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Article

Syringoleosides A–H, Secoiridoids from *Syringa dilatata* Flowers and Their Inhibition of NO Production in LPS-Induced RAW 264.7 Cells

Jung Eun Gwag, Yeong-Geun Lee, Hyoung-Geun Kim, Dong-Sung Lee, Dae Young Lee, and Nam-In Baek*

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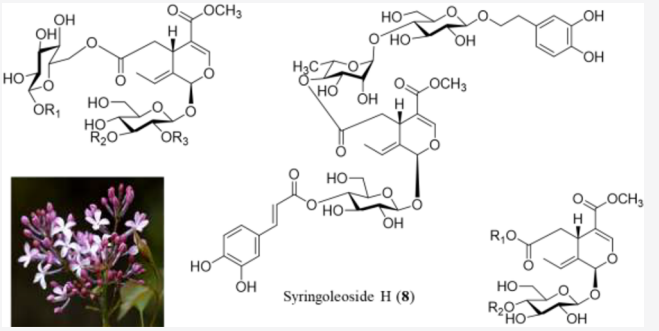
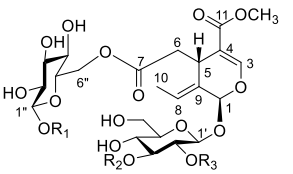
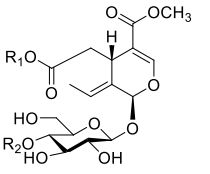
ABSTRACT: Repeated column chromatography of *Syringa dilatata* flowers, a native shrub to Korea, led to the isolation of eight new oleoside-type secoiridoids, syringoleosides A–H (**1**–**8**), as well as five known secoiridoids (**9**–**13**). The new chemical structures were identified through spectroscopic data analysis, as well as the application of chemical methods. Compounds **1**, **2**, **6**, **7**, **11**, and **13** showed suppression effects on NO production in LPS-induced RAW 264.7 cells, with IC_{50} values ranging from 32.5 ± 9.8 to 65.7 ± 11.0 μM, and no visible toxicity. The content of the major secoiridoids in *S. dilatata* flowers, compounds **1**, **4**, **5**, **8**, **9**, **12**, and **13**, were determined through HPLC analysis.



Protecting the sovereignty of biological resources in the country of origin is important, as stated in the Nagoya protocol. This protocol was established for the fair and equitable distribution of profits resulting from the use of natural resources[1] and is intended to contribute to the conservation and sustainable use of biodiversity. With this protocol in force, research using domestic native species has become a key issue. Within the *Syringa* genus, *S. dilatata* Nakai is a native Korean species. *Syringa* means "blooming like a sorghum flower" in Korean. The genus *Syringa*, belonging to the family Oleaceae, contains more than 20 species,[2] which produce many secondary metabolites, particularly secoiridoids.[3] In general, secologanin-type secoiridoids are cleaved at C-7 and C-8, so they have an exomethylene functional group between C-8 and C-10. However, secoiridoids in Oleaceae are characterized by an olefin functional group between C-8 and C-9. This type of secoiridoid, called the oleoside type, occurs only in the Oleaceae family.[4] The oleoside-type secoiridoid oleuropein is reported to show higher anti-oxidant activity than the secologanin-type secoiridoid oleuroside.[4] Various pharmacological efficacies, such as anti-inflammation, anti-tumor, and anti-microbial effects, have been reported for secoiridoids.[5−7] However, no phytochemical or pharmacological study of the flowers of *S. dilatata* has been reported to date. Thus, the present study isolated and identified oleoside-type secoiridoids from *S. dilatata* flowers and then evaluated their pharmacological potential.

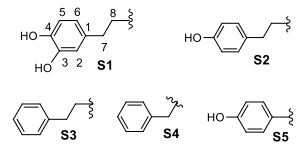
Eight of the isolated compounds are new oleoside-type secoiridoids, here named syringoleosides A–H (**1**–**8**), and five of them were previously known (**9**–**13**). A quantitative HPLC experiment was conducted for major secoiridoids (**1**, **4**, **5**, **8**, **9**, **12**, and **13**) in 80% aqueous MeOH extract. The potential of all the secoiridoids to suppress NO production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages was determined.





syringoleoside A (**1**) R_1 = **S3**, R_2, R_3 = H
syringoleoside B (**2**) R_1 = **S4**, R_2, R_3 = H
syringoleoside C (**3**) R_1 = coniferyl alcohol (-4), R_2, R_3 = H
syringoleoside D (**4**) R_1 = **S2**, R_2 = OS11Me (-7‴), R_3 = H
syringoleoside E (**5**) R_1 = **S2**, R_2 = H, R_3 = OS11Me (-7‴)
syringeleoside F (**6**) R_1 = **S5**, R_2, R_3 = H
neuzhenide (**12**) R_1 = **S2**, R_2, R_3 = H
neonuezhenide (**13**) R_1 = **S1**, R_2, R_3 = H

syringoleoside G (**7**) R_1 = **S6**, R_2 = H,
syringoleoside H (**8**) R_1 = **S7**, R_2 = caffeoyl (-9)
oleoside 11-methyl ester (**9**) (OS11Me) R_1, R_2 = H
oleoside dimethyl ester (**10**) R_1, = CH_3, R_2 = H
oleouropein (**11**) R_1 = **S1**, R_2 = H



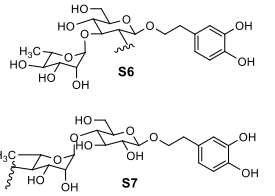


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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−8 (600 MHz, δ_H, Coupling Patterns, J in Hz)[a]

position	1	2	3	4		5		6	7	8
1	5.94, s	6.32, s	6.31, s	6.44, s	6.23, s	6.43, s	6.30, s	6.31, s	5.98, s	5.94, s
3	7.54, s	7.69, s	7.68, s	7.70, s	7.67, s	7.72, s	7.68, s	7.67, s	7.55, s	7.54, s
5	4.03, m	4.29, m	4.30, m	4.24, overlapped	4.24, overlapped	4.28, m	4.21, m	4.31, m	4.03, m	4.03, m
6	2.77, dd, 14.4, 4.2	2.78, dd, 14.4, 4.2	2.90, dd, 14.4, 4.8	2.86, overlapped	2.86, overlapped	2.80, dd, 14.4, 2.4	2.80, dd, 14.4, 2.4	2.85, dd, 15.0, 8.4	2.73, dd, 15.6, 4.2	2.77, dd, 14.4, 4.2
	2.52, dd, 14.4, 8.4	2.50, dd, 14.4, 8.8	2.48, dd, 14.4, 8.4	2.48, dd, 14.4, 7.8	2.48, dd, 14.4, 7.8	2.49.dd, 14.4, 8.4	2.49, dd, 14.4, 8.4	2.48, dd, 15.0, 5.4	2.66, dd, 15.6, 9.0	2.52, dd, 14.4, 8.4
8	6.11, q, 6.0	6.14, q, 7.2	6.14, q, 7.2	6.14, overlapped	6.14, overlapped	6.18, q, 7.2	6.13, q, 7.2	6.13, q, 7.2	6.13, q, 7.2	6.11, q, 6.0
10	1.74, d, 6.0	1.70, d, 7.2	1.68, d, 7.2	1.71, d, 6.6	1.70, d, 6.0	1.73, d, 7.2	1.69, d, 7.2	1.68, d, 7.2	1.80, d, 7.2	1.74, d, 6.0
OCH_3-11	3.70, s	3.60, s	3.64, s	3.63, s	3.62, s	3.66, s	3.64, s	3.61, s	3.70, s	3.70, s
1-Glc[b]-1′	4.85, d, 7.8	5.46, d, 7.8	5.47, d, 7.2	5.47, d, 7.8	5.46, d, 7.8	5.48, d, 7.8	5.46, d, 7.8	5.49, d, 7.8	4.82, d, 7.8	4.83, d, 7.8
1-Glc-2′	3.32, overlapped	4.13, m	4.31, overlapped	3.96, overlapped	3.84, m	5.42, dd, 9.6, 7.8	5.41, overlapped	4.11, m	3.32, overlapped	3.32, overlapped
1-Glc-3′	3.41, m	4.28, overlapped	4.31, overlapped	5.70, dd, 9.6, 9.0	3.96, overlapped	4.29, overlapped	4.13, overlapped	4.31, overlapped	3.43, dd, 6.6, 6.6	3.41, m
1-Glc-4′	3.31, overlapped	4.28, overlapped	4.31, overlapped	4.22, overlapped	4.22, overlapped	4.21, overlapped	3.85, overlapped	4.31, overlapped	3.33, overlapped	3.31, overlapped
1-Glc-5′	3.34, overlapped	4.16, m	4.30, overlapped	3.96, overlapped	3.96, overlapped	4.13, overlapped	4.21, overlapped	4.27, overlapped	3.30, overlapped	3.34, overlapped
1-Glc-6′	3.90, br d, 12.0	4.37, overlapped	4.47, dd, 12.0, 2.4	4.50, overlapped	4.50, overlapped	4.49, overlapped	4.49, overlapped	4.50, dd, 12.0, 2.0	3.89, overlapped	3.90, br d, 12.0
	3.68, m	4.24, dd, 12.0, 5.4	4.32, overlapped	4.28, m	4.28, overlapped	4.30, overlapped	4.30, overlapped	4.33, dd, 12.0, 5.4	3.69, overlapped	3.68, m
7-Glc-1″	4.37, d, 7.8	4.89, d, 7.8	5.56, d, 7.8	4.77, d, 7.2		4.85, d, 7.8		5.39, d, 7.8	4.31, d, 7.8	4.39, d, 7.8
7-Glc-2″	3.32, overlapped	4.03, overlapped	4.16, m	4.18, overlapped		4.27, overlapped		4.27, overlapped	4.92, d, 9.6	3.57, overlapped
7-Glc-3″	3.33, overlapped	3.96, m	3.99, m	3.95, overlapped		4.13, overlapped		4.01, m	3.52, dd, 9.0, 9.0	3.92, overlapped
7-Glc-4″	3.32, overlapped	4.04, overlapped	4.10, m	4.22, overlapped		4.27, overlapped		4.11, overlapped	3.89, overlapped	3.49, overlapped
7-Glc-5″	3.34, overlapped	3.94, m	4.20, m	3.95, overlapped		4.21, overlapped		4.16, m	3.36, overlapped	3.49, overlapped
7-Glc-6″	4.37, m	4.93, dd, 11.4, 1.2	4.93, dd, 12.0, 1.8	4.87, dd, 10.4, 9.0		4.54, br d, 11.4		4.94, br d, 12.0	3.89, overlapped	3.66, overlapped
	4.24, dd, 12.0, 5.4	4.67, dd, 11.4, 6.0	4.63, dd, 12.0, 6.6	4.61, dd, 10.4, 5.4				4.50, d, 12.0, 2.4	3.68, overlapped	3.56, overlapped
1″-PA[c]-2‴	7.27, br s	7.30, m	7.16, d, 1.8	7.22, d, 8.4		7.25, d, 8.4		7.36, d, 9.0	6.70, d, 2.4	6.72, d, 1.2
1″-PA-3‴	7.57, br s	7.55, br s		7.11, d, 8.4 Hz		7.16, d, 8.4		7.18, d, 9.0		
1″-PA-4‴	7.19, m	7.24, m								
1″-PA-5‴	7.57, br s	7.55, br s	7.53, br d, 8.4	7.11, d, 8.4		7.16, d, 8.4		7.18, d, 9.0	6.69, d, 7.8	6.71, d, 7.8
1″-PA-6‴	7.27, br s	7.30, m	7.29, dd, 8.4, 1.8	7.22, d, 8.4		7.25, d, 8.4		7.36, d, 9.0	6.57, dd, 7.8, 2.4	6.57, dd, 7.8, 1.2
1″-PA-7‴	2.95, m	4.30, overlapped	6.90, d, 15.6	2.94, m		3.05, m			2.80, m	2.80, m
		3.78, dd, 15.0, 6.6								
1″-PA-8‴	5.18, br d, 12.0		6.62, dt, 15.6, 5.4	4.77, br d, 16.8		4.21, overlapped 3.84, m			3.71, overlapped	3.92, overlapped
	4.85, br d, 12.0			3.87, dd, 16.8, 7.2						
1″-PA-9‴			4.57, d, 5.4							
OCH_3			3.71, s (CoA[e])							
3″-Rhm[d]-1									5.23, br s	5.50, br s
3″-Rhm-2									3.31, overlapped	3.66, overlapped
3″-Rhm-3									3.97, overlapped	3.40, overlapped
3″-Rhm-4									3.33, overlapped	4.84, overlapped
3″-Rhm-5									4.20, dq, 9.6, 6.0	3.76, overlapped
3″-Rhm-6									1.14, d, 6.0	1.09, d, 6.6
4′-Caff[f]-2										7.07, d, 1.2
4′-Caff-5										6.77, d, 7.8

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Table 1. continued

position	1	2	3	4	5	6	7	8
4′-Caff-6								6.98, dd, 7.8, 1.2
4′-Caff-7								7.61, d, 15.6
4′-Caff-8								6.27, d, 15.6

[a]**1**, **7**, and **8** in CD_3OD; **2**–**6** in pyridine-d_5. [b]β-D-Glucopyranosyl. [c]Phenylalkanoid. [d]α-L-Rhamanopyranosyl. [e]Coniferyl alcohol. [f]*trans*-Caffeoyl. **1**, syringoleoside A; **2**, syringoleoside B; **3**, syringoleoside C; **4**, syringoleoside D; **5**, syringoleoside E; **6**, syringoleoside F; **7**, syringoleoside G; **8**, syringoleoside H.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1–8 (150 MHz, δ_C)[a]

position	1	2	3	4		5		6	7	8
1	94.8	94.7	94.7	94.9	94.4	94.4	94.5	94.6	95.7	95.7
3	154.8	154.1	154.0	154.0	153.9	153.8	153.9	154.0	155.4	155.4
4	109.0	108.6	108.6	108.4	108.3	108.4	108.3	108.7	109.7	109.3
5	31.1	30.8	32.2	30.7	30.6	30.6	30.6	30.8	31.4	30.8
6	40.6	40.7	42.1	40.6	40.1	40.3	40.1	40.8	41.0	41.1
7	172.6	171.6	171.4	171.5	171.3	170.6	171.3	171.4	173.0	173.1
8	124.6	123.1	123.1	123.8	124.1	123.7	123.7	123.9	125.2	124.9
9	130.1	130.3	130.2	130.2	130.1	129.7	130.1	130.2	130.7	130.9
10	13.0	13.4	14.7	13.3	13.2	13.2	13.2	13.3	13.9	18.3
11	168.3	166.9	166.9	167.1	167.0	166.8	166.8	166.9	168.9	169.0
OCH_3-11	52.1	51.2	52.6	51.1	51.1	51.1	51.0	51.2	52.1	52.3
1-Glc[b]-1′	100.5	101.3	101.2	101.0	101.1	101.0	101.0	101.2	101.1	101.2
1-Glc-2′	74.6	75.0	74.6	74.5	74.8	74.5	74.5	75.0	76.0	75.5
1-Glc-3′	78.0	78.3	78.2	78.0	78.0	78.5	78.0	78.3	78.1	78.2
1-Glc-4′	71.4	71.3	71.2	71.2	71.4	70.7	71.2	71.2	70.2	70.3
1-Glc-5′	77.6	78.2	78.2	78.0	78.0	78.0	78.0	78.2	78.0	78.2
1-Glc-6′	62.3	62.4	62.3	62.4	62.2	62.2	62.2	62.3	62.8	62.9
7-Glc-1″	104.0	103.7	102.2	104.5		101.3		103.6	104.3	101.1
7-Glc-2″	74.8	74.8	74.7	74.5		74.6		74.8	76.1	75.8
7-Glc-3″	77.7	78.8	78.8	78.6		78.6		78.8	83.8	78.0
7-Glc-4″	71.3	71.5	71.3	72.4		71.1		71.4	70.4	76.8
7-Glc-5″	77.7	75.0	75.1	78.6		75.4		74.8	78.5	77.9
7-Glc-6″	64.6	64.9	64.8	64.3		64.4		64.8	62.9	62.4
1″-PA[c]-1‴	139.6	138.7	132.5	129.1		129.3		151.6	131.6	131.7
1″-PA-2‴	129.0	128.6	110.7	140.3		130.3		119.0	117.2	117.4
1″-PA-3‴	129.6	128.4	149.4	115.9		116.0		116.8	146.3	146.2
1″-PA-4‴	126.8	127.8	147.2	157.0		157.1		154.2	144.8	144.7
1″-PA-5‴	129.6	128.4	116.7	115.9		116.0		116.8	116.4	117.0
1″-PA-6‴	129.0	128.6	119.8	140.3		130.3		119.0	121.4	121.6
1″-PA-7‴	36.6	70.9	129.3	35.6		35.5			36.7	36.7
1″-PA-8‴	70.8		130.0	71.1		71.1			72.3	72.5
1″-PA-9‴			62.8							
OCH_3			52.5							
3″-Rhm[d]-1									102.4	104.2
3″-Rhm-2									71.7	70.1
3″-Rhm-3									72.6	71.6
3″-Rhm-4									74.9	74.9
3″-Rhm-5									67.9	67.9
3″-Rhm-6									18.0	14.0
4′-Caff[e]-1										127.6
4′-Caff-2										115.7
4′-Caff-3										147.1
4′-Caff-4										150.1
4′-Caff-5										116.6
4′-Caff-6										123.3
4′-Caff-7										148.4
4′-Caff-8										114.4
4′-Caff-9										168.3

[a]**1**, **7**, and **8** in CD_3OD, and **2**–**6** in pyridine-d_5. [b]β-D-Glucopyranosyl. [c]Phenylalkanoid. [d]α-L-Rhamanopyranosyl. [e]*trans*-Caffeoyl. **1**, syringoleoside A; **2**, syringoleoside B; **3**, syringoleoside C; **4**, syringoleoside D; **5**, syringoleoside E; **6**, syringoleoside F; **7**, syringoleoside G; **8**, syringoleoside H.

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RESULTS AND DISCUSSION

Dried *S. dilatata* flowers were extracted in MeOH, and the concentrate was divided into H_2O (SDW), *n*-BuOH (SDB), and EtOAc (SDE) fractions (Fr). Repetition of silica gel (SiO_2), octadecyl SiO_2 (ODS), and Sephadex LH-20 column chromatography (CC) of SDB yielded eight new secoiridoids (compounds **1**−**8**) and five known ones (compounds **9**−**13**). The chemical structures of the 13 oleoside-type secoiridoids (**1**−**13**) were identified based on their physical and spectroscopic data, as well as the application of chemical methods. The new secoiridoids were named syringoleosides A−H (**1**−**8**). The five known compounds were identified as oleoside 11-methyl ester (OS11Me, **9**),[8] oleoside dimethyl ester (**10**),[9] oleuropein (**11**),[10] nuezhenide (**12**),[11] and neonuezhenide (**13**)[12] through interpretation of the spectroscopic data and comparison with literature values. Furthermore, the ability of the isolated secoiridoids to inhibit NO suppression in RAW 264.7 cells was tested, and a quantitative HPLC analysis of the major secoiridoids in the MeOH extract was conducted.

The UV and IR spectra of compounds **1**−**8** suggested the presence of an iridoid enol ester system conjugated with a carbonyl group: bands at 233 nm, 1705 to 1697 cm^{-1}, and 1630 to 1615 cm^{-1}, are typical absorption bands of secoiridoids, and an additional absorption (279 nm) caused by a phenolic group was observed.[13] All the oleoside-type secoiridoids were isolated as amorphous powders and developed into a dark brown zone on TLC by spraying with 10% H_2SO_4 and heating.

Compound **1**, a pale yellow powder, exhibited a molecular ion peak at *m/z* 693.2378 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{16}Na$, 693.2366) in the positive high-resolution fast bombardment mass spectrum (positive HRFABMS) and was determined as $C_{31}H_{42}O_{16}$ (Figure S1, Supporting Information). The NMR spectra (Tables 1 and 2) displayed signals typical of an oleoside-type secoiridoid moiety.[8] In the ^1H NMR spectrum (Figure S2, Supporting Information), signals of an oxygenated olefinic methine (δ_H 7.54, s, H-3), an allylic acetal (δ_H 5.94, br s, H-1), an allylic methine (δ_H 4.03, m, H-5), a methylene (δ_H 2.77, dd, J = 14.4, 4.2 Hz, H-6a; 2.52, dd, J = 14.4, 8.4 Hz, H-6b), and an ethylidene group (δ_H 6.11, q, J = 6.0 Hz, H-8; 1.74, d, J = 6.0 Hz, H-10) were observed, indicating an aglycone characteristic of an oleoside-type secoiridoid. In the ^{13}C NMR spectrum (Figure S3, Supporting Information) of the aglycon moiety, 10 carbons along with a methoxy group (δ_C 52.1, OCH_3-11) signal were detected, constituted by two esters (δ_C 172.6, C-7; 168.3, C-11), an oxygenated olefinic methine (δ_C 154.8, C-3), two olefinic quaternary carbons (δ_C 130.1, C-9; 109.0, C-4), an olefinic methine (δ_C 124.6, C-8), an acetal (δ_C 94.8, C-1), a methylene (δ_C 40.6, C-6), a methine (δ_C 31.1, C-5), and a methyl (δ_C 13.0, C-10).[8] Additionally, proton signals of two hemiacetals (δ_H 4.85, d, J = 7.8 Hz, H-1′; 4.37, d, J = 7.8 Hz, H-1″), eight oxygenated methines from δ_H 3.31 to 3.41, and two oxygenated methylenes (δ_H 3.90, br d, J = 12.0 Hz, H-6′a; 3.68, m, H-6′b; 4.37, overlapped, H-6″a; 4.24, dd, J = 12.0, 5.4 Hz, H-6″b) were detected due to two hexose moieties. Signals of an oxygenated methylene (δ_H 5.18, br d, J = 15.6, 6.6 Hz, H-8‴a; 4.85, br d, J = 15.6, 6.6 Hz, H-8‴b), a methylene (δ_H 2.95, m, H-7‴), and five olefinic methines (δ_H 7.57, br s, H-3‴, 5‴; 7.27, br s, H-2‴, 6‴; 7.19, m, H-4‴) were observed due to a phenylethanol moiety. Carbon signals corresponding to the chemical shifts of two hemiacetals (δ_C 100.5, C-1′; 104.0, C-1″), eight oxygenated methines (δ_C 78.0, C-3′; 77.6, C-5′; 74.6, C-2′; 71.4, C-4′; 77.7, C-3″; 77.7, C-5″; 74.8, C-2″; 71.3, C-4″), and two oxygenated methylenes (δ_C 62.3, C-6′; 64.6, C-6″) caused by two hexoses led to the identification of both sugars as β-glucopyranose moieties. The β-configuration of the anomeric hydroxy groups was confirmed from the coupling constant of the anomeric proton signals (J = 7.8 and 7.8 Hz). An olefinic quaternary carbon (δ_C 139.6, C-1‴), an oxygenated methylene (δ_C 70.8, C-8‴), a methylene (δ_C 36.6, C-7‴), and five olefinic methine (δ_C 129.6, C-3‴, 5‴; 129.0, C-2‴, 6‴; 126.8, C-4‴) carbon signals were concluded to compose a phenylethanol unit. The oxygenated methylene proton resonance of the β-glucopyranose (H-6″) was observed at a relatively low magnetic field, δ_H 4.37 and 4.24, because of an esterification effect, which is commonly detected at δ_H 3.82,[14] indicating the oxygenated methylene of the β-glucopyranose (C-6″) to be linked to a carboxyl group of the secoiridoid moiety (C-7). This determination was supported by a cross-peak between H-6″ (δ_H 4.37, H-6″a; 4.24, H-6″b) and C-7 (δ_C 172.6) in the gHMBC spectrum (Figure S4, Supporting Information). Another glucopyranose was shown to be linked to C-1 from a cross-peak between H-1′ (δ_H 4.85) and C-1 (δ_C 94.8) in the gHMBC spectrum. The oxygenated methylene carbon signal of the phenylethanoid unit was observed at a lower magnetic field, δ_C 70.8 (C-8‴), than the commonly reported chemical shift δ_C 62.3,[15] because of a glycosidation shift, verifying that the phenylethanol is linked to the anomer position, C-1″, of the β-glucopyranose. This inference was supported by the cross-peak between H-8‴ (δ_H 4.85) and C-1″ (δ_C 104.0) in the gHMBC spectrum. The positive FABMS showed fragmentation peaks *m/z* 509 $[M + H - 180\ (\text{hexose} + H_2O)]^+$ and *m/z* 369 $[M + H - 300\ (\text{phenylethyl} + \text{hexose} + H_2O)]^+$, consistent with the partial structure proposed for this compound (Figure S5, Supporting Information). The absolute configuration of β-glucose was determined as D, through GC analysis of a monosaccharide produced by hydrolysis of compound **1** and derivatization using L-cysteine methyl ester hydrochloride and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, see Experimental Section; Figure S6, Supporting Information). Consequently, compound **1** was assigned as a new oleoside-type secoiridoid glycoside, named syringoleoside A, with the structure as shown.

Compound **2**, a pale yellow powder, displayed a molecular ion peak at *m/z* 679.2209 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{16}Na$, 679.2209) in the positive HRFABMS and was determined as $C_{30}H_{40}O_{16}$ (Figure S7, Supporting Information). The molecular weight of compound **2** was 14 Da less than that of compound **1** (670 Da). The ^1H NMR and ^{13}C NMR spectra of compound **2** were closely comparable to those of compound **1**, with the exception of the absence of one methylene unit (Figures S8 and S9, Supporting Information). In the ^{13}C NMR spectrum, signals of one olefinic quaternary carbon (δ_C 138.7, C-1‴), one oxygenated methylene (δ_C 70.9, C-7‴), and five olefinic methines (δ_C 128.6, C-2‴, 6‴; 128.4, C-3‴, 5‴; 127.8, C-4‴) were found to belong to a phenylmethanol moiety. The positive FABMS showed fragmentation peaks *m/z* 517 $[M + Na - 162\ (\text{hexose} - H_2O)]^+$, *m/z* 388 $[M - 268\ (\text{benzyl} + \text{hexose})]^+$, and *m/z* 90 $[M - 566\ (2\ \text{hexoses} - 2\ H_2O - \text{oleoside aglycone})]^+$ (Figure S12, Supporting Information), confirming the partial structure of this compound. Consequently, compound **2** (syringoleoside B) was revealed as a

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new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **3**, a white powder, exhibited a molecular ion peak at m/z 751.2426 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{18}Na$,751.2420) in the positive HRFABMS and was assigned as $C_{33}H_{44}O_{18}$ (Figure S13, Supporting Information). The ^1H NMR and ^{13}C NMR spectra of compound **3** (Figures S14 and S15, Supporting Information) were analogous to those of compounds **1** and **2**, but their phenylethanoid moiety was replaced by a coniferyl alcohol moiety. In the ^1H NMR spectrum, signals of three olefinic methines (δ_H 7.53, d, J = 8.4 Hz, H-5‴; 7.29, dd, J = 8.4, 1.8 Hz, H-6‴; 7.16, d, J = 1.8 Hz, H-2‴) representing a 2,3,5-trisubstituted benzene ring structure; two olefinic methines (δ_H 6.90, d, J = 15.6 Hz, H-7‴; 6.62, dt, J = 15.6, 5.4 Hz, H-8‴) representing a double bond with a *trans*-configuration; an oxygenated methylene (δ_H 4.57, d, J = 5.4 Hz, H-9‴); and a methoxy group (δ_H 3.71, s, H−OCH_3) were detected. In the ^{13}C NMR spectrum, signals of two oxygenated olefinic quaternary carbons (δ_C 149.4, C-3‴; 147.2, C-4‴), an olefinic quaternary carbon (δ_C 132.5, C-1‴), and three olefinic methines (δ_C 119.8, C-6‴; 116.7, C-5‴; 110.7, C-2‴) representing a dioxygenated phenyl moiety; two olefinic methines (δ_C 130.0, C-8‴; 129.3, C-7‴) and an oxygenated methylene (δ_C 62.8, C-9‴) representing a prop-2-enol moiety; and a methoxy group (δ_C 52.5, C-OCH_3) were observed, confirming the presence of a coniferyl alcohol unit. The gHMBC spectrum (Figure S16, Supporting Information) exhibited a correlation between H-1″ (δ_H 5.56) and C-4‴ (δ_C 147.2), which suggested that the anomeric carbon (C-1″) is linked to OH-4‴. In the positive FABMS, the fragmentation peak at m/z 180 [hexose or coniferyl alcohol]$^+$ (Figure S17, Supporting Information) confirmed the partial structure of this compound. Consequently, compound **3** (syringoleoside C) was revealed to be a new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **4**, a white powder, exhibited a molecular ion peak at m/z 1095.3534 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{27}Na$, 1095.3528) in the positive HRFABMS and was determined as $C_{48}H_{64}O_{27}$ (Figure S18, Supporting Information). The ^1H NMR and ^{13}C NMR spectra showed signals (Figures S19 and S20, Supporting Information) derived from two sets of oleoside 11-methyl esters (OS11Me). The ^1H NMR spectrum exhibited peaks characteristic of these two OS11Me as follows: two oxygenated olefinic methines (δ_H 7.70, s, H-3; 7.67, s, H-3‴′), two olefinic methines (δ_H 6.14, overlapped, H-8, 8‴′), two allylic acetals (δ_H 6.44, s, H-1; 6.23, H-1‴′), two allylic methyls (δ_H 1.71, d, J = 6.6 Hz, H-10; 1.70, d, J = 6.0 Hz, H-10‴′), two hemiacetals (δ_H 5.47, d, J = 7.8 Hz, H-1′; 5.46, d, J = 7.8 Hz, H-1‴′), and two methoxy groups (δ_H 3.63, s; 3.62, s), as well as the signals of two β-glucopyranosyl moieties. The ^{13}C NMR spectrum included the following diagnostic signals: four esters (δ_C 171.5, C-7; 171.3, C-7‴′; 167.1, C-11; 167.0, C-11‴′), two oxygenated olefinic methines (δ_C 154.0, C-3; 153.9, C-3‴′), two olefinic methines (δ_C 123.8, C-8; 124.1, C-8‴′), two acetals (δ_C 94.9, C-1; 94.4, C-1‴′), two methyls (δ_C 13.3, C-10; 13.2, C-10‴′), two methoxy groups (δ_C 51.1; 51.1), and two hemiacetals (δ_C 108.4, C-1′; 108.3, C-1‴″). Additionally, the ^1H NMR signals of the hexose and phenylethanoid moieties were observed. In the ^1H NMR spectrum, signals of a hemiacetal (δ_H 4.77, d, J = 7.2 Hz, H-1″), four oxygenated methines (δ_H 4.22, overlapped, H-4″; 4.18, overlapped, H-2″; 3.95, overlapped, H-3″, 5″), and an oxygenated methylene (δ_H 4.87, dd, J = 10.4, 9.0 Hz, H-6″a; 4.61, dd, J = 10.4, 5.4 Hz, H-6″b) representing a hexose moiety and four olefinic methines (δ_H 7.22, d, J = 8.4 Hz, H-2‴, 6‴; 7.11, d, J = 8.4 Hz, H-3‴, 5‴) from a *p*-disubstituted benzene unit, an oxygenated methylene (δ_H 4.77, br d, J = 7.2 Hz, H-8‴a; 3.87, dd, J = 16.8, 7.2 Hz, H-8‴b), and a methylene (δ_H 2.94, m, H-7‴) representing a phenylethanol with a *p*-disubstituted benzene structure were detected. In the ^{13}C NMR spectrum, signals owing to a hemiacetal (δ_C 104.5, C-1″), four oxygenated methines (δ_C 78.6, C-3″, 5″; 74.5, C-2″; 72.4, C-4″), and an oxygenated methylene (δ_C 64.3, C-6″) revealed the hexose to be a β-glucopyranose moiety. Additionally, signals from an oxygenated olefinic quaternary carbon (δ_C 157.0, C-4‴), an olefinic quaternary carbon (δ_C 129.1, C-1‴), four olefinic methines (δ_C 140.3, C-2‴, 6‴; 115.9, C-3‴, 5‴), an oxygenated methylene (δ_C 71.1, C-8‴), and a methylene (δ_C 35.6, C-7‴) confirmed the phenylethanoid unit to be a *p*-hydroxyphenyl ethanol. The downfield-shifted oxygenated methylene proton signal of a β-glucopyranose (δ_H 4.87, H-6″a; 4.61, H-6″b) caused by an esterification effect, as well as the correlation of the H-6″ proton signal with an ester carbon signal (δ_C 171.5, C-7) in the gHMBC spectrum, gave evidence for a linkage at C-7 and C-6″ (Figure S21, Supporting Information). The downfield-shifted oxygenated methine proton signal of a β-glucopyranose (δ_H 5.70, H-3′), as well as the correlation of the proton H-3′ with an ester carbon (δ_C 171.3, C-7‴′) in the gHMBC spectrum, showed a linkage between C-7‴′ and C-3′. The downfield-shifted oxygenated methylene carbon (δ_C 71.1, C-8‴), caused by a glycosidation shift, as well as the gHMBC correlations between δ_H 4.77 (H-1″) and δ_C 71.1 (C-8‴) and between δ_C 101.1 (C-1″) and δ_H 4.77 and δ_H 3.87 (H-8‴), confirmed that the phenylethanol unit was linked to the anomeric carbon of the glucopyranose. In the positive FABMS spectrum, fragmentation peaks were detected at m/z 933 $[M + Na - 162$ (hexose − $H_2O)]^+$, produced through the loss of a hexose from the parent molecule (m/z 1072); m/z 709 $[M + Na - 386$ (OS11Me − $H_2O)]^+$, produced by the loss of an OS11Me; m/z 388 $[OS11Me - OH + H]^+$; and m/z 136 $[M - 935$ (two OS11Me + hexose − OH + H)$]^+$, produced via the loss of two OS11Me and a hexose (Figure S22, Supporting Information). Consequently, compound **4** (syringoleoside D) was revealed to be a new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **5**, a white powder, showed a molecular ion peak at m/z 1095.3536 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{27}Na$, 1095.3528) in the positive HRFABMS, consistent with the elemental formula $C_{48}H_{64}O_{27}$ (Figure S23, Supporting Information), which was the same as compound **4**. The ^1H NMR and ^{13}C NMR spectra of **5** (Figures S24 and S25, Supporting Information) were nearly identical to those of **4**, except for the binding site of a secoiridoid glycoside unit. An oxygenated methine resonance of β-glucopyranose (H-2′), which is commonly detected at δ_H 3.17, was detected at a lower magnetic field, δ_H 5.42, because of an esterification effect.[14] In the gHMBC spectrum (Figure S26, Supporting Information), the oxygenated methine proton signal of β-glucopyranose (H-2′) exhibited a cross-peak with the ester carbon of the oleoside signal at δ_C 170.6 (C-7‴′), suggesting that a β-glucopyranose unit was linked to C-7‴′. The secoiridoid moiety has two chiral centers, C-1 and C-5. In the NOESY spectrum (Figure S27, Supporting Information), H-1 showed no correlation with H-5 but did show a correlation with H-6, suggesting the *trans*-orientation for H-1

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and H-5. The NaOMe alkaline hydrolysis of compound **5** produced an oleoside dimethyl ester (**10**) (Figure S28, Supporting Information). The specific rotation value of the hydrolysate, $[\alpha]_D$ −109 ($[\alpha]_D$ −115 in the literature),[8] suggested the absolute configurations of C-1 and C-5 to be *S* and *S*. In a NOESY experiment, no cross-peak between H-1 and H-5 was found (Figure S29, Supporting Information). In addition, the ECD spectrum of oleoside dimethyl ester (**10**) (230 nm, $\Delta\varepsilon$ −14.1) was almost the same as that of the 1*S*,5*S*-oleoside dimethyl ester (227 nm, $\Delta\varepsilon$ −8.3), obtained from flowers of *Syringa vulgaris*[8] (Figure S30, Supporting Information). In the NOESY spectrum, a doublet methyl proton signal (δ_H 1.64, H-10) showed a correlation with a methine proton signal (δ_H 3.72, H-5), but not with an acetal proton signal (δ_H 5.83, H-1) (Figure S27, Supporting Information), confirming the double bond between C-8 and C-9 to have an *E* geometry. Consequently, compound **5** (syringoleoside E) represents a new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **6**, a pale yellow powder, showed a molecular ion peak at *m/z* 681.2010 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{17}Na$, 681.2002) in the positive HRFABMS, determined as $C_{29}H_{28}O_{17}$ (Figure S31, Supporting Information). The ^1H NMR and ^{13}C NMR spectra of **6** (Figures S32 and S33, Supporting Information) were analogous to those of compounds **1**−**3**, with the exception of the phenylethanoid unit. Four olefinic methine proton signals (δ_H 7.36, d, *J* = 9.0 Hz, H-2‴, 6‴; 7.11, d, *J* = 9.0 Hz, H-3‴, 5‴) representing a *p*-disubstituted benzene moiety, the carbon signals of two oxygenated olefinic quaternary carbons (δ_C 119.0, C-2‴, 6‴; 116.8, C-3‴, 5‴), and four olefinic methines (δ_C 154.2, C-4‴; 151.6, C-1‴), representing a 1,4-benzenediol moiety, were observed. The positive FABMS showed fragmentation peaks at *m/z* 180 $[hexose]^+$ and *m/z* 481 [M − 177 (methyl hexose − OH)]$^+$ (Figure S35, Supporting Information), supporting the partial structure proposed for this compound. Consequently, compound **6** (syringoleoside F) was revealed as a new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **7**, a white powder, exhibited a molecular ion peak at *m/z* 871.2850 $[M + Na]^+$ (calcd for $C_{37}H_{52}O_{22}Na$, 871.2843) in the positive HRFABMS, with the elemental formula $C_{37}H_{52}O_{22}$ (Figure S36, Supporting Information). The ^1H NMR and ^{13}C NMR spectra (Figures S37 and S38, Supporting Information) revealed that **7** consisted of an OS11Me, two hexoses, and one phenylethanoid moiety. In the ^1H NMR spectrum, the peaks from two hemiacetals (δ_H 5.23, s, H-1‴′; 4.31, d, *J* = 7.8 Hz, H-1″), eight oxygenated methines (δ_H 4.92, d, *J* = 9.6 Hz, H-2″; 4.20, dd, *J* = 9.6, 6.0 Hz, H-5‴′; 3.97, overlapped, H-3‴′; 3.89, overlapped, H-4″; 3.52, dd, *J* = 9.0, 9.0 Hz, H-3″, 3.36, overlapped, H-5″; 3.33, overlapped, H-4‴′; 3.31, overlapped, H-2‴′), an oxygenated methylene (δ_H 3.89, overlapped, H-6″a; 3.68, overlapped, H-6″b), and a methyl (δ_H 1.14, d, *J* = 6.0 Hz, H-6‴′) were detected, suggesting the sugars to be one aldohexose and one 6-deoxyaldohexose. The ^{13}C NMR spectrum exhibited the peaks of two hemiacetals (δ_C 104.3, C-1‴; 102.4, C-1″), eight oxygenated methines (δ_C 83.8, C-3″; 78.5, C-5″; 76.1, C-2″; 74.9, C-4‴′; 72.6, C-3‴′; 71.7, C-2‴′; 70.4, C-4″; 67.9, C-5‴′), an oxygenated methylene (δ_C 62.9, C-6″), and a methyl (δ_C 18.0, C-6‴′), indicating that the sugars present were a *β*-glucose and an *α*-rhamnose. The coupling constants of the anomeric protons (7.8 Hz and br s) confirmed that their anomeric hydroxy group configurations were *β* and *α*. The ^1H NMR spectrum displayed three olefinic methines (δ_H 6.70, d, *J* = 2.4 Hz, H-2‴; 6.69, d, *J* = 7.8 Hz, H-5‴; 6.57, dd, *J* = 7.8, 2.4 Hz, H-6‴) representing a 1,2,4-trisubstituted benzene unit, a methylene (δ_H 2.80, m, H-6‴), and an oxygenated methylene (δ_H 3.71, overlapped, H-8‴), suggesting that the phenylalkanoid unit is 3,4-dihydroxyphenylethanol. Likewise, the ^{13}C NMR spectrum exhibited peaks for a 3,4-dihydroxyphenylethanol moiety: two oxygenated olefinic quaternary carbons (δ_C 146.3, C-3‴; 144.8, C-4‴), one olefinic quaternary carbon (δ_C 131.6, C-1‴), three olefinic methines (δ_C 121.4, C-6‴; 117.2, C-2‴; 116.4, C-5‴), one methylene (δ_C 36.7, C-7‴), and one oxygenated methylene (δ_C 72.3, C-8‴). The downfield-shifted oxygenated methine proton of *β*-glucose, which is commonly detected at δ_H 3.17, was observed at δ_H 4.92 (H-2″), because of an esterification effect.[14] This observation, along with a correlation of the H-2″ proton and an ester carbon, δ_C 173.0 (C-7) in the gHMBC spectrum, demonstrated an ester bond linkage at C-7 and C-2″. Furthermore, the downfield-shifted oxygenated methine carbon signal of *β*-glucopyranose, δ_C 83.8 (C-3″), and the oxygenated methylene carbon signal of phenylethanol, δ_C 72.3 (C-8‴), which are commonly detected at δ_C 78.2 (C-3″) and δ_C 62.3 (C-8‴′),[14,16] respectively, showed the linkage position proposed for the terminal sugar, *α*-rhamnopyranose, and the 3,4-dihydroxyphenyl ethanol moieties. These were confirmed from the gHMBC correlations of H-3″/C-1‴′, C-3″/H-1‴′, H-1″/C-8‴, and C-1″/H-8‴ (Figure S39, Supporting Information). The absolute configurations of *β*-glucose and *α*-rhamnose were determined to be D and L, respectively, through a GC study of the diastereomers of the monosugars acquired by the hydrolysis of **7** and derivatization with L-cysteine methyl ester hydrochloride and MSTFA. Additionally, the positive FABMS spectrum showed fragmentation peaks *m/z* 735 [M + Na − 136 (phenylethanoid − H_2O)]$^+$, produced through the release of a phenylethanol moiety from the parent molecule (*m/z* 848), and *m/z* 391 [M + Na − 480 (2 hexoses + phenylethanoid − 2 OH)]$^+$, produced by the loss of two hexoses and one phenylethanol moiety (Figure S40, Supporting Information). Consequently, compound **7** (syringoleoside G) was revealed to be a new oleoside-type secoiridoid glycoside, with the structure as shown.

Compound **8**, a white powder, exhibited a molecular ion peak at *m/z* 1009.3193 $[M - H]^-$ (calcd for $C_{46}H_{57}O_{25}$, 1009.3185) in the negative HRFABMS, consistent with the elemental formula $C_{46}H_{57}O_{25}$ (Figure S41, Supporting Information). The molecular weight of compound **8** was 162 Da larger than that of compound **7** (848 Da). The ^1H NMR and ^{13}C NMR spectra of **8** (Figures S42 and S43, Supporting Information) were analogous to those of **7**, with the exception of the presence of a caffeoyl moiety. The ^1H NMR signals of three olefinic methines (δ_H 7.07, d, *J* = 1.2 Hz, H-2⁗; 6.98, dd, *J* = 7.8, 1.2 Hz, H-6⁗; 6.77, d, *J* = 7.8 Hz, H-5⁗) representing a 1,2,4-trisubstituted benzene unit and two olefinic methines (δ_H 7.61, d, *J* = 15.6 Hz, H-7⁗ 6.27, d, *J* = 15.6 Hz, H-8⁗), representing a double bond with a *trans*-configuration, were detected. The ^{13}C NMR spectrum also displayed the peaks of a caffeoyl moiety: an ester (δ_C 168.3, C-9⁗), two oxygenated olefinic quaternary carbons (δ_C 150.1, C-4⁗; 147.1, C-3⁗), five olefinic methines (δ_C 148.4, C-7⁗; 123.3, C-6⁗; 116.6, C-5⁗; 115.7, C-2⁗; 114.4, C-8⁗), and an olefinic quaternary carbon (δ_C 127.6, C-1⁗). A downfield-shifted oxygenated methine proton signal from the terminal

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Table 3. Inhibitory Effects of Secoiridoids (1−8, 10−13) from *S. diliatata* Flowers on NO Production in RAW 264.7 Macrophage Cells[a]

	1	2	3	4	5	6	7	8	10	11	12	13
IC_{50} (μM)	65.7 ± 11.0	32.5 ± 9.8	>80	>80	>80	58.3 ± 17.3	42.1 ± 14.3	>80	>80	>80	59.8 ± 15.8	48.9 ± 17.7

[a]The cells were treated with LPS (1 μg/mL) and the compounds for 24 h. Nitrite levels were quantified in the culture medium of LPS-stimulated cells using the Griess reaction. Data are presented as the means ± standard deviation of three independent experiments.

sugar, an α-rhamnopyranose, at δ_H 4.84 (H-4‴′), caused by an esterification effect, as well as a correlation of the ester carbon of the secoiridoid moiety at δ_C 173.1 (C-7) and the proton signal (H-4‴′) in the gHMBC spectrum, supported the linkage positions of the two sugars with a phenylethanoid and the secoiridoid aglycone. The downfield-shifted hemiacetal proton signal of the OS11Me sugar, a β-glucopyranose, δ_H 5.00 (H-4′), caused by an esterification effect, as well as a cross-peak between the proton signal (H-4′) and the ester carbon of the caffeoyl moiety at δ_C 168.3 (C-9⁗″) in the gHMBC spectrum, demonstrated that the caffeoyl group is linked to C-4′. Additionally, the negative FABMS spectrum showed fragmentation peaks *m/z* 847 $[M - H - 162 (caffeoyl - H_2O)]^-$, suggesting the loss of a caffeoyl group from the parent molecule (*m/z* 1010); *m/z* 461 $[M - H - 548 (OS11Me + caffeoyl - H_2O)]^-$, obtained from the loss of OS11Me and caffeoyl moieties; *m/z* 315 $[M - H - 694 (OS11Me + caffeoyl + hexose - H_2O)]^-$, obtained from the loss of OS11Me, caffeoyl, and hexose moieties; *m/z* 241 $[OS11Me aglycone - H]^-$; *m/z* 162 $[caffeoyl - H_2O]^-$; *m/z* 155 [3,4-dihydroxyphenylethanol − H]$^-$; and *m/z* 135 [vinylcatechol − H]$^-$ (Figure S45, Supporting Information). Consequently, compound **8** (syringoleoside H) was revealed to be a new oleoside-type secoiridoid glycoside, with the structure as shown.

Inflammation, as a reaction to injury, infection, or internal stress, induces symptoms of redness, swelling, heat, and often pain. Long-lasting inflammation raises the risk of chronic disease. Nitric oxide (NO) is a pro-inflammatory mediator that induces inflammation due to overproduction in abnormal situations.[17,18] Prior research has indicated that iridoids exhibit promising anti-inflammatory activity.[19] Therefore, the secoiridoids isolated from *S. dilatata* flowers were assayed for their potential to suppress NO production in LPS-provoked RAW 264.7 cells. These cells were treated with the fractions and isolated compounds (10, 20, 40, 50, 80, and 100 μM). The isolated secoiridoids showed no cytotoxicity at concentrations lower than 100 (**1**, **4**, **5**, **7**, **8**, **10**, **11**, **13**) or 50 (**2**, **3**, **6**, **12**) μM. Table 3 shows that the LPS-treated compound group included suppressed NO formation. The positive control, butein, markedly decreased NO production at 10 μM. Compounds **1**, **2**, **6**, **7**, **12**, and **13** dose-dependently suppressed NO formation in LPS-treated RAW 264.7 cells without any apparent toxicity, with IC_{50} values of 65.7 ± 11.0, 32.5 ± 9.8, 58.3 ± 17.3, 42.1 ± 14.3, 59.8 ± 15.8, and 48.9 ± 17.7 μM, respectively. Compounds **3**, **4**, **5**, and **8** suppressed NO formation at 80 μM.

HPLC was conducted to quantify the major secoiridoids isolated from *S. dilatata* flowers, compounds **1**, **4**, **5**, **8**, **9**, **12**, and **13**. The chromatogram showed the clear separation of each secoiridoid. The calibration curves were built using various concentrations of each compound, for which the equations and correlation coefficients (r^2 = 0.999) are presented in Figure S46 (Supporting Information). The large value of r^2 confirmed the experiment to be reliable. The quantitative analysis concluded that the amounts of compounds **1**, **4**, **5**, **8**, **9**, **12**, and **13** in the *S. dilatata* flower extract were 2.06 ± 0.02, 5.44 ± 0.06, 8.91 ± 0.07, 14.17 ± 0.19, 3.11 ± 0.06, 4.99 ± 0.10, and 3.68 ± 0.07 (%), respectively. Notably, the total content of the major secoiridoids in the extract was very high, 42.4%. It has been reported that the content of secondary metabolites in the floral organs may often be very high when compared with those in other parts of a plant,[20,21] indicating that flowers can be a good source of bioactive compounds.

EXPERIMENTAL SECTION

General Experimental Procedures. The adsorbents for CC and TLC analysis were the same as those previously used.[20] Melting point, optical rotation, IR, ECD, NMR (600 MHz), and HRFABMS measurements were performed as previously reported.[22]

Plant Material. The flowers of *S. dilatata* were collected at the campus of Kyung Hee University, Yong-In, Korea, in April 2017, and Dr. Jin Hee Park, Animal & Plant Resources Research Division, Freshwater Bioresources Research Bureau, NNIBR, Sangju, Korea, confirmed the plant identity. A voucher specimen (KHU-NPCL-201704) is deposited at the laboratory of natural products chemistry, Kyung Hee University.

Extraction and Isolation. Dried *S. dilatata* flowers (705 g) were soaked in 80% MeOH (22 L × 4, room temperature, 24 h), filtered, and evaporated in vacuo to give 419 g of residue. This extract (415 g) was poured into H_2O (500 mL) and extracted with EtOAc (500 mL × 3) and *n*-BuOH (450 mL × 4), successively. The partitioned fractions were evaporated to obtain the residues of H_2O (SDH, 266 g), *n*-BuOH (SDB, 116 g), and EtOAc (SDE, 37 g). Fr SDB (116 g) was subjected to SiO_2 CC (ϕ 12 × 20 cm) using $CHCl_3$−MeOH−H_2O (15:3:1 → 8:3:1 → 7:3:1 → 6:4:1, 2.4 L of each) as the eluting solvent to furnish 19 fractions (SDB-1 to SDB-19), as monitored using TLC. Fr SDB-6 [2.7 g, elution volume/total volume (Ev/Tv) 0.192−0.240] was subjected to ODS CC (ϕ 4 × 15 cm) using acetone−MeOH−H_2O (1:1:3, 1.9 L) as the eluting solvent to afford 15 fractions (SDB-6-1 to SDB-6-15) and pure compound **4** [SDB-6-9, 22 mg, Ev/Tv 0.600−0.642, TLC (SiO_2) R_f = 0.50, $CHCl_3$−MeOH−H_2O (12:3:1), (ODS) R_f = 0.32, acetone−MeOH−H_2O (1:1:3)]. Fr SDB-6-5 (399 mg, Ev/Tv 0.321−0.368) was subjected to SiO_2 CC (ϕ 3 × 15 cm) using $CHCl_3$−MeOH−H_2O (20:3:1, 2.7 L) as the eluting solvent to give 14 fractions (SDB-6-5-1 to SDB-6-5-14) and pure compound **2** [SDB-6-5-10, 12 mg, Ev/Tv 0.656−0.806, TLC (SiO_2) R_f = 0.40, $CHCl_3$−MeOH−H_2O (10:3:1), (ODS) R_f = 0.50, acetone−MeOH−H_2O (1:1:2)]. Fr SDB-6-5-6 (171 mg, Ev/Tv 0.211−0.372) was subjected to ODS CC (ϕ 2 × 5 cm) using acetone−H_2O (1:2, 500 mL) to give 10 fractions (SDB-6-5-6-1 to SDB-6-5-6-10). Fr SDB-6-5-6-2 (107 mg, Ev/Tv 0.080−0.120) was subjected to Sephadex LH-20 CC (ϕ 1.5 cm × 60 cm) using 100% MeOH (500 mL) to give six fractions (SDB-6-5-6-2-1 to SDB-6-5-6-2-6) and pure compound **5** [SDB-6-5-6-2-5, 18 mg, Ev/Tv 0.946−1.000, TLC (SiO_2) R_f = 0.50, $CHCl_3$−MeOH−H_2O (13:3:1), (ODS) R_f = 0.30, acetone−H_2O (1:2)]. Fr SDB-10 (3.9 g, Ev/Tv 0.495−0.579) was subjected to ODS CC (ϕ 5 × 15 cm) using MeOH−H_2O (1:3, 7.1 L) to afford 25 fractions (SDB-10-1 to SDB-10-25) and pure compounds **3**, **7**, and **12** [**3**, SDB-10-19, 55 mg, Ve/Vt 0.707−0.831, TLC (SiO_2) R_f = 0.54, $CHCl_3$−MeOH−H_2O (8:3:1), (ODS) R_f = 0.30, MeOH−H_2O (1:1); **7**, SDB-10-21, 98 mg, Ev/Tv 0.865−0.899, TLC (SiO_2) R_f = 0.51, $CHCl_3$−MeOH−H_2O (8:3:1), (ODS) R_f = 0.23, MeOH−H_2O (1:1); **12**, SDB-10-14, 633 mg, Ev/Tv 0.319−

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0.506, TLC (SiO_2) R_f = 0.45, $CHCl_3$−MeOH−H_2O (8:3:1), (ODS) R_f = 0.55, MeOH−H_2O (1:1)]. Fr SDB-10-16 (181 mg, Ev/Tv 0.618−0.730) was subjected to SiO_2 CC (ϕ 3 × 15 cm) using EtOAc−*n*-BuOH−H_2O (10:3:1, 500 mL) as the eluting solvent to afford 14 fractions (SDB-10-16-1 to SDB-10-16-14) and pure compounds **1** and **10** [**1**, SDB-10-16-11, 13 mg, Ev/Tv 0.590−0.810, TLC (SiO_2) R_f = 0.30, EtOAc−*n*-BuOH−H_2O (10:3:1), (ODS) R_f = 0.55, MeOH−H_2O (1:1); **10**, SDB-10-16-2, 30 mg, Ev/Tv 0.070−0.100, TLC (SiO_2) R_f = 0.54, EtOAc−*n*-BuOH−H_2O (10:3:1), (ODS) R_f = 0.50, MeOH−H_2O (1:1)]. Fr SDB-13 (26.1 g, Ev/Tv 0.763−0.850) was subjected to ODS CC (ϕ 7 × 10 cm) using MeOH−H_2O (1:3 → 1:2 → 1:1, 1.5 L of each) as the eluting solvent to give 21 fractions (SDB-13-1 to SDB-13-21). Fr SDB-13-5 (1.9 g, Ev/Tv 0.078−0.1000) was subjected to SiO_2 CC (ϕ 4.5 × 15 cm) using EtOAc−*n*-BuOH−H_2O (27:3:1 → 23:3:1 → 20:3:1, 1.5 L of each) as the eluting solvent to give 18 fractions (SDB-13-5-1 to SDB-13-5-18). Fr SDB-13-5-8 (117 mg, Ev/Tv 0.156−0.179) was subjected to ODS CC (ϕ 2 × 10 cm) and eluted with MeOH−H_2O (1:3, 750 mL) to afford 10 fractions (SDB-13-5-8-1 to SDB-13-5-8-10) and pure compound **11** [SDB-13-5-8-8, 7 mg, Ev/Tv 0.309−0.444, TLC (SiO_2) R_f = 0.50, EtOAc−*n*-BuOH−H_2O (7:3:1), (ODS) R_f = 0.62, MeOH−H_2O (1:1)]. Fr SDB-13-5-13 (294 mg, Ev/Tv 0.429−0.598) was subjected to ODS CC (ϕ 4 × 10 cm) and eluted with MeOH−H_2O (1:3, 1.7 L) to give 19 fractions (SDB-13-5-13-1 to SDB-13-5-13-19) and pure compound **13** [SDB-13-5-13-15, 9 mg, Ev/Tv 0.796−0.822, TLC (SiO_2) R_f = 0.50, EtOAc−*n*-BuOH−H_2O (7:3:1), (ODS) R_f = 0.65, MeOH−H_2O (1:1)]. Fr SDB-13-9 (1.8 g, Ev/Tv 0.121−0.174) was subjected to ODS CC (ϕ 4 × 10 cm) and eluted with acetone−MeOH−H_2O (1:1:5, 1.1 L) to afford 10 fractions (SDB-13-9-1 to SDB-13-9-10). Fr SDB-13-9-3 (557 mg, Ev/Tv 0.155−0.272) was subjected to SiO_2 CC (ϕ 3 cm × 15 cm) and eluted with EtOAc−*n*-BuOH−H_2O (20:3:1 → 18:3:1, 5.0 L of both) to give 15 fractions (SDB-13-9-3-1 to SDB-13-9-3-15). Fr SDB-13-9-3-8 (62 mg, Ev/Tv 0.194−0.282) was subjected to Sephadex LH-20 CC (ϕ 1.5 × 50 cm) and eluted with 100% MeOH (450 mL) to give 10 fractions (SDB-13-9-3-8-1 to SDB-13-9-3-8-10) along with pure compounds **6** and **9** [**6**, SDB-13-9-3-8-5, 9 mg, Ev/Tv 0.322−0.368, TLC (SiO_2) R_f = 0.60, EtOAc−*n*-BuOH−H_2O (7:3:1), (ODS) R_f = 0.60, MeOH−H_2O (1:1); **9**, SDB-13-9-3-8-3, 3 mg, Ev/Tv 0.195−0.276, TLC (SiO_2) R_f = 0.30, EtOAc−*n*-BuOH−H_2O (7:3:1), (ODS) R_f = 0.60, MeOH−H_2O (1:1)]. Fr SDB-13-14 (6.2 g, Ev/Tv 0.437−0.512) was subjected to ODS CC (ϕ 7 × 5 cm) and eluted with acetone−MeOH−H_2O (1:1:6 → 1:1:5, 3.2 L of both) to give 10 fractions (SDB-13-14-1 to SDB-13-14-10). Fr SDB-13-14-8 (279 mg, Ev/Tv 0.764−0.922) was subjected to Sephadex LH-20 CC (ϕ 2 × 55 cm) using 100% MeOH (500 mL) to give eight fractions (SDB-13-14-8-1 to SDB-13-14-8-8), including pure compound **8** [SDB-13-14-8-3, 209 mg, Ev/Tv 0.400−0.480, TLC (SiO_2) R_f = 0.60, EtOAc−*n*-BuOH−H_2O (7:3:1), (ODS) R_f = 0.45, MeOH−H_2O (1:1)].

*Syringoleoside A (**1**):* pale yellow powder; mp 158−159 °C; $[\alpha]_D^{25}$ −81.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2950, 1702, 1629 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 1 and 2; positive HRFABMS *m/z* 693.2378 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{16}Na$, 693.2366).

*Syringoleoside B (**2**):* pale yellow powder; mp 149−150 °C; $[\alpha]_D^{25}$ −25.2 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2360, 1706, 1630 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data, see Tables 1 and 2; positive FABMS *m/z* 517 $[M + Na - 162 (hexose - H_2O)]^+$, *m/z* 388 $[M - 268 (benzyl + hexose)]^+$, *m/z* 90 $[M - 566 (2 hexose - 2 H_2O - oleoside aglycone)]^+$; positive HRFABMS *m/z* 679.2209 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_{16}Na$, 679.2209).

*Syringoleoside C (**3**):* white powder; mp 143−144 °C; $[\alpha]_D^{25}$ −205.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2024, 1705, 1628 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data, see Tables 1 and 2; positive FABMS *m/z* 180 [hexose or coniferyl alcohol]$^+$; positive HRFABMS *m/z* 751.2426 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{18}Na$, 751.2420).

*Syringoleoside D (**4**):* white powder; mp 146−147 °C; $[\alpha]_D^{25}$ −217.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2923, 1702, 1630 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data, see Tables 1 and 2; positive HRFABMS *m/z* 1095.3534 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{27}Na$, 1095.3528).

*Syringoleoside E (**5**):* white powder; mp 141−142 °C; $[\alpha]_D^{25}$ −94.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2358, 1701, 1629 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data, see Tables 1 and 2; positive HRFABMS *m/z* 1095.3536 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{27}Na$, 1095.3528).

*Syringoleoside F (**6**):* pale yellow powder; mp 151−152 °C; $[\alpha]_D^{25}$ −40.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2025, 1704, 1615 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data, see Tables 1 and 2; positive FABMS *m/z* 180 $[hexose]^+$, *m/z* 481 $[M - 177 (methyl hexose - OH)]^+$; positive HRFABMS *m/z* 681.2010 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{17}Na$, 681.2002).

*Syringoleoside G (**7**):* white powder; mp 147−148 °C; $[\alpha]_D^{25}$ −152.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2360, 1701, 1628 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 1 and 2; positive FABMS *m/z* 735 $[M + Na - 136 (phenylethanoid - H_2O)]^+$, *m/z* 391 $[M + Na - 480 (2 hexose + phenylethanoid - 2 OH)]^+$; positive HRFABMS *m/z* 871.2850 $[M + Na]^+$ (calcd for $C_{37}H_{52}O_{22}Na$, 871.2843).

*Syringoleoside H (**8**):* white powder; mp 157−158 °C; $[\alpha]_D^{25}$ −181.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 2358, 1697, 1628 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Tables 1 and 2; negative FABMS 847 $[M - H - 162 (caffeoyl - H_2O)]^-$, *m/z* 461 $[M - H - 548 (OS11Me + caffeoyl - H_2O)]^-$, *m/z* 315 $[M - H - 694 (OS11Me - caffeoyl + hexose - H_2O)]^-$, *m/z* 241 $[OS11Me aglycone - H]^-$, *m/z* 135 [vinylcatechol − H]$^-$; negative HRFABMS *m/z* 1009.3193 $[M - H]^-$ (calcd for $C_{46}H_{57}O_{25}$, 1009.3185).

Acid Hydrolysis of Syringoleosides and Identification of the Absolute Configuration of the Sugars. The absolute configuration of the monosaccharides, produced via acid hydrolysis of the syringoleosides, was determined by applying the method described previously.[23] Each compound (3 mg) was treated with 2 N HCl (2 mL) at 80 °C for 6 h, followed by neutralization with Ag_2CO_3 and filtering. The filtrates were concentrated under reduced pressure to produce a sugar fraction. The residue was treated with pyridine (100 μL) and 0.1 M L-cysteine methyl ester hydrochloride (150 μL) and stirred at 60 °C for 90 min. The reaction mixture was dried under reduced pressure; MSTFA (100 μL) was added, and the mixture was incubated at 60 °C for 60 min. Then, the resultant mixture was used for a GC experiment as follows: capillary column, HP-5MS (30 m × 0.32 mm × 0.25 μm); detector, FID; detector temperature, 280 °C; injector temperature, 250 °C; carrier, N_2 gas (20.4 mL/min); oven temperature, 170−250 °C with a rate of 5 °C/min; 1 μL of each sample injected directly into the injection port (splitless mode). By comparing the retention time (t_R) of the sugar derivatives with those of the standards (D-glucose, t_R = 15.01 min; L-glucose, t_R = 15.59 min; L-rhamnose, t_R = 13.04 min; Sigma), the absolute configurations of the monosaccharides were confirmed to be D-glucose (t_R = 15.01 min) and L-rhamnose (t_R = 13.03 min).

Alkaline Hydrolysis of Syringoleoside H (8) and Determination of the Absolute Configuration of the Chiral Centers. A total of 1.5 g of crude syringoleoside H (**8**) was dissolved in MeOH solution containing 30 mmol of sodium methoxide (100 mL), hydrolyzed at room temperature for 8 h.[24] Then, H_2O (50 mL) was added to the reaction solution, concentrated until 50 mL, extracted with *n*-BuOH (50 mL × 2), and concentrated. The *n*-BuOH extract (241 mg) was purified using SiO_2 CC (ϕ 3 × 12 cm, $CHCl_3$−MeOH−H_2O = 15:3:1, 2.5 L) to give 174 mg of oleoside dimethyl ester (**10**). It was measured to determine its specific rotation value, $[\alpha]_D$ −174 (lit. −176)[8] and ECD (MeOH) 230 ($\Delta\varepsilon$ −14.1).

Preparation of 1*S*,5*S*-Oleoside Dimethyl Ester from the Flowers of *Syringa vulgaris*. Dried *S. vulgaris* flowers (50 g) were extracted with 80% MeOH (1 L, room temperature, 24 h), filtered, and evaporated in vacuo to give a dried concentrate (27 g). The extract was poured into H_2O (100 mL) and extracted with *n*-BuOH (90 mL × 2), and the organic phase was evaporated to obtain the residue of *n*-BuOH (12 g). The *n*-BuOH fraction was treated with the

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same method as that above-described to give 1*S*,5*S*-oleoside dimethyl ester (52 mg).[8] The ECD of the compound was measured: (MeOH) 227 ($\Delta\varepsilon$ −8.3).

RAW 264.7 Cell Viability Assay and Evaluation of NO Production. The cell viability assay and determination of NO production were performed by the same methodology as reported in the literature.[25] Butein, an iNOS inhibitor, was used as a positive control.

Statistical Analysis. Data are expressed as the means ± SEM (n = 3).

Quantitative Analysis of Major Secoiridoids in *Syringa dilatata* Flowers. The filtered secoiridoids (10 000 ppm) were diluted to various concentrations to establish calibration curves (**1**: 500, 250, 125, 62.5; **4**, **5**, and **13**: 2000, 1000, 500, 250, 125; **8**: 4000, 2000, 1000, 500, 250; **9** and **12**: 1000, 500, 250 ppm). HPLC (Shimadzu Corporation, Kyoto, Japan) was conducted to separate the secoiridoids using a Shim-Pack C_{18} column (Shimadzu, 250 × 4.6 mm, 5 μm) with a flow rate of 1.0 mL/min. A 10 μL aliquot of each sample was injected into the HPLC system. The column temperature was set at 40 °C. Analysis was achieved using a Shimadzu 20AD at 203 nm (Shimadzu Corporation). The mobile phase consisted of a combination of 0.1% FA in H_2O (solvent A) and acetonitrile (solvent B) with the following gradient elution: solvent B, 10% (0.01 min) → 20% (5 min) → 25% (18 min) → 35% (25 min) → 50% (50 min) → 100% (60 min). The 80% MeOH extract was analyzed to determine the content of each secoiridoid. The quantitative analysis was repeated three times.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00490.

^{1}H NMR, ^{13}C NMR, HRFABMS, and ECD spectra, MS fragmentation patterns of compounds **1**−**8**, and GC-MS spectra of sugars (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Nam-In Baek − *Graduate School of Biotechnology and Department of Oriental Medicinal Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea;* orcid.org/0000-0002-9438-2817; Email: nibaek@khu.ac.kr

Authors

Jung Eun Gwag − *Graduate School of Biotechnology and Department of Oriental Medicinal Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea*

Yeong-Geun Lee − *Graduate School of Biotechnology and Department of Oriental Medicinal Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea*

Hyoung-Geun Kim − *Graduate School of Biotechnology and Department of Oriental Medicinal Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea*

Dong-Sung Lee − *College of Pharmacy, Chosun University, Gwangju 61452, Republic of Korea*

Dae Young Lee − *Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Eumseong 27709, Republic of Korea*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c00490

Notes

The authors declare no competing financial interest.

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