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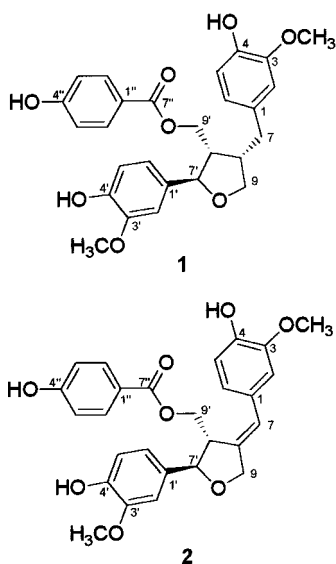
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Investigation of the whole plant of *Agastache rugosa* resulted in the isolation of two new lignan compounds. Their structures were elucidated as (8*S*,7'*R*,8'*S*)-4-hydroxybenzoic acid 4-(4-hydroxy-3-methoxybenzyl)-2-(4-hydroxy-3-methoxyphenyl)tetrahydrofuran-3-ylmethyl ester (agastinol, **1**) and (7'*R*,8'*S*)-4-hydroxybenzoic acid 4-(hydroxy-3-methoxybenzylidene)-2-(4-hydroxy-3-methoxyphenyl)-tetrahydrofuran-3-ylmethyl ester (agastenol, **2**). Agastinol and agastenol inhibited etoposide-induced apoptosis in U937 cells with IC₅₀ values of 15.2 and 11.4 μg/mL, respectively.

Apoptosis, programmed cell death, is involved in a wide range of biological and pathological processes such as embryogenesis, immune responses, neurodegenerative disorders, and the progression of cancer.^{1–3} Accordingly, apoptosis modulators represent a new type of bioactive natural products. Caspase-3 protease plays important roles in the signaling pathway controlling mammalian apoptosis. Proteolytic cleavage and activation of caspase-3 may be functionally important in the induction of apoptosis.¹ We recently reported dykellic acid,⁵ petasiphenol,⁶ terrein,⁷ and two new compounds⁸ from *Isodon excisus* as inhibitors of etoposide-induced apoptosis in U937 cells. In the course of screening medicinal plants, the MeOH extract of the whole plant of *Agastache rugosa* showed potent inhibitory activity against apoptosis induced by etoposide in the U937 cell line. *Agastache rugosa* is widely distributed in Korea, where the leaf parts have been used for food and traditional medicine.⁹ In this paper, we report the isolation and characterization of two new lignans (**1** and **2**) as apoptosis inhibitors from the plant source.



Compound **1** was obtained as an amorphous powder that analyzed for C₂₇H₂₈O₈ by HRESIMS ([M + H]⁺ at 481.1868, calcd 481.1862) and ¹³C NMR data (Table 1). The UV

absorption at λ_{max} 258 nm was indicative of a phenolic moiety. The ¹³C NMR spectrum showed signals for 27 carbons, including two methoxyls and 18 aromatic carbons. The ¹³C NMR (Table 1) and DEPT spectra indicated the presence of (i) three aromatic rings where carbons were attached to 10 protons, five oxygen atoms, and three carbon atoms, (ii) one carbonyl carbon (166.5 ppm), (iii) two methoxy carbons (56.3, 56.3 ppm), (iv) three methylene carbons (34.0, 63.7, 73.3 ppm), and (v) three methyne carbons (43.8, 50.6, 84.2 ppm).

The ¹H NMR spectrum of **1** revealed that the 10 aromatic protons (6.70, 6.74, 6.78, 6.84, 6.87, 6.90, 6.90, 6.97, 7.82, 7.82 ppm) comprised two 1,3,4-trisubstituted aromatic rings and a 1,4-disubstituted aromatic ring. These assignments and the linkage of the side chain were determined using a combination of 2D NMR methods, particularly by interpretation of ¹³C NMR, DEPT, HMQC, and HMBC data, which allowed all protons and carbons to be assigned. The HMBC correlations between the signals of H-9' (4.39, 4.59 ppm), H-2'' (7.82 ppm), and H-6'' (7.82 ppm) and the signal at 166.5 ppm suggested that the carbonyl group must be located at 7''. The points of attachment of the methoxy groups were also deduced from HMBC as C-3 and C-3'. The chemical shift of the H-7' signal of **1** (4.85 ppm) agreed with the assignment of the *trans* configuration when compared with literature data.^{10,11} The NOESY spectrum displayed correlations between H-8 and H-8', and H-7' and H-9', but no correlation was observed between H-7' and H-8'. This led to the assignment of *trans* and *cis* orientations for H-7'/H-8' and H-8'/H-8, respectively. The optical rotation of **1** was found to be -30°, establishing the structure of **1** as *S*, *R*, *S* relative configuration at the C-8, C-7', and C-8' chiral centers, respectively.^{12,13} As a result, the structure of **1** was elucidated as (8*S*,7'*R*,8'*S*)-4-hydroxybenzoic acid 4-(4-hydroxy-3-methoxybenzyl)-2-(4-hydroxy-3-methoxyphenyl)tetrahydrofuran-3-ylmethyl ester. This novel compound was named agastinol.

The spectral data of **2** closely resembled those of **1**, which thus suggested it to be a derivative of **1**. Compound **2** was obtained as an amorphous powder that analyzed for C₂₇H₂₆O₈ by HRESIMS ([M + Na]⁺ at 501.1535, calcd 501.1525) and ¹³C NMR data (Table 1). The UV absorption at λ_{max} 264 nm was indicative of a phenolic moiety. The ¹³C NMR spectrum showed signals for 27 carbons, including two methoxyls and 18 aromatic carbons. The ¹³C NMR (Table 1) and DEPT spectra indicated the presence of (i) three aromatic rings where carbons were attached to 10

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Table 1. ^1H , ^{13}C , and HMBC Data for Compounds **1** and **2** (CD_3OD)

1				2			
position	$^{13}\text{C}^a$	$^1\text{H}^b$	HMBC ^c (H→C)	position	$^{13}\text{C}^a$	$^1\text{H}^b$	HMBC ^c (H→C)
1	132.9 (C)			1	137.9 (C)		
2	113.2 (CH)	6.87 (d, 1.8)	3, 4, 6, 9	2	112.5 (CH)	7.05 (d, 2.0)	3, 4, 6, 7, 8
3	148.3 (C)			3	148.3 (C) ^e		
4	145.8 (C)			4	146.9 (C) ^f		
5	115.8 (CH)	6.74 (d, 7.8)	1, 3, 4	5	116.0 (CH)	6.80 (d, 8.1)	2, 3, 4, 8
6	121.9 (CH)	6.70 (dd, 7.8, 1.8)	2, 4, 9	6	122.5 (CH)	6.97 (dd, 8.1, 2.0)	2, 4, 7
7	73.3 (CH_2)	3.75 (dd, 8.5, 6.7), 4.04 (dd, 8.5, 6.7)	9, 7', 9'	7	123.6 (CH)	6.50 (d, 1.8)	2, 9, 8'
8	43.8 (CH)	2.84 (m)	9	8	129.4 (C)		
9	33.9 (CH_2)	2.63 (dd, 13.5, 10.9), 2.96 (dd, 13.5, 5.1)	1, 2, 6, 7, 8, 8'	9	73.5 (CH_2)	4.63 (s)	7, 8, 7', 8'
1'	135.8 (C)			1'	134.8 (C)		
2'	110.6 (CH)	6.97 (d, 1.8)	1', 3', 4', 6', 7'	2'	110.6 (CH)	6.94 (d, 1.8)	1', 3', 4', 6', 7',
3'	148.4 (C)			3'	148.4 (C) ^e		
4'	146.8 (C)			4'	147.0 (C) ^f		
5'	115.5 (CH)	6.78 (d, 7.8)	1', 3', 4'	5'	115.6 (CH)	6.74 (d, 8.1)	1', 2', 3', 4', 6'
6'	119.7 (CH)	6.84 (d, d, 1.8, 7.8)	2', 4', 7'	6'	119.7 (CH)	6.82 (dd, 8.1, 1.8)	8, 2', 4', 7'
7'	84.2 (CH)	4.85 (d, 6.6)	7, 8, 1', 2', 6', 8', 9'	7'	85.7 (CH)	5.14 (d, 3.5)	9, 1', 2', 6', 7', 8', 9'
8'	50.6 (CH)	2.67 (m)	9, 7', 9'	8'	46.7 (CH)	3.80 (m)	1'
9'	63.7 (CH_2)	4.39 (dd, 11.2, 6.7), 4.59 (dd, 11.2, 7.3)	8, 7', 8', 7''	9'	64.5 (CH_2)	4.20 (dd, 11.1, 4.6), 4.70 (dd, 11.1, 9.3)	7', 8', 7''
1''	122.5 (C)			1''	122.3 (C)		
2''	132.6 (CH)	7.82 (d, 7.8)	4'', 7''	2''	132.7 (CH)	7.82 (dd, 7.8, 1.8)	
3''	111.6 (CH)	6.90 (d, 7.8)	1'', 4'', 5''	3''	116.1 (CH)	6.80 (d, 7.8)	1'', 2'', 4'', 5''
4''	162.8 (C)			4''	162.9 (C)		
5''	111.6 (CH)	6.90 (d, 7.8)	1'', 3'', 5''	5''	116.1 (CH)	6.80 (d, 7.8)	1'', 3'', 4'', 6''
6''	132.6 (CH)	7.82 (d, 7.8)	2'', 4'', 7''	6''	132.7 (CH)	7.82 (dd, 7.8, 1.8)	1'', 2'', 4'', 5'', 7''
7''	166.5 (C)			7''	166.6 (C)		
OMe-3	56.3 (CH_3)	3.78 (s) ^d	3	OMe-3	56.4 (CH_3)	3.84 (s)	3
OMe-3'	56.3 (CH_3)	3.81 (s) ^d	3'	OMe-3'	56.2 (CH_3)	3.72 (s)	3'

^a Recorded at 150 MHz. ^b Recorded at 600 MHz. ^c Recorded at 600 MHz. ^{d-f} Chemical shifts are interchangeable.

protons, five oxygen atoms, and three carbon atoms, (ii) one carbonyl carbon (166.6 ppm), (iii) one quaternary carbon (129.4 ppm), (iv) two methoxy carbons (56.2, 56.4 ppm), (v) two methylene carbons (64.5, 73.5 ppm), and (vi) three methyne carbons (46.7, 85.7, 123.6 ppm).

These assignments and the linkage of the side chain were determined using a combination of 2D NMR methods, as described for **1**. The molecular weight and NMR data differences at C-7 and C-8 between **1** and **2** gave evidence that the C-7 and C-8 linkage was a double bond in **2** rather than a single bond in **1**. The *E*-geometry of the C-7–8 double bond was established by NOE between H-7 and H-9. The points of attachment of the methoxy groups were also deduced from HMBC as C-3 and C-3'. The NOESY spectrum displayed a correlation between H-7' and H-9', but no correlation was observed between H-7' and H-8'. This led to the assignment of *trans* for H-7'/H-8'. The optical rotation of (–)-magnofargesin (7'*S*, 8'*R*)¹⁴ was -33° . The optical rotation of **2** was found to be $+45^\circ$, establishing the structure of **2** as *R*, *S* absolute configuration at the C-7' and C-8' chiral centers, respectively. As a result, the structure of **2** was elucidated as (7'*R*, 8'*S*)-4-hydroxybenzoic acid 4-(hydroxy-3-methoxybenzylidene)-2-(4-hydroxy-3-methoxyphenyl)tetrahydrofuran-3-ylmethyl ester. This novel compound was named agastenol.

Compounds **1** and **2** inhibited etoposide-induced apoptosis in U937 cells with similar potency with IC_{50} values of 15.2 and 11.4 $\mu\text{g}/\text{mL}$, respectively (Table 2). From these results, agastinol (**1**) and agastenol (**2**) seem to be worthy candidates for further research as potential anti-apoptotic agents.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. UV spectra were recorded using a Shimadzu UV-260 spectropho-

Table 2. Inhibitory Effects of **1** and **2** on Etoposide-Induced Caspase-3 Induction in U937 Leukemia Cells

compound	IC_{50} ($\mu\text{g}/\text{mL}$)
1	15.2
2	11.4
pyrrolidine dithiocarbamate (PDTC)	8.3

tometer. IR spectra were obtained with a Jasco Report-100 spectrophotometer. The NMR spectra were taken on Varian UNITY 300 and JEOL JNM-A600 spectrometer. ESIMS were obtained on a Fisons VG Quattro 400 mass spectrometer. Column chromatography was performed over Si gel 60 (Merck, particle size 230–400 mesh) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden).

Collection. The whole plant of *Agastache rugosa* was collected at Jin-ju, Kyung-sang-dam-do, Korea, in September 2000. The plant was identified by Dr. Sangmyung Lee, KRIBB. A voucher specimen (IMP0009-A) is retained at the herbarium of KRIBB.

Extraction and Isolation. Air-dried whole plants of *Agastache rugosa* (3 kg) were percolated with MeOH at 25 °C for 3 weeks. The residue obtained after removal of the solvent (50 g) was diluted with H_2O (1 L) and extracted with EtOAc (1 L \times 3). The EtOAc extract on concentration left a dark syrup (20 g), which was chromatographed on a column of Si gel with CHCl_3 and MeOH mixtures of increasing polarity. The active fractions were further purified by Sephadex LH-20 column chromatography using as solvent system MeOH– H_2O (8:2). Final purification was effected by HPLC (C_{18} column) with an acetonitrile– H_2O gradient solvent system, yielding pure compounds **1** (4 mg) and **2** (6 mg).

Agastinol (1): yellow amorphous powder; $[\alpha]_{\text{D}}^{25} -30^\circ$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (4.32), 259 (4.34), 334 (3.26) nm; IR (KBr) ν_{max} 3430, 3011, 2920, 1720, 1590, 1510, 1460, 1340, 1250, 1150, 1000 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (positive) m/z 481.1868 [$\text{M} + \text{H}$]⁺ for $\text{C}_{27}\text{H}_{29}\text{O}_8$ (calcd 481.1862).

Agastenol (2): yellow amorphous powder; $[\alpha]_{\text{D}}^{25} +45^\circ$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (4.50), 300 (3.82) nm;

IR (KBr) ν_{\max} 3400, 2910, 1700, 1600, 1510, 1450, 1380, 1270, 1160, 1120, 980 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (positive) m/z 501.1535 $[\text{M} + \text{Na}]^+$ for $\text{C}_{27}\text{H}_{26}\text{O}_8\text{-Na}$ (calcd 501.1525).

Apoptosis Inhibition Assay. Etoposide-induced caspase induction assay was conducted in U937 leukemia cells using pyrrolidine dithiocarbamate (PDTC) as a standard apoptosis inhibitor. Etoposide (10 μM) was added to U937 cells in the presence or absence of various concentrations of compound. The solution was incubated for 7 h at 37 $^\circ\text{C}$ in a 5% CO_2 -95% air atmosphere. After observing apoptotic body generation on cells by microscopy, the caspase 3-like protease activity was estimated from cell lysates using DEVD-AFC (Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin) as a substrate.^{15,16} These activities were determined in duplicate for each treatment.

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