, and the MeOH extract was analyzed by HPLC under the following conditions: column, YMC pack Polyamine II (YMC Co., Ltd., 4.6 mm i.d.×250 mm); solvent, 80% CH_3CN; flow rate, 1.0 ml/min; column temperature, 35 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; and column oven, JASCO CO-2060. The retention time and optical activity of each of the samples were identical to those [t_R (min), 13.7; optical activity, positive] of D-glucose.

Assay of Scavenging Effect on DPPH The method of Uchiyama *et al.*[17] was applied in a slightly modified manner. The EtOH solution (1.00 ml) of each testing sample was added to a mixture of 0.1 M acetic acid buffer (pH 5.5, 1.00 ml) and 0.5 mM DPPH EtOH solution (0.50 ml) in a test tube and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. α-Tocopherol and L-cysteine were used as standard samples.

Acknowledgments We express our appreciation to Mr. K. Takeda and Mr. T. Iriguchi of Kumamoto University for their measurement of the NMR and MS spectra.

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