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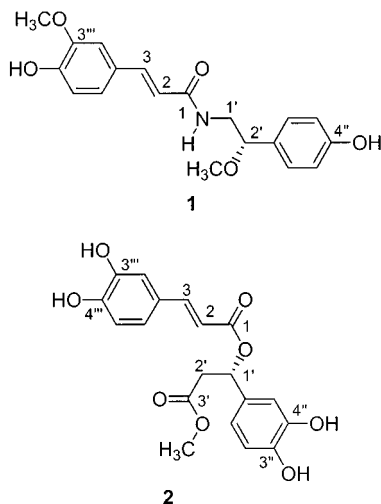
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Investigation of the whole plant of *Isodon excisus* resulted in the isolation of two new apoptosis inhibitors (**1** and **2**). Compounds **1** and **2** inhibited etoposide-induced apoptosis in U937 cells with IC₅₀ values of 10.2 and 52.4 µg/mL, respectively. The structures of **1** and **2** were determined by spectral data interpretation.

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.⁴ We recently reported dykellic acid,⁵ petasiphenol,⁶ and terrein⁷ as inhibitors of etoposide-induced apoptosis in U937 cells. In the course of screening medicinal plants, the MeOH extract of the whole plant of *Isodon excisus* showed potent inhibitory activity against apoptosis induced by etoposide in the U937 cell line. *Isodon excisus* is widely distributed in Korea, where the aerial parts have been used for detoxification.⁵ In this paper, we report the isolation and characterization of two new compounds (**1** and **2**) as apoptosis inhibitors from this plant source.



Compound **1** was obtained as an amorphous powder that analyzed for C₁₉H₂₁NO₅ by HRESIMS ([M + H]⁺ at 344.1501, calcd 344.1498) and its ¹³C NMR data (Table 1). The UV absorption at λ_{max} 294 nm was indicative of a phenolic moiety. The ¹³C NMR spectrum showed signals for 19 carbons, including two methoxys, and 12 aromatic carbons. The ¹³C NMR (Table 1) and DEPT spectra indicated the presence of two aromatic rings containing seven protons, three oxygen atoms, and two carbon atoms,

one conjugated double bond (118.7, 142.2 ppm), one amide carbon (169.2 ppm), two methoxy carbons (56.4, 56.8 ppm), one methylene carbon (47.0 ppm), and one methine carbon (82.3 ppm).

The ¹H NMR spectrum of **1** revealed that the seven aromatic protons (6.80, 6.80, 6.80, 7.03, 7.13, 7.17, 7.17 ppm) comprised a 1,3,4-trisubstituted aromatic ring 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 position of the side chain was established from the HMBC correlations between H-1' (3.41, 3.50 ppm) and C-2' (82.3 ppm), C-1'' (131.5 ppm), and C-1 (168.2 ppm). The point of attachment of the methoxy group was also deduced from HMBC as C-2'. The optical rotation of **1** was found to be –2°, establishing the *S*(–) absolute configuration at the C-2' chiral center.^{9,10} The large coupling constants at 6.47 (d, 15.9) and 7.44 (d, 15.9) ppm of the double bond between C-2 and C-3 showed its configuration is *trans*. As a result, the structure of **1** was elucidated as 3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide.

The spectral data of **2** closely resembled those of **1**. Compound **2** was obtained as an amorphous powder that analyzed for C₁₉H₁₈O₈ by HRESIMS ([M + H]⁺ at 375.1070, calcd 375.1080) and its ¹³C NMR data (Table 1). The UV absorption at λ_{max} 291 nm was indicative of a phenolic moiety. The ¹³C NMR spectrum showed signals for 19 carbons, including one methoxy, and 12 aromatic carbons. The ¹³C NMR (Table 1) and DEPT spectra indicated the presence of two aromatic rings containing six protons, four oxygen atoms, and two carbon atoms, one conjugated double bond (114.2, 147.9 ppm), two ester carbons (168.3, 172.1 ppm), one methoxy carbon (52.7 ppm), one methylene carbon (39.8 ppm), and one methyne carbon (74.3 ppm).

The ¹H NMR spectrum of **2** revealed that the seven aromatic protons (6.57, 6.69, 6.71, 6.78, 6.96, 7.04 ppm) comprised two 1,3,4-trisubstituted aromatic rings. These assignments and the linkage of the side chain were determined using a combination of 2D NMR methods, as described for **1**. The position of the side chain was established from the HMBC correlations between H-1' (5.18 ppm) and C-3' (168.3 ppm), C-2' (37.9 ppm), C-6'' (121.8 ppm), and C-1'' (128.8 ppm). The large coupling constants at 6.25 (d, 15.8) and 7.55 (d, 15.8) ppm show that the C-2 and C-3 double bond is *trans*. The optical rotation of **2** was found to be +26°, establishing the *R*(+) absolute configuration at the C-1' chiral center.^{9,10} As a result, the

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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 \rightarrow C)	position	$^{13}\text{C}^a$	$^1\text{H}^b$	HMBC c (H \rightarrow C)
1	169.2 (C)			1	168.3 (C)		
2	118.7 (CH)	6.47 (d, 15.9)	1.3, 1'''	2	114.2 (CH)	6.25 (d, 15.8)	1, 3, 1'''
3	142.2 (CH)	7.44 (d, 15.9)	1, 2, 1''', 2''', 6'''	3	147.9 (CH)	7.55 (d, 15.8)	1, 2, 1''', 2''', 6'''
1'	47.0 (CH_2)	3.41 (dd, 13.8, 8.1)	1, 2', 1''	1'	74.7 (CH)	5.18 (dd, 7.2, 5.7)	1, 2', 3', 1''
		3.50 (dd, 13.8, 4.5)		2'	37.9 (CH_2)	3.02 (d, 7.2)	1', 3', 1''
2'	82.3 (CH)	4.25 (dd, 8.1, 4.5)	1', OMe-2', 1''			3.02 (d, 5.7)	
			2'', 6''	3'	172.1 (C)		
1''	131.5 (C)			1''	128.8 (C)		
2'', 6''	129.2 (CH)	7.17 (d, 8.4)	2', 3'', 4'', 5''	2''	117.5 (CH)	6.71 (d, 2)	1'', 4'', 6''
3'', 5''	116.3 (CH)	6.80 (d, 8.4)	1''	3''	146.2 (CH)		
4''	158.5 (C)			4''	145.4 (C)		
1'''	128.3 (C)			5''	116.3 (CH)	6.69 (d, 8.0)	1'', 3'', 4'', 6''
2'''	111.6 (CH)	7.13 (d, 2.0)	3, 1''', 3''', 6'''	6''	121.8 (CH)	6.57 (dd, 8.0, 2.0)	1'', 4'', 5''
3'''	149.3 (C)			1'''	127.6 (C)		
4'''	149.9 (C)			2'''	115.3 (CH)	7.04 (d, 2.1)	3, 3''', 4''', 6'''
5'''	116.5 (CH)	6.80 (d, 8.1)	1''', 3'''	3'''	146.8 (C)		
6'''	123.3 (CH)	7.03 (dd, 8.1, 2.0)	3, 2'', 4'''	4'''	149.8 (C)		
OMe-2'	56.8 (CH_3)	3.21 (s)	2'	5'''	116.5 (CH)	6.78 (d, 7.8)	1''', 3''', 4'''
OMe-3'''	56.4 (CH_3)	3.88 (s)	3'''	6'''	123.2 (CH)	6.96 (dd, 7.8, 2.1)	3, 2''', 4'''
				OMe-1'	52.7 (CH_3)	3.69 (s)	3'

^a Recorded at 75 MHz. ^b Recorded at 300 MHz. ^c Recorded at 600 MHz.

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

compound	IC_{50} ($\mu\text{g/mL}$)
1	10.2
2	52.4
pyrrolidine dithiocarbamate (PDTC)	8.3

structure of **2** was elucidated as 3-(3,4-dihydroxyphenyl)-acrylic acid 1-(3,4-dihydroxyphenyl)-2-methoxycarbonyl-ethyl ester.

Compound **1** inhibited etoposide-induced apoptosis in U937 cells with an IC_{50} value of 10.2 $\mu\text{g/mL}$ (Table 2), while compound **2** showed weak activity (IC_{50} = 52.4 $\mu\text{g/mL}$). From these results, compound **1** seems to be a worthy candidate for further research as a potential anti-apoptotic agent. Compound **1** is the first nitrogen-containing compound to have been found thus far in the genus *Isodon*.

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 spectrophotometer. IR spectra were obtained with a JASCO Report-100 spectrophotometer. The NMR spectra were taken on Varian UNITY 300 and JEOL JNM-A600 spectrometer. ES-IMS 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).

Plant Material. The whole plant of *Isodon excisus* was collected at O-Dae Mountain, Kang-won-do, Korea, in September 1999. The plant was identified by Dr. Sangmyung Lee, KRIBB. A voucher specimen (IMP9909-I) is retained at the herbarium of KRIBB.

Extraction and Isolation. Air-dried whole plants of *I. excisus* (3 kg) were percolated with MeOH at 25 °C for 3 weeks. The residue obtained after removal of the solvent (48 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 (6 g), which was chromatographed on a column of Si gel with CHCl_3 and MeOH mixtures with increasing polarity. The active fractions were further purified by Sephadex LH-20 column chromatography using as solvent system MeOH– H_2O (8:2). Final purifications were effected by HPLC (C_{18} column) with an acetonitrile– H_2O gradient solvent system, yielding pure compounds **1** (12 mg) and **2** (6 mg).

3-(4-Hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (1): yellow amorphous powder; $[\alpha]_D^{25}$ -2° (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.44), 294 (4.36), 318 (4.43) nm; IR (KBr) ν_{max} 2900, 1650, 1600, 1520, 1280 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (positive) m/z 344.1501 $[\text{M} + \text{H}]^+$ for $\text{C}_{19}\text{H}_{22}\text{NO}_5$ (calcd 344.1498).

3-(3,4-Dihydroxyphenyl)acrylic acid 1-(3,4-dihydroxyphenyl)-2-methoxycarbonyl-ethyl ester (2): yellow amorphous powder; $[\alpha]_D^{25}$ $+26^\circ$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.35), 291 (4.20), 329 (4.31) nm; IR (KBr) ν_{max} 2900, 1700, 1600, 1520, 1450, 1290, 1150 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS (positive) m/z 375.1070 $[\text{M} + \text{H}]^+$ for $\text{C}_{19}\text{H}_{19}\text{O}_8$ (calcd 375.1080).

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 °C in a 5% CO_2 –95% air atmosphere. After observing apoptotic 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.^{11,12}

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