Neuroprotective Limonoids of Root Bark of *Dictamnus dasycarpus*

Jeong Seon Yoon, Sang Hyun Sung, and Young Choong Kim*

College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, San 56-1, Sillim-Dong, Gwanak-Gu, Seoul 151-742, Korea

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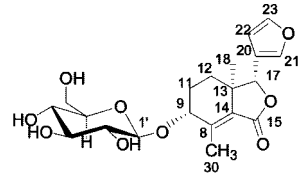
A methanolic extract of *Dictamnus dasycarpus* root bark afforded four new degraded limonoids, 9α-hydroxyfraxinellone-9-*O*-β-D-glucoside (**1**), dictamnusine (**2**), dictamdiol A (**3**), and dictamdiol B (**4**), together with eight known compounds, dictamdiol (**5**), fraxinellone (**6**), fraxinellonone (**7**), 9β-hydroxyfraxinellone (**8**), calodendrolide (**9**), obacunone (**10**), limonin (**11**), and rutaevin (**12**). Compounds, **2**, **3**, **6**, **9**, **10**, and **11** showed significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells at a concentration of 0.1 μM.

Glutamate is known to be associated with central excitatory neurotransmission as occurs in neuronal survival, synaptogenesis, neuronal plasticity, learning, and memory processes in the brain.[1] However, high concentration of glutamate causes neuronal cell death within the central nervous system and may be involved in neuropsychiatric and neuropathological disorders such as Alzheimer's disease, Parkinson's disease, ischemic stroke, and spinal cord trauma.[2,3] Thus, neuroprotection against glutamate-induced neurotoxicity has been a therapeutic strategy to treat neurodegenerative disease.[4]

In the course of searching for neuroprotective compounds from natural sources using primary cultures of rat cortical cells injured by glutamate as an *in vitro* assay system, it was found that a methanolic extract of the root bark of *Dictamnus dasycarpus* Turcz. (Rutaceae) showed significant neuroprotective activity. *D. dasycarpus* is widely distributed in Asia, and root bark of this plant has been used for treatment of various ailments such as skin inflammation, eczema, rubella, scabies, acute rheumatoid arthritis, jaundice, cold, and headache in Korean traditional medicine.[5] Known constituents of *D. dasycarpus* root bark include limonoids,[6,7] furoquinoline alkaloids,[6,8] flavonoids,[9,10] coumarins,[11] sesquiterpenes,[12] sesquiterpene glycosides,[13,14] and phenolic glycosides.[15] To date, however, there has been no report related to neuroprotective constituents of this plant. Thus, we have attempted to isolate compounds having neuroprotective activity from a methanolic extract of *D. dasycarpus* root bark using a bioactivity-guided fractionation technique. As a result, four new degraded limonoids (**1**–**4**), five known degraded limonoids (**5**–**9**), and three limonoid derivatives (**10**–**12**) were obtained. In the present study, we report the isolation and structural elucidation of compounds **1**–**4** and the neuroprotective activities of compounds **1**–**12**.



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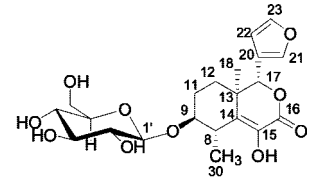

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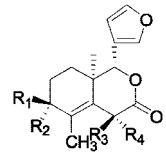
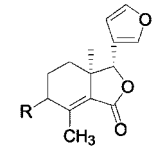


3 : R_1 = OH, R_2 = H, R_3 = H, R_4 = OH

4 : R_1 = H, R_2 = OH, R_3 = OH, R_4 = H

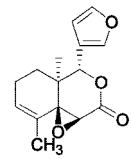
5 : R_1 = OH, R_2 = H, R_3 = OH, R_4 = H



6 : R = H

7 : R = O

8 : R = β-OH



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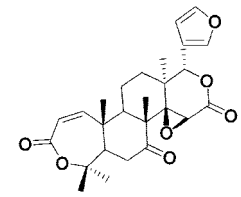

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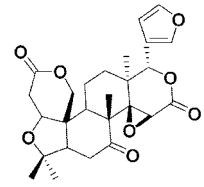

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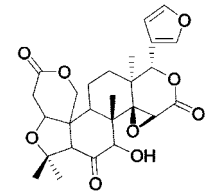

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Results and Discussion

The methanolic extract of *D. dasycarpus* root bark was dissolved in water and successively partitioned into *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O fractions. Each fraction was evaluated for its activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells. The $CHCl_3$ and EtOAc fractions showed significant neuroprotective activity. Thus, these two fractions were further subjected to repeated column chromatography to yield four new degraded limonoids (**1**–**4**), five known degraded limonoids (**5**–**9**), and three limonoid derivatives (**10**–**12**).

Compound **1** was isolated as a yellowish oil. The molecular formula was determined to be $C_{20}H_{26}O_9$ from the HRFABMS at *m*/*z* 433.1475 $[M + Na]^+$ (calcd *m*/*z* 433.1474). The IR spectrum of **1** indicated the presence of γ-lactone (1752 cm^{-1}) and furan ring (2924, 1509 cm^{-1}) moieties. The ^1H NMR spectrum showed the presence of two tertiary methyl singlets (δ_H 2.17 and 0.76) and a β-substituted furan ring [δ_H 7.71 (2H, brs, H-21, 23) and 6.51 (1H, brs, H-22)]. In addition, the ^{13}C NMR spectrum had signals

* To whom correspondence should be addressed. Tel: 82-2-880-7842. Fax: 82-2-888-2933. E-mail: youngkim@snu.ac.kr.

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Table 1. ^{1}H and ^{13}C NMR of Compounds **1** and **2** in DMSO-d_6[a]

	1		**2**	
pos.	δ_H (500 MHz)	δ_C (125 MHz)	δ_H (400 MHz)	δ_C (100 MHz)
8		145.3	3.24 (1H, d, 7.6)	31.7
9	4.01 (1H, d, 3.7)	75.9	3.71 (1H, brs)	76.9
11_{ax}	2.26 (1H, d, 14.3)	25.7	1.76 (2H, m)	27.4
11_{eq}	1.79 (1H, td, 14.3, 2.4)			
12_{ax}	1.63 (1H, td, 12.0, 2.4)	26.6	1.76 (1H, m)	21.4
12_{eq}	1.49 (1H, dt, 12.5, 3.2)		0.89 (1H, m)	
13		42.7		36.2
14		129.8		135.3
15		168.9	8.43 (OH, brs)	135.5
16				162.2
17	5.00 (1H, s)	82.1	5.22 (1H, s)	80.8
20		120.1		120.0
21	7.71 (1H, brs)	143.8	7.72 (1H, brs)	141.4
22	6.51 (1H, brs)	109.0	6.52 (1H, brs)	110.2
23	7.71 (1H, brs)	140.2	7.68 (1H, d, 1.5)	143.1
18-CH_3	0.76 (3H, s)	18.7	1.00 (3H, s)	19.7
30-CH_3	2.17 (3H, s)	15.0	1.06 (3H, d, 7.6)	18.3
1′	4.38 (1H, d, 7.6)	105.7	4.20 (1H, d, 7.7)	101.8
2′	2.97 (1H, m)	73.7	2.93 (1H, m)	73.3
3′	3.15 (1H, m)	76.7	3.10 (1H, m)	76.7
4′	3.05 (1H, m)	70.0	3.04 (1H, m)	70.1
5′	3.15 (1H, m)	76.7	3.10 (1H, m)	76.7
6′	3.68 (1H, dd, 11.3, 2.2)	61.1	3.68 (1H, d, 11.4, 2.2)	61.1
	3.45 (1H, dd, 11.3, 5.7)		3.42 (1H, dd, 11.4, 5.5)	

[a] All assignments were made by extensive analysis of 1D and 2D NMR (COSY, HMQC, HMBC, and NOESY).

indicating two oxymethine groups [δ_C 75.9 (C-9) and 82.1 (C-17)], one sp^3 quaternary carbon [δ_C 42.7 (C-13)], a conjugated ester carbonyl [δ_C 168.9 (C-15)], and a tetrasubstituted double bond [δ_C 129.8 (C-14) and 145.3 (C-8)], together with signals characteristic for a glucose unit. The NMR spectra indicated that **1** was a 9α-hydroxyfraxinellone derivative by comparing its spectroscopic data with previously reported information.[7,16] Hydrolysis of **1** with β-glucosidase yielded D-glucose ($[\alpha]^{20}_D$ +48.2).[17] The glucose was determined to be at C-9 from the HMBC correlation between H-1′ and C-9. The relative configuration of **1** was assigned using a NOESY experiment, in which H-9 (δ_H 4.01) showed a β-orientation as judged by the correlation with H-11_{ax} and CH_3-30 (Supporting Information). Thus, compound **1** was determined to be 9α-hydroxyfraxinellone-9-*O*-β-D-glucoside.

Compound **2** showed a molecular ion peak at *m*/*z* 441.1761 [M + H]$^+$ ($C_{21}H_{28}O_{10}$). The ^{1}H NMR spectrum showed the presence of a β-substituted furan ring [δ_H 7.72 (1H, brs, H-21), 7.68 (1H, d, J = 1.5 Hz, H-23), and 6.52 (1H, brs, H-22)], one angular and one secondary methyl [δ_H 1.00 (s) and 1.06 (d, J = 7.6)], and signals of a glucose unit (Table 1). These data were similar to those of fagaropsine[18] including the C14−C15 tetrasubstituted double bond [δ_C 135.3 and 135.5] adjacent to the lactone carbonyl [δ_C 162.6] except for the location of a glucose unit. Enzymatic hydrolysis of **2** gave β-D-glucose ($[\alpha]^{20}_D$ +41.3), and the glucose unit was placed at C-9 from the HMBC correlation between H-1′ and C-9. HMBC correlation from the OH group to C-15 and C-16 indicated that the OH group was at C-15. NOESY correlation between H-9 and CH_3-30 suggested that H-9 was β-oriented. Thus, the structure of **2** (named dictamnusine) was deduced to be 7-*O*-β-D-glucopyranosyl-(4α,4aα,7β,8α,)-4-(3′-furanyl)-1-hydroxy-4a,8-dimethyl-4,4a,5,6,7,8-hexahydro-2*H*-3-benzopyran-2-one, as the nomenclature system adopted refers to its limonoid origin.

Compound **3** had the molecular formula $C_{15}H_{18}O_5$ by HREIMS (molecular ion peak at *m*/*z* 278.1146). The ^{1}H NMR spectrum showed the presence of two tertiary methyl singlets (δ_H 1.79 and 0.88), a β-substituted furan ring [δ_H 7.70 (1H, brs, H-21), 7.67 (1H, d, J = 1.4 Hz, H-23), and 6.50 (1H, d, J = 0.9 Hz, H-22)], and two OH groups [δ_H 5.72 (d, J = 5.4 Hz) and 4.81 (d, J = 5.8 Hz)]. In addition, the ^{13}C NMR spectrum contained three oxymethine signals [δ_C 65.8 (C-15), 66.6 (C-9), and 80.6 (C-17)], a quaternary carbon [δ_C 38.8 (C-13)], an ester carbonyl [δ_C 174.3 (C-16)], and a tetrasubstituted double bond [δ_C 134.6 (C-14) and 135.8 (C-8)]. HMBC correlations from OH-15 to C-15 and C-14 and from OH-9 to C-9 and C-8 suggested that **3** was a stereoisomer of dictamdiol (**5**)[19] (Table 2). NOESY correlations (H-15 with H-17 and CH_3-30; H-9 with H-11_{eq} and CH_3-18) indicated that OH-15 and OH-9 were α- and β- oriented, respectively. The relative configuration of the limonoid core in **3** was confirmed by the NOESY spectrum (Table 2). Thus, compound **3** was determined to be as shown and was named dictamdiol A.

The HREIMS of **4** gave [M + H]$^+$ at *m*/*z* 278.1146 corresponding to molecular formula $C_{15}H_{18}O_5$. According to the ^{1}H, ^{13}C, HMQC, and HMBC spectra (Table 2), **4** was a stereoisomer of **3**. In the NOESY spectrum, the correlation of H-15 with H-17 was absent and the correlations of H-9 with H-11_{ax} and H-12_{ax} and of H-17 with H-12_{ax} were observed, indicating that OH-15 and OH-9 were β- and α-oriented, respectively. Thus, the sturucture of **4** was determined to be as shown and was named dictamdiol B.

Eight known compounds were also isolated and were identified as dictamdiol (**5**),[19] fraxinellone (**6**),[20,21] fraxinellonone (**7**),[22] 9β-hydroxyfraxinellone (**8**),[7,16] calodendrolide (**9**),[23] obacunone (**10**),[24] limonin (**11**),[25] and rutaevin (**12**),[26] respectively, by comparison of spectroscopic data with those previously reported.

Neuroprotective activities of compounds **1**−**12** against glutamate-induced neurotoxicity in primary cultures of rat cortical cells were evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as described in previous reports.[27,28] All the compounds isolated except **7** showed protection against glutamate-induced neurotoxicity in primary cultures of rat cortical cells at a concentration of 0.1 μM. Compounds **2**, **3**, **6**, **9**, **10**, and **11** exhibited greater neuroprotective activity than MK-801, a positive control at a concentration of 0.1 μM. The potency of neuroprotective activity of **2**, **3**, **6**, **9**, **10**, and **11** was in the order limonin (**11**) ≥ calodendrolide (**9**) ≥ fraxinellone (**6**) > obacunone (**10**) ≥ dictamnusine (**2**) = dictamdiol A (**3**) (Table 3).

Experimental Section

General Experimental Procedures. Optical rotation was measured with a Jasco DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzu UV-201 spectrometer using MeOH as a solvent. FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrometer. ^{1}H and ^{13}C NMR measurements were carried out in Bruker AMX 400

Table 2. NMR Data of Compounds **3** and **4** in DMSO-d_6

	3				**4**			
pos.	δ_H	δ_C	HMBC[a](H→C#)	NOESY[a](H→H#)	δ_H	δ_C	HMBC[a](H→C#)	NOESY[a](H→H#)
8		135.8				138.9		
9	3.74 (1H, brs)	66.6	8, 12, 14	11_{eq}, 18-CH_3, 30-CH_3	3.84 (1H, brt, 7.1)	69.2	8, 14	11_{ax} 12_{ax}, 30-CH_3
9-OH	4.81 (1H, d, 5.8)		8, 9		4.82 (1H, 6.1)		8, 9	
11_{ax}	1.62–1.65(1H,m)	27.7	12	17	1.90 (1H, td, 9.8, 3.5)	28.6	8, 9, 12, 13,	9, 11_{eq}, 12_{ax}
11_{eq}	1.55–1.62 (1H, m)		8, 9, 12, 13	9	1.60 (1H, brd, 13.1)		9, 12	11_{ax}, 18-CH_3
12_{ax}	1.53–1.58 (1H, m)	27.3	18-CH_3, 13, 17	12_{eq},17	1.41 (1H, td, 11.8, 2.7)	32.0	18-CH_3, 11, 13, 17	9, 11_{ax}, 12_{eq}, 17, 22
12_{eq}	0.83 (1H, brd, 13.8)		9, 11, 13, 14	12_{ax}, 22	0.96 (1H, dt, 9.5, 3.4)		9, 11, 13, 14	12_{ax}
13		38.8				39.5		
14		134.6				134.4		
15	4.85 (1H, d, 5.4)	65.8	8, 13, 14, 16	17, 30-CH_3,	4.64 (1H, s)	67.0	8, 13, 14, 16	30-CH_3
15-OH	5.72 (1H, d, 5.4)		14, 15		6.37 (1H, brd, 3.9)		14, 15	
16		174.3				171.6		
17	5.03 (1H, s)	80.6	18-CH_3, 12, 14, 20, 21, 22	12_{ax}, 15, 21, 22	5.44 (1H, s)	79.3	18-CH_3, 12, 13, 14, 20, 21, 22	12_{ax}, 21, 22
20		121.7				120.8		
21	7.70 (1H, brs)	142.2	20, 22, 23	17, 23	7.73 (1H, brs)	142.4	20, 22, 23	17
22	6.50 (1H, d, 0.9)	110.9	17, 20, 21, 23	17, 23	6.52 (1H, d, 1.0)	110.9	17, 20, 21, 23	12_{ax}, 17, 23
23	7.67 (1H, d, 1.4)	144.2	20, 21, 22	21, 22	7.68 (1H, d, 1.5)	144.2	20, 21, 22	22
18-CH_3	0.88 (3H, s)	16.3	8, 9, 13, 14, 17,	9	0.94 (3H, s)	19.2	8, 9, 13, 14, 17	11_{eq}
30-CH_3	1.79 (3H, s)	17.9	12, 13, 14, 17	9, 15	1.73 (3H, s)	15.0	12, 13, 14, 17	9, 15

[a] Main observed HMBC and NOESY correlations are presented.

Table 3. Neuroprotective Activity of Compounds **1**–**12** Isolated from *D. dasycarpus* Root Bark against Glutamate-Induced Toxicity in Primary Cultures of Rat Cortical Cells

compound	EC_{50} (μM)	E_{max} (%)[a]	
1	0.705 ± 0.187	53.0 ± 2.4**	at 1.0 μM
2	0.042 ± 0.001	62.1 ± 3.6**	at 0.1 μM
3	0.068 ± 0.001	68.1 ± 2.5***	at 0.1 μM
4	ND	45.6 ± 2.5*	at 0.1 μM
5	ND	35.3 ± 0.7*	at 0.1 μM
6	0.022 ± 0.009	70.4 ± 3.4***	at 0.1 μM
7	ND	47.6 ± 0.9**	at 1.0 μM
8	0.098 ± 0.005	51.1 ± 1.3***	at 0.1 μM
9	0.019 ± 0.003	71.9 ± 1.1***	at 0.1 μM
10	0.039 ± 0.004	63.9 ± 1.1**	at 0.1 μM
11	0.018 ± 0.001	75.9 ± 3.5***	at 0.1 μM
12	3.065 ± 0.121	65.4 ± 1.4***	at 10.0 μM
MK-801[b]	0.480 ± 0.020	83.8 ± 1.7***	at 10.0 μM

[a] Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate-treated culture − OD of glutamate-treated culture]/[OD of control culture − OD of glutamate-treated culture]. The ODs of control and glutamate-injured cultures were 1.07 ± 0.01 and 0.77 ± 0.01, respectively. Cell viabilities of control and glutamate-treated cells were represented as 100 and 0%, respectively. Glutamate-injured cells differ significantly from the control at a level of $p < 0.001$. [b] MK-801: dizocipline maleate, a noncompetitive antagonist of NMDA receptor. The values expressed as mean ± SD of triplicate experiments. ND = not determined. (*P < 0.05, **P < 0.01, ***P < 0.001).

and 500 spectrometers. Solvent signals were used as internal standards. $^1H-^1H$ COSY, HMQC, HMBC, and NOESY NMR experiments were performed on the same spectrometer. EI-mass spectra were obtained on a VG Trio 2 spectrometer with a 70 eV ionizing potential. TLC and column chromatography were carried out on precoated silica gel F_{254} plates (Merck, art. 5715), RP-18 F_{254} plates (Merck, art. 15423), silica gel 60 (230–400 mesh, Merck), Sephadex LH 20 (18–110 μm, Pharmacia Co. Ltd.), and LiChroprep RP-18 (40–63 μm, Merck).

Plant Material. Root bark of *D. dasycarpus* was purchased from Kyungdong Oriental Herbal Market, Seoul, Korea, in April 2006 and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNU-0189) has been deposited in Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. Root bark of *D. dasycarpus* (21 kg) was extracted with 80% MeOH three times in an ultrasonic apparatus. Upon removal of the solvent under vacuum, the methanolic extract yielded 2.18 kg of material (10.4% by dry weight). The methanolic extract was suspended in H_2O and partitioned successively with *n*-hexane, $CHCl_3$, ethyl acetate (EtOAc), and *n*-butanol. Each fraction was evaluated for its neuroprotective activity against glutamate-induced toxicity in primary cultures of rat cortical cells. Among the five fractions, the $CHCl_3$ and EtOAc fractions showed significant neuroprotective activity (72.4% and 55.0% at 50 μg/mL, respectively). The $CHCl_3$ fraction (220.2 g) was subjected to column chromatography (CC) over silica gel eluted with a $CHCl_3$/MeOH gradient (100:0 to 0:100) to obtain 10 fractions (1–10). Neuroprotective activity of these 10 fractions was monitored using the primary cultures of rat cortical cells injured by glutamate, and fractions 2 and 3 showed significant neuroprotective activity (72.3% and 50.6% at 10 μg/mL, respectively). Compounds **6** (1.2 g), **9** (120 mg), and **10** (11.3 g) were purified from fraction 2 by recrystallization from *n*-hexane or MeOH. Fraction 3 was chromatographed on a silica gel column eluting with a mixture of *n*-hexane/EtOAc to afford five fractions (3-1–3-5). Crystallization of fraction 3-4 from MeOH afforded compound **11** (1.4 g). The residue from fraction 3-4 was subjected to reversed-phase (RP) CC with a MeOH/H_2O step gradient (40% to 100% MeOH) to yield 17 subfractions (3-4-1–3-4-17). Subfraction 3-4-3 was subjected to reversed-phase HPLC (YMC-Pack Pro C_{18}, 10 × 250 mm) using CH_3CN/H_2O (60:40, 2 mL/min) as an eluent to give compound **8** (30 mg). The EtOAc fraction (36 g) was subjected to silica gel CC eluting with a gradient of $CHCl_3$/MeOH (100:0 to 0:100) to yield 10 fractions (e1−e10). Neuroprotective activity of these 10 fractions was monitored using the primary cultures of rat cortical cells injured by glutamate, and fractions 2, 5, and 8 showed significant neuroprotective activity (41.7%, 50.1%, and 81.4% at 10 μg/mL, respectively). Fractions e8 and e9 were combined and chromatographed over RP to give 15 subfractions (e8-1−e8-15). Subfraction e8-8 was subjected to purification with HPLC (YMC-Pack Pro C_{18}, 10 × 250 mm) using CH_3CN/H_2O (23:77, 2 mL/min) as an eluent, affording compounds **1** (14.7 mg) and **2** (20.2 mg). Subfraction e5 was subjected to RP CC with a MeOH/H_2O step gradient (10% MeOH → 100% MeOH) to yield 19 subfractions (e5-1−e5-19). Compounds **3** (11.2 mg), **4** (35.3 mg), and **5** (13.8 mg) were obtained from e5-3 by additional C_{18} HPLC using CH_3CN/H_2O (25:75, 2 mL/min) as an eluent. Subfractions e2 and e3 were combined and chromatographed over silica gel to obtain nine subfractions (e2-1−e2-9), of which e2-4 was subjected to HPLC using CH_3CN/H_2O (40:60, 2 mL/min) as an eluent, affording compound **7** (4.5 mg). Subfraction e4 was subjected to CC over Sephadex LH-20 using MeOH to yield 14 subfractions (e4-1−e4-14). Compound **12** (11.9 mg) was obtained from e4-6 by crystallization from MeOH.

9α-Hydroxyfraxinellone-9-*O*-β-D-glucoside (1): yellowish oil; $[\alpha]^{20}_D$ +30.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (2.15), 258 (2.16) nm; IR νKBr max cm^{-1} 3396, 2924, 1752, 1456, 1211, 1078, 1028, 876, 766; ^1H and ^{13}C NMR, see Table 1; HRFABMS (positive) *m/z* 433.1475 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_9Na$, 433.1474).

Dictamnusine (2): white, amorphous powder, mp 172−173 °C; $[\alpha]^{20}_D$ +21.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (1.62), 238 (1.64) nm; IR νKBr max cm^{-1} 3389, 2921, 1710, 1509, 1458, 1364, 1191, 1024, 826; ^1H and ^{13}C NMR, see Table 1; HRFABMS (positive) *m/z* 441.1761 $[M + H]^+$ (calcd for $C_{21}H_{28}O_{10}$, 441.1760).

Dictamdiol A (3): yellowish oil; $[\alpha]^{20}_D$ −4.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (2.12) nm; IR νKBr max cm^{-1} 3391, 2921, 1737, 1453, 1195, 1158, 1024, 875, 822, 758; NMR data, see Table 2; HREIMS (positive) *m/z* 278.1146 $[M]^+$ (calcd for $C_{15}H_{18}O_5$, 278.1154).

Dictamdiol B (4): yellowish oil; $[\alpha]^{20}_D$ −5.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (2.08) nm; IR νKBr max cm^{-1} 3396, 2921, 1744, 1457, 1281, 1024, 766; NMR data, see Table 2; HREIMS (positive) *m/z* 278.1146 $[M]^+$ (calcd for $C_{15}H_{18}O_5$, 278.1154).

Enzymatic Hydrolyses of 1 and 2. Compounds **1** (4.3 mg), and **2** (4.0 mg) were individually hydrolyzed with 8.0 mg of β-glucosidase (Almonds Lot 1264252, Sigma-Aldrich) in 1.5 mL of H_2O at 37 °C for 12 h. The reaction mixtures of **1** and **2** were extracted with $CHCl_3$ (3 × 3 mL), respectively. The aqueous phase of the hydrolysates of **1** and **2** were dried using a stream of N_2 and then subjected to CC over silica gel eluted with $CHCl_3$/MeCN (3:1) to yield glucose (1.8 mg) from **1**, $[\alpha]^{20}_D$ +48.2 (*c* 0.5, H_2O), and glucose (1.5 mg) from **2** $[\alpha]^{20}_D$ +41.3 (*c* 0.38, H_2O).

Cell Culture. Primary cultures of rat cortical cells containing both neurons and non-neuronal cells were prepared from 17- to 19-day-old fetal rats (Sprague–Dawley) as reported previously.[27] Cortical cells were seeded onto a collagen-coated 48-well plate at a density of 1×10^6 cells/mL. The cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air−5% CO_2. Cytosine-β-D-arbinofuranoside (1 μM) was added to the culture medium 3 days after plating to inhibit the proliferation of non-neuronal cells. Cultures were allowed to mature for 15 days before being used for experiments.

Assessment of Neuroprotective Activity. All tested compounds were dissolved in DMSO (final culture concentration, 0.1%). Cortical cell cultures were pretreated with a test compound for 1 h and then exposed to 100 μM glutamate. After incubation for an additional 24 h, cell viability of the cultures was assessed by the MTT assay, which reflects the mitochondrial enzyme function of cells. Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate-treated culture − OD of glutamate-treated culture]/[OD of control culture − OD of glutamate-treated culture].

Statistical Analysis. Data were evaluated for statistical significance using an analysis of variance (ANOVA) with a computerized statistical package. The data were considered to be statistically significant if the probability value was <0.05.

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Supporting Information Available: NMR spectra of compounds **1**–**4** and structure of fagaropsine. This information is available free of charge via the Internet at http://pubs.acs.org.

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