Four New Neuroprotective Iridoid Glycosides from *Scrophularia buergeriana* Roots

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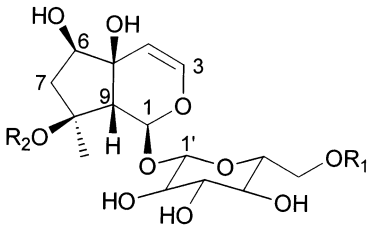
Four new iridoid glycosides were isolated from a 90% MeOH extract of *Scrophularia buergeriana* roots and characterized as 8-*O*-*E*-*p*-methoxycinnamoylharpagide (**1**), 8-*O*-*Z*-*p*-methoxycinnamoylharpagide (**2**), 6′-*O*-*E*-*p*-methoxycinnamoylharpagide (**3**), and 6′-*O*-*Z*-*p*-methoxycinnamoylharpagide (**4**), respectively. In addition, three known iridoids were identified as *E*-harpagoside (**5**), *Z*-harpagoside (**6**), and harpagide (**7**). Compounds **1**−**7** significantly attenuated glutamate-induced neurotoxicity when added to primary cultures of rat cortical cells at concentrations ranging from 100 nM to 10 μM. The results obtained indicate that the iridoid glycosides isolated from *S. buergeriana* have significant protective effects against glutamate-induced neurodegeneration in primary cultures of rat cortical neurons.

During a search for compounds that protect against glutamate-induced injury on primary cultures of rat cortical cells from natural sources,[1] it was found that a 50% methanolic $CHCl_3$ extract of *Scrophularia buergeriana* Miquel (Scrophulariaceae) roots exhibited significant neuroprotective activity.[2] Glutamate is widely known to be associated with central excitatory neurotransmission.[3] However, it is also recognized as being a cause of neuronal cell loss,[4,5] which is involved in neurodegenerative disorders such as seizures,[6] ischemia, spinal cord trauma,[7,8] Alzheimer's disease,[9] and Parkinson's disease.[10]

The genus *Scrophularia* is represented by over 300 species all over the world.[11] The dried roots of *Scrophularia* species have been used in Asian medicine for the treatment of fever, swelling, constipation, pharyngitis, neuritis, and laryngitis.[11−13] A number of iridoid glycosides, phenylpropanoids, terpenoids, and flavonoids have been reported as constituents of *Scrophularia* species.[14−17] We have previously isolated 10 phenylpropanoids from a 90% MeOH extract of *S. buergeriana* roots and demonstrated their neuroprotective activity against glutamate-induced toxicity in primary cultured rat cortical cells.[2] In the present study, further bioactivity-guided fractionation of the 90% MeOH extract of *S. buergeriana* has resulted in the isolation of four new iridoid glycosides, **1**−**4**, and three known iridoids, **5**−**7**. These compounds (**1**−**7**) showed significant protective activity on primary cultures of rat cortical cells after exposure to the excitotoxin glutamate.

The 50% methanolic $CHCl_3$ extract (1.5 kg) of *S. buergeriana* roots was suspended in water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was evaporated subsequently in vacuo. The resultant CH_2Cl_2 fraction was suspended in 90% MeOH and extracted with *n*-hexane. The residual 90% MeOH fraction was evaporated, subjected to silica gel column chromatography, and yielded seven iridoid glycosides (**1**−**7**).

Compound **1** was obtained as a pale brown amorphous powder. The molecular formula of **1** was determined as $C_{25}H_{32}O_{12}$ by HRFABMS. The positive FABMS of **1** exhibited significant fragment peaks at *m*/*z* 547 $[M + Na]^+$, 525 $[M + H]^+$, and 179 [methoxycinnamic acid + H]$^+$. The IR spectrum of **1** showed absorption bands for hydroxyl groups (3400 cm^{-1}, br), an α,β-unsaturated ester ($\nu_{C=O}$ 1690, $\nu_{C=C}$ 1625, ν_{C-O} 1025 cm^{-1}), and an aromatic moiety (1600, 1515 cm^{-1}). The ^1H and ^{13}C NMR (Table 1) data of **1** were similar to those of **5** (*E*-harpagoside)[14] except for the signals due to an *E*-cinnamic acid residue. The ^1H NMR signals of **1** at δ 7.55 (d, J = 16.0 Hz, H-β), 7.48 (d, J = 8.8 Hz, H-2″ and 6″), 6.89 (d, J = 8.8 Hz, H-3″ and 5″), 6.30 (d, J = 16.0 Hz, H-α), and 3.71 (s, OMe) suggested the presence of a *E*-*p*-methoxycinnamic acid unit in the molecule instead of an *E*-cinnamic acid residue as found in *E*-harpagoside. In the ^1H NMR spectrum, the downfield shifts of H-7 at δ 2.20 and 2.02 (ca. 0.5 ppm) and H-10 at δ 1.47 (ca. 0.4 ppm) by comparison with harpagide (**7**) itself proved that the hydroxyl group at the C-8 position of the harpagide unit was acylated with an *E*-*p*-methoxycinnamoyl group. Thus, the structure of **1** was assigned as 8-*O*-*E*-*p*-methoxycinnamoylharpagide.



1 R_1=H, R_2=*E*-*p*-methoxycinnamoyl
2 R_1=H, R_2=*Z*-*p*-methoxycinnamoyl
3 R_1= *E*-*p*-methoxycinnamoyl, R_2=H
4 R_1= *Z*-*p*-methoxycinnamoyl, R_2=H
5 R_1=H, R_2=*E*-cinnamoyl
6 R_1=H, R_2=*Z*-cinnamoyl
7 R_1=R_2=H

Compound **2** was also obtained as a pale brown amorphous powder. The spectroscopic data of **2** closely resembled those of **1** except for two olefinic proton signals at δ 6.83 (d, J = 12.7 Hz, H-β) and 5.81 (d, J = 12.7 Hz, H-α).

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Table 1. ^{13}C NMR Data of Compounds **1**−**4**

carbon	δ_C ppm **1**	**2**	**3**	**4**
aglycon				
1	94.8	94.6	93.2	93.3
3	144.1	143.7	141.0	142.7
4	106.9	107.3	108.6	108.6
5	73.6	73.3	72.9	72.9
6	77.7	77.8	78.5	78.5
7	46.4	46.2	47.2	47.2
8	88.2	88.3	90.0	89.1
9	55.9	55.8	59.7	59.7
10	22.9	22.7	23.5	25.2
glucose				
1′	100.1	99.9	99.6	99.5
2′	74.6	74.7	74.6	74.6
3′	77.8	77.8	78.1	78.3
4′	71.9	71.9	71.8	71.8
5′	77.8	77.8	56.1	56.0
6′	63.1	63.0	62.9	64.6
acyl moiety				
1″	128.4	131.1	130.2	129.4
2″	131.1	133.3	131.2	133.6
3″	115.5	114.7	116.0	114.7
4″	163.2	162.1	162.4	162.1
5″	115.5	114.7	116.0	114.7
6″	131.1	133.3	131.2	133.6
α	117.5	119.4	119.3	117.4
β	146.0	143.9	146.6	145.1
COOH	169.2	168.5	168.1	167.5
OMe	56.0	55.9	56.0	56.0

Besides these olefinic protons, four aromatic protons and three protons of a methoxyl group were attributed to a *Z*-*p*-methoxycinnamic acid moiety. The esterification site was determined to be the C-8 hydroxyl groups from the observed acylation-induced shifts of H-7 at δ 2.18 and 1.98 and H-10 at δ 1.50, respectively. Thus, the structure of **2** was assigned as 8-*O*-*Z*-*p*-methoxycinnamoylharpagide.

Compound **3** was obtained as a pale brown amorphous powder and had the same molecular formula, $C_{25}H_{32}O_{12}$, as **1** and **2**. The IR spectrum of **3** also showed absorption bands similar to **1**. The ^1H and ^{13}C NMR (Table 1) spectral data indicated that **3** contains an *E*-*p*-methoxycinnamic acid unit. In the ^1H NMR spectrum, a downfield shift of the H-6′ signal (δ 4.50 and 4.47) by comparison with harpagide suggested that the hydroxyl group at C-6′ of glucopyranoside in the structure was substituted. Thus, the site of esterification by the *E*-*p*-methoxycinnamoyl group was determined to be the C-6′ of the glucopyranose unit, which was confirmed from the HMBC correlations observed between the signals at δ_C 168.1 (carbonyl of *E*-*p*-methoxycinnamoyl group) and δ_H 4.50 and 4.47 (H-6′). Thus, the structure of **3** was determined as 6′-*O*-*E*-*p*-methoxycinnamoylharpagide.

Compound **4** was also obtained as a pale brown amorphous powder. The spectroscopic data of **4** were essentially similar to those of **3** except for two olefinic proton signals at δ 6.93 (d, J = 12.66 Hz, H-*β*) and 5.83 (d, J = 12.66 Hz, H-α). Besides these olefinic protons, four aromatic protons and three protons of a methoxyl group were attributed to a *Z*-*p*-methoxycinnamic acid unit. Thus, the structure of **4** was assigned as 6′-*O*-*Z*-*p*-methoxycinnamoylharpagide.

The three known compounds (**5**−**7**) also displayed a characteristic TLC pattern of iridoid glycosides and gave a deep blue color by spraying with anisaldehyde-sulfuric reagent. In addition, their ^1H and ^{13}C NMR spectra showed typical signals of H-1 (1H, br s, ca. 6 ppm) and C-1 (near to 100 ppm) of iridoid glycosides, respectively.[14] These compounds were identified as *E*-harpagoside (**5**),[14] *Z*-harpagoside (**6**),[18] and harpagide (**7**),[14] respectively, by spectral data comparison with literature values.

The neuroprotective activity of compounds **1**−**7** was quantified by measuring the release of LDH into the culture media from primary cultures of rat cortical cells injured with glutamate (Table 2). At concentrations of 0.1−10.0 μM, compounds **1**−**4** significantly blocked the release of LDH from glutamate-injured primary cultures of rat cortical cells. Compounds **5**−**7** also reduced the release of LDH in a dose-dependent manner. Among the seven compounds tested, **1** and **3**, with an *E*-*p*-methoxycinnamoyl group in the molecule, showed the most potent neuroprotective activity in the in vitro system used. However, the site of acylation by the *E*-*p*-methoxycinnamoyl group of **1** and **3** proved not to have an effect on neuroprotectivity since both compounds showed similar protective potencies against glutamate-induced neurotoxicity. In a previous report, we observed similar results for flavonoids and iridoids containing *E*-*p*-methoxycinnamoyl substituents in terms of their resultant neuroprotective activities.[19] Although an *E*-*p*-methoxycinnamic acid unit was revealed to be a crucial structural moiety for neuroprotective compounds described in our previous reports,[19,20] iridoids **5**−**7**, which have no *E*-*p*-methoxycinnamoyl moiety in their structure, exhibited significant neuroprotective activity. On the basis of these results, it can be concluded that the iridoid aglycon unit, as well as the *E*-*p*-methoxycinnamic acid unit, leads to significant neuroprotective effect on primary cultures of rat cortical cells injured by glutamate. The neuroprotective activity of harpagide (**7**), the aglycon of harpagide-type iridoids **1**−**6**, against glutamate-induced neuronal toxicity in primary cultured rat cortical cells is reported herein for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 1000 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer. FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer. ^1H and ^{13}C NMR spectra were performed on a JEOL GSX 400 spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard. ^1H NMR spectra were obtained on a Bruker AMX 500 spectrometer at 500 MHz. FABMS and HRFABMS were obtained on a VG 70-VSEQ mass spectrometer with a direct inlet system using PEG 600/glycerol as the matrix. HPLC separations were performed with a Hitachi HPLC system (L-6200 Pump, L-4000 UV−vis detector) on A-323 ODS C_{18} (4 μm, 250 × 10 mm, YMC), with detection at 230 nm. TLC was carried out on silica gel precoated plates (Art. No. 5715, Merck).

Plant Material. The roots of *S. buergeriana* were purchased from Kyongdong Oriental Herbal Market, Seoul, Korea, in 1996 and identified by Dr. Dae S. Han, Professor Emeritus, College of Pharmacy, Seoul National University. Voucher specimens (SNUPH-0303) documenting this purchase have been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. Dried roots of *S. buergeriana* (20 kg) were extracted for 3 h with $CHCl_3$−MeOH (1:1) (3×, each 10 L) using a reflux apparatus that yielded an extract (1.5 kg) upon removal of the solvent in vacuo. This extract was suspended in water and extracted with CH_2Cl_2, with the organic layer evaporated to dryness in vacuo. The resultant CH_2Cl_2 extract (327.3 g) was suspended in 90% MeOH and extracted with *n*-hexane. The residual 90% MeOH suspension was dried in vacuo to yield 250 g of a 90% MeOH extract. The 90% MeOH extract, which showed significant neuroprotective activity (67.4% protection against glutamate-induced neurotoxicity at 10 μg/mL, p <0.001), was chromatographed on a silica gel column (2.5 kg, 230−400 mesh, column size 12 ×

Table 2. Neuroprotective Activities of Compounds **1**−**7** on Primary Cultures of Rat Cortical Cells Injured by Glutamate[a]

compound	cell viability (%)[b,c] 0.1 μM	1.0 μM	10.0 μM
8-*O*-*E*-*p*-methoxycinnamoylharpagide (**1**)	54.9 ± 5.9***	61.8 ± 4.1***	58.9 ± 5.3***
8-*O*-*Z*-*p*-methoxycinnamoylharpagide (**2**)	40.7 ± 2.9**	48.2 ± 5.7***	49.6 ± 9.6**
6′-*O*-*E*-*p*-methoxycinnamoylharpagide (**3**)	39.1 ± 8.3**	60.9 ± 5.9***	60.3 ± 9.0***
6′-*O*-*Z*-*p*-methoxycinnamoylharpagide (**4**)	33.3 ± 3.3**	47.6 ± 3.6***	46.6 ± 2.2***
E-harpagoside (**5**)	38.2 ± 2.9**	38.5 ± 5.1**	44.7 ± 3.0***
Z-harpagoside (**6**)	26.4 ± 2.6*	38.8 ± 3.9**	42.5 ± 1.1***
harpagide (**7**)	41.4 ± 6.2**	42.2 ± 9.4**	46.2 ± 8.4***
MK-801[d]	31.8 ± 7.1*	61.5 ± 2.7***	83.6 ± 4.2***
APV[e]	5.7 ± 1.9	27.8 ± 4.4*	43.6 ± 3.2***
CNQX[f]	28.1 ± 5.6*	47.3 ± 3.6***	61.6 ± 2.7***

[a] Rat cortical cultures were washed with DMEM and incubated with test compounds for 1 h. The cultures were then exposed to 50 μM glutamate for 24 h. After the incubation, the cultures were assessed for the extent of neuronal damage (treatment throughout). The values shown are the mean ± SEM of three experiments (5−6 cultures per experiment). *p <0.05, **p <0.01, ***p <0.001 vs glutamate-treated cultures (ANOVA and Tukey). [b] LDH released from control and glutamate-treated cultures were 110.9 ± 3.3 and 397.6 ± 4.2 mU/mL, respectively. Cell viabilities of control and glutamate-treated cells were represented as 100 and 0%, respectively. [c] Glutamate-treated value differed significantly from the untreated control at a level of p <0.001. [d] MK 801: dizocilpine maleate, a noncompetitive antagonist of the NMDA receptor. [e] APV: DL-2-amino-5-phosphonovaleric acid, a competitive antagonist of the NMDA receptor. [f] CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione, non-NMDA receptor antagonist.

130 cm), eluted with a stepwise gradient from $CHCl_3$−MeOH (100:1) to MeOH to give 250 fractions (1 L each). Fractions were combined to give eight subfractions after monitoring by TLC using mixtures of $CHCl_3$−acetone−MeOH (100:1:1 → 2:1:1) as developing solvent systems. Fraction 5 (eluted with $CHCl_3$−acetone−MeOH, 100:1:1) showed significant neuroprotective activity against glutamate-induced neurotoxicity (74.0% protection at 10 μg/mL, p < 0.001). Therefore, this fraction (120 g) was rechromatographed on a silica gel column (2.4 kg, 230−400 mesh, column size 10 × 160 cm) eluted with a stepwise gradient using $CHCl_3$−acetone−MeOH (100:1:1) and pure MeOH to obtain 125 fractions (500 mL each), which were further combined into 12 fractions (90V-1−90V-12). Among these 12 fractions, fractions 90V-7 and 90V-11 showed significant neuroprotective activity against glutamate-induced neurotoxicity (78.0% and 72.3% protection at 5 μg/mL, respectively, p <0.001). Fraction 7 (90V-7) was further separated by semipreparative HPLC (YMC J'sphere, A-323 ODS C_{18}, 4 μm, 250 × 10 mm, 2 mL/min) in CH_3CN−MeOH−H_2O (24:22:54) to obtain compounds **1** (120 mg, t_R 28.8 min), **2** (83 mg, t_R 22.3 min), **5** (64 mg, t_R 25.4 min), and a mixture of **3** and **4** (10 mg, t_R 18.1 min). Compounds **3** (6 mg, t_R 26.8 min) and **4** (4 mg, t_R 29.4 min) were obtained by further HPLC on an ODS column eluted with CH_3CN−MeOH−H_2O (15:15:70). Fraction 11 (90V-11) was purified by semipreparative HPLC (YMC J'sphere, A-323 ODS C_{18}, 4 μm, 250 × 10 mm, 2 mL/min) using CH_3CN−H_2O (23:77) as solvent to give *Z*-harpagoside (**6**; 9 mg, t_R 27.6 min) and harpagide (**7**; 8 mg, t_R 8.0 min).

8-*O*-*E*-*p*-Methoxycinnamoylharpagide (1): pale brown amorphous powder; $[\alpha]^{15}_D$ −37.4° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226.0 (1.13), 286.5 (1.70), 306.5 (1.35) nm; IR ν_{max} 3400, 1690, 1625, 1600, 1515, 1100−1000 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.55 (1H, d, J = 16.0 Hz, H-β), 7.48 (2H, d, J = 8.8 Hz, H-2″, 6″), 6.89 (2H, d, J = 8.8 Hz, H-3″, 5″), 6.37 (1H, d, J = 6.4 Hz, H-3), 6.30 (1H, d, J = 16.0 Hz, H-α), 6.14 (1H, br s, H-1), 4.88 (1H, dd, J = 6.3, 1.4 Hz, H-4), 4.59 (1H, d, J = 7.8 Hz, H-1′), 4.00 (1H, dd, J = 11.0, 2.1 Hz, H-6b′), 3.76 (1H, dd, J = 5.8, 11.0 Hz, H-6a′), 3.71 (3H, s, OMe), 3.70 (1H, m, H-6), 3.39 (1H, dd, J = 5.8, 2.1 Hz, H-5′), 3.30 (1H, t, J = 8.3 Hz, H-4′), 3.23 (1H, t, J = 8.0 Hz, H-2′), 2.89 (1H, s, H-9), 2.20 (1H, d, J = 15.1 Hz, H-7β), 2.02 (1H, dd, J = 15.1, 4.4 Hz, H-7α), 1.47 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; FABMS (glycerol) m/z 547 [M + Na]$^+$, 525 [M + H]$^+$, 179 [methoxycinnamic acid + H]$^+$; HRFABMS m/z 547.1831 (calcd for $C_{25}H_{32}O_{12}Na$, 547.1894).

8-*O*-*Z*-*p*-Methoxycinnamoylharpagide (2): pale brown amorphous powder; $[\alpha]^{15}_D$ −54.3° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226.5 (1.24), 286.2 (1.96), 306.0 (1.64) nm; IR ν_{max} 3400, 1690, 1625, 1600, 1515, 1100−1000 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.64 (2H, d, J = 8.8 Hz, H-2″, 6″), 6.90 (2H, d, J = 8.8 Hz, H-3″, 5″), 6.83 (1H, d, J = 12.7 Hz, H-β), 6.38 (1H, d, J = 6.3 Hz, H-3), 6.11 (1H, d, J = 1.0 Hz, H-1), 5.81 (1H, d, J = 12.7 Hz, H-α), 4.92 (1H, dd, J = 6.3, 1.5 Hz, H-4), 4.59 (1H, d, J = 7.8 Hz, H-1′), 3.82 (3H, s, OMe), 3.73 (1H, m, H-6), 3.72 (1H, dd, J = 12.2, 2.2 Hz, H-6b′), 3.64 (1H, dd, J = 12.2, 5.7 Hz, H-6a′), 3.54 (1H, t, J = 8.5 Hz, H-3′), 3.34 (1H, dd, J = 5.7, 2.2 Hz, H-5′), 3.31 (1H, t, J = 8.3 Hz, H-4′), 3.22 (1H, t, J = 8.0 Hz, H-2′), 2.91 (1H, s, H-9), 2.18 (1H, d, J = 15.2 Hz, H-7β), 1.98 (1H, dd, J = 15.2, 4.6 Hz, H-7α), 1.50 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; FABMS (glycerol) m/z 547 [M + Na]$^+$, 525 [M + H]$^+$, 179 [methoxycinnamic acid + H]$^+$; HRFABMS m/z 547.1834 (calcd for $C_{25}H_{32}O_{12}Na$, 547.1894).

6′-*O*-*E*-*p*-Methoxycinnamoylharpagide (3): pale brown amorphous powder; $[\alpha]^{15}_D$ −26.7° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226.4 (sh), 286.5 (2.80), 306.5 (sh) nm; IR ν_{max} 3400, 1690, 1625, 1600, 1515, 1100−1000 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.70 (1H, d, J = 16.0 Hz, H-β), 7.58 (2H, d, J = 8.8 Hz, H-2″, 6″), 6.98 (2H, d, J = 8.8 Hz, H-3″, 5″), 6.44 (1H, d, J = 16.0 Hz, H-α), 6.34 (1H, d, J = 6.4 Hz, H-3), 5.68 (1H, br s, H-1), 4.97 (1H, dd, J = 6.8, 1.4 Hz, H-4), 4.62 (1H, d, J = 7.8 Hz, H-1′), 4.50 (1H, dd, J = 11.2, 1.9 Hz, H-6b′), 4.47 (1H, dd, J = 11.2, 5.9 Hz, H-6a′), 3.82 (3H, s, OMe), 3.70 (1H, m, H-6), 3.2−3.4 (4H, m, H-2′, 3′, 4′, 5′), 2.56 (1H, s, H-9), 1.90 (1H, d, J = 15.1 Hz, H-7β), 1.81 (1H, dd, J = 15.1, 4.4 Hz, H-7α), 1.17 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; FABMS (glycerol) m/z 547 [M + Na]$^+$, 525 [M + H]$^+$, 179 [methoxycinnamic acid + H]$^+$; HRFABMS m/z 547.1807 (calcd for $C_{25}H_{32}O_{12}Na$, 547.1894).

6′-*O*-*Z*-*p*-Methoxycinnamoylharpagide (4): pale brown amorphous powder; $[\alpha]^{15}_D$ −29.09 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226.4 (sh), 286.0 (2.31), 306.2 (sh) nm; IR ν_{max} 3400, 1690, 1625, 1600, 1515, 1100−1000 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.70 (2H, d, J = 8.8 Hz, H-2″, 6″), 6.93 (1H, d, J = 12.7 Hz, H-β), 6.91 (2H, d, J = 8.8 Hz, H-3″, 5″), 6.31 (1H, d, J = 6.4 Hz, H-3), 5.83 (1H, d, J = 12.7 Hz, H-α), 5.64 (1H, br s, H-1), 4.94 (1H, dd, J = 6.8, 1.4 Hz, H-4), 4.57 (1H, d, J = 7.8 Hz, H-1′), 4.46 (1H, dd, J = 11.2, 1.9 Hz, H-6b′), 4.29 (1H, dd, J = 11.2, 5.9 Hz, H-6a′), 3.81 (3H, s, OMe), 3.69 (1H, m, H-6), 3.2−3.4 (4H, m, H-2′, 3′, 4′, 5′), 2.53 (1H, s, H-9), 1.87 (1H, d, J = 15.1 Hz, H-7β), 1.81 (1H, dd, J = 15.1, 4.4 Hz, H-7α). 1.19 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; FABMS (glycerol) m/z 547 [M + Na]$^+$, 525 [M + H]$^+$, 179 [*Z*-*p*-methoxycinnamic acid + H]$^+$; HRFABMS m/z 547.1808 (calcd for $C_{25}H_{32}O_{12}Na$, 547.1894).

Identification of Compounds 5−7. The known compounds were identified as *E*-harpagoside (**5**),[14] *Z*-harpagoside (**6**),[18] and harpagide (**7**)[14] by IR, MS, and ^{1}H and ^{13}C NMR spectral data interpretation and confirmed by comparison with reference data.

Cell Culture. Primary cultures of mixed cortical cells containing both neuronal and glial cells were prepared from 17- to 19-day-old fetal rats (Sprague Dawley) as described previously.[1] The cortical cells were grown in Dulbecco's modi-

fied Eagle's medium (DMEM; Gibco) containing 10% heat-inactivated fetal bovine serum (Gibco) with penicillin (100 IU/mL; Sigma) and streptomycin (10 μg/mL; Sigma) at 37 °C in a humidified atmosphere of 95% air−5% CO_2. After 3 days in culture, nonneuronal cell division was halted by adding 5-fluoro-2′-deoxyuridine (50 μM; Sigma). Cultures were allowed to mature for 2 weeks.

Assessment of Neurotoxicity. All compounds were dissolved in DMSO (final culture concentration, 0.1%). Preliminary studies indicated that the solvent had no effect on cell viability at the concentration used. Cortical cell cultures were washed with DMEM and incubated with test compounds for 1 h. The cultures were then exposed to 50 μM glutamate for 24 h in the presence of the test compounds. After incubation, the cultures were assessed for the extent of neuronal damage.

Neuronal integrity was assessed by spectrophotometric measurement of the efflux of lactate dehydrogenase (LDH) into the culture medium by the methods described previously.[1,19] In brief, 30 μL of the medium was collected and assayed for LDH release using the modified method of Koh and Choi.[21] Optical densities were read by an automated spectrophotometric plate reader at wavelength of 405 nm. Data were expressed as percent cell viability relative to vehicle-treated control cultures. Cell viability was calculated as 100 × (LDH released from glutamate-treated cultures − LDH released from glutamate + test compound-treated cultures)/(LDH released from glutamate-treated cultures − LDH released from control cultures).

Statistical Analysis. The evaluation of statistical significance was determined by one-way ANOVA, and if significant, group means were compared by post hoc analysis using Tukey multiple comparison of means.

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Supporting Information Available: Six figures of NMR data and a table showing the ^{1}H and ^{13}C NMR data of **5**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org

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