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Two New Chemical Constituents from Leaves of *Perilla frutescens* var. *acuta*

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Perilla frutescens var. *acuta* (Labiatae) is an annual plant that is widely distributed throughout Korea, China, and Japan. In Korea, its leaves are used as a garnish and colorant for foods and as a traditional medicine for the treatment of asthma, cough, sore throat, dyspersia, insomnia, and diabetes.[1,2] The EtOH extract of *P. frutescens* var. *acuta* was reported to have anti-allergic and anti-bacterial effects.[3,4] Terpenoids, and phenolic compounds were also reported.[5-7] In our continuing search for bioactive secondary metabolites from Korean medicinal plants, we investigated the constituents of the leaves of *P. frutescens* var. *acuta*. Repeated column chromatographic separation of the MeOH extract led to the isolation of two new chemical constituents, including one phenolic compound, named perillascens (**1**) and one monoterpene glycoside, named perillaside (**36**), together with 34 known compounds (Figure 1). The structures of these new compounds were determined by spectroscopic methods, including 1D and 2D NMR (COSY, HMQC, and HMBC), HR-FAB-MS, optical rotation, and chemical reaction.

Compound **1** was isolated as a colorless gum with positive optical rotation ($[\alpha]_D^{25}$ + 1.4). The molecular formula of **1** was determined to be $C_{19}H_{21}NO_5$ on the basis of a $[M + H]^+$ peak at *m/z* 344.1497 (calcd. for $C_{19}H_{22}NO_5$: 344.1498) in the HR-FAB-MS. The ^{1}H NMR spectrum (Table 1) of **1** showed two olefinic protons at δ_H 6.65 (1H, d, *J* = 13.0 Hz, H-3) and 5.86 (1H, d, *J* = 13.0 Hz, H-2), seven aromatic protons at δ_H 7.40 (1H, d, *J* = 2.0 Hz, H-2‴), 7.14 (2H, d, *J* = 8.5 Hz, H-2″, 6″), 6.97 (1H, dd, *J* = 8.0, 2.0 Hz, H-6‴), 6.79 (1H, d, *J* = 8.5 Hz, H-5‴), and 6.77 (2H, d, *J* = 8.5 Hz, H-3″, 5″), methylene protons at δ_H 3.47 (1H, dd, *J* = 14.0, 4.0 Hz, H-1′a), and 3.35 (1H, m, H-1′b), and two methoxy groups at δ_H 3.89 (3H, s, OCH_3-3‴) and 3.19 (3H, s, OCH_3-2′). The ^{13}C NMR spectra demonstrated the presence of 19 carbon signals, including a carbonyl carbon at δ_C 168.8, two olefinic carbons at δ_C 137.3 and 120.1 and two methoxy carbons at d 55.3 and 54.9, an oxygenated methine carbon at δ_C 81.6, twelve aromatic carbons, and methylene carbons. These data of **1** were similar to those of 3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide isolated from *Isodon excises*,[8] except for the coupling constants of H-2 (δ_H 5.86 (d, *J* = 13.0 Hz)) and H-3 (δ_H 6.65 (d, *J* = 13.0 Hz)), which indicated that the (*E*)-form of the double bond at C-2 in **2** was replaced with the (*Z*)-form in **1**.[10] The full NMR assignments were determined by COSY, HMQC, and HMBC (Figure 2). The absolute configuration at C-2′ for **1** was established as *R* by comparison of its optical rotation value ($[\alpha]_D^{25}$ + 1.4).[8,9] Thus, the structure of **1** was determined to be (*Z*)-3-(4‴-hydroxy-3‴-methoxyphenyl)-*N*-((*R*)-2-(4″-hydroxyphenyl)-2′-methoxyethyl)acrylamide, and named perillascens.

Compound **36** was isolated as a colorless gum. The molecular formula was determined to be $C_{16}H_{24}O_8$ on the basis of the $[M + H]^+$ peak at *m/z* 345.1548 (calcd. for $C_{16}H_{25}O_8$: 345.1549) in the HR-FAB-MS. The ^{1}H NMR (Table 2) of **36** displayed signals for the two methyl groups at δ_H 1.32 (3H, s, H-11) and 1.31 (3H, s, H-10), two methylene protons at δ_H 3.06 (1H, m, H-7a), 2.99 (1H, m, H-7b), and 1.94 (2H, m), and three furan ring protons at δ_H 8.41 (1H, brs, H-2), 7.60 (1H, brs, H-5), and 6.80 (1H, brs, H-4). In the ^{13}C-NMR spectrum, ten carbon signals showed, including two methyl carbons at δ_C 27.2 and 27.0, two methylene carbons at δ_C 36.9 and 36.2, two quaternary carbons at δ_C 129.0 and 78.3, and one carbonyl carbon at δ_C 198.7, and three furan ring carbons at δ_C 150.3, 145.9, and 109.4. These spectral data implied that **36** was to be a monoterpene derivative.[11] The NMR spectral data of **36** were similar to those of 5-(3-furyl)-2-methyl-5-oxo-2-pentanol,[12] except for additional sugar moiety [δ_H 4.87 (1H, d, *J* = 8.0 Hz, H-1'), 3.82 (1H, dd, *J* = 12.0, 2.9 Hz, H-6a'), 3.65 (1H, dd, *J* = 12.0, 5.0 Hz, H-6b'), 3.38 (1H, m, H-3'), 3.33 (1H, m, H-4'), 3.32 (1H, m, H-5'), and 3.16 (1H, dd, *J* = 9.0, 8.0 Hz, H-2') in the ^{1}H NMR spectrum; δ_C 98.8, 78.2, 77.8, 75.4, 71.8, and 62.9 in the ^{13}C NMR spectrum]. The position of glucose was confirmed by an HMBC experiment, in which a correlation was observed between the H-1' (δ_H 4.87) and C-10 (δ_C 78.3) as shown in Figure 2. Acid hydrolysis of **36** afforded β-D-glucopyranose, which was identified by co-TLC with authentic sugars and by GC analysis.[13] Thus, the structure of **36** was established as shown in Figure 1 and the compound was named perillaside.

The other known compounds were identified as (*R*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (**2**),[14] rosmarinic acid (**3**), rosmarinic methyl ester (**4**),[15] protocatechuic acid (**5**),[16] 1-β-D-glucopyranosyl-3,4,5-trimethoxybenzen (**6**),[17] 3,4,5-trimethoxyphenyl 1-O-β-apiofuranosyl (1"→6')-β-glucopyranoside (**7**),[18] protocatechualdehyde (**8**),[19] vanillic acid (**9**),[20] 4-hydroxybenzoic

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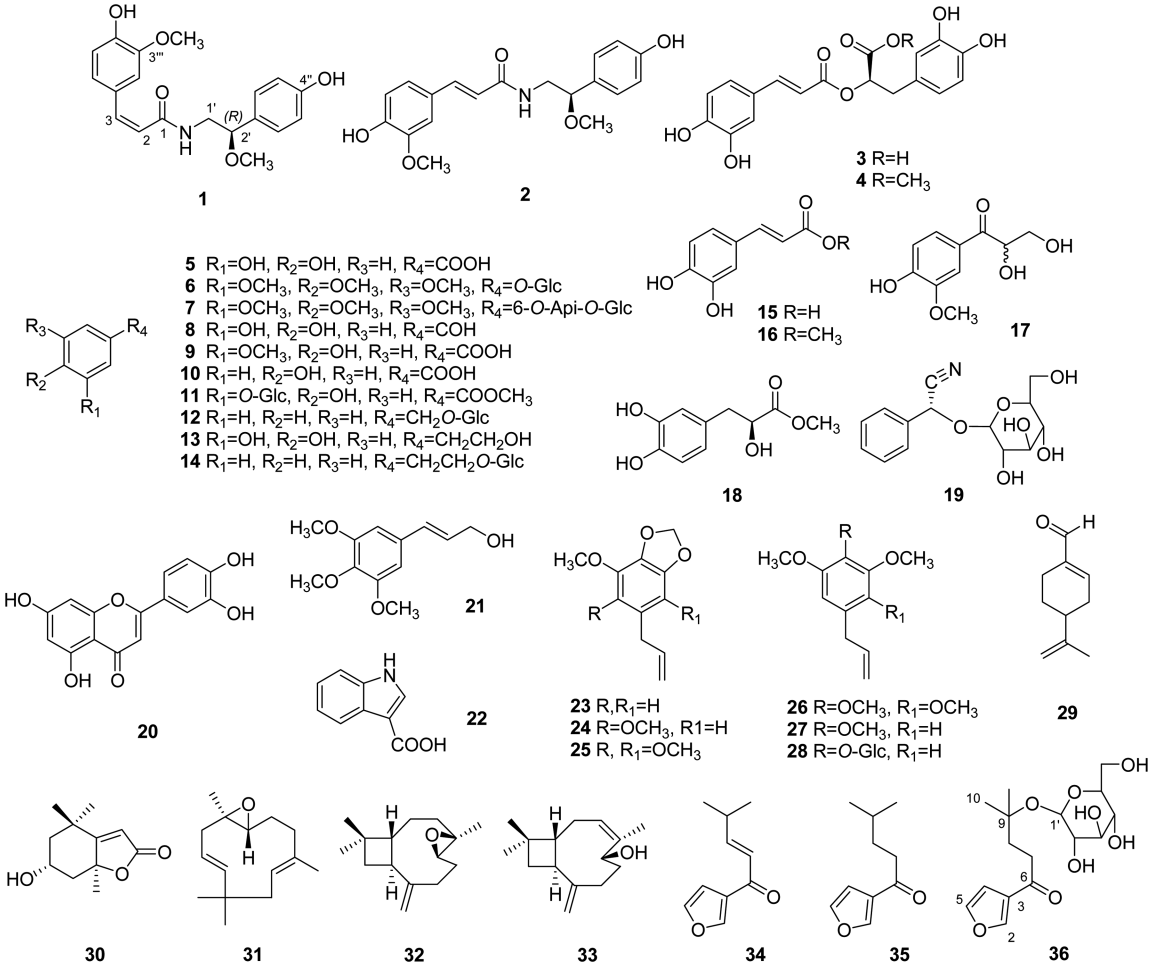

Figure 1. Chemical structures of compounds **1**-**36**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** in CD_3OD[a]

Position	**1**	
	^{1}H (J = Hz)	^{13}C
1	-	168.8
2	5.86 d (13.0)	120.1
3	6.65 d (13.0)	137.3
1a′	3.47 dd (14.0, 4.0)	45.4
1b′	3.35 m	
2′	4.19 dd (8.0, 4.0)	81.6
1″	-	129.9
2″	7.14 d (8.5)	127.7
3″	6.77 d (8.5)	114.9
4″	-	157.1
5″	6.77 d (8.5)	114.9
6″	7.14 d (8.5)	127.7
1‴	-	127.1
2‴	7.40 d (2.0)	112.6
3‴	-	147.1
4‴	-	147.1
5‴	6.79 d (8.5)	114.4
6‴	6.97 dd (8.0, 2.0)	123.4
OCH_3-2′	3.19 s	55.3
OCH_3-3‴	3.89 s	54.9

[a]NMR data were obtained at 700 MHz for ^{1}H and 175 MHz for ^{13}C.

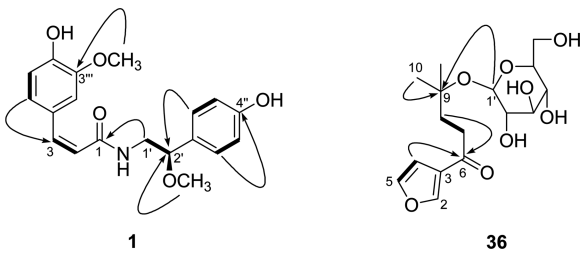

Figure 2. Key ^{1}H-^{1}H COSY(▬) and HMBC (→) correlations of **1** and **36**.

acid (**10**),[21] woodorien (**11**),[22] benzyl alcohol glucoside (**12**),[23] hydroxytyrosol (**13**),[20] 2-phenylethyl β-D-glucopyranoside (**14**),[24] caffeic acid (**15**),[20] methyl caffeate (**16**),[25] 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (**17**),[26] orebiusin A (**18**),[27] (2*R*)-prunasin (**19**),[23] luteolin (**20**),[15] *trans*-3,4,5-trimethoxycinnamyl alcohol (**21**),[28] indole-3-carboxylic acid (**22**),[29] myristicin (**23**), dillapiole (**24**),[30] nothoapiole (**25**),[31] allyltetramethoxybenzene (**26**),[32] elemicin (**27**),[33] 4-allyl-2,6-dimethoxyphenyl glucopyranoside (**28**),[34] perilladehyde (**29**),[35] (−)-loliolide (**30**),[25] humulene epoxide II (**31**),[36] 4α,β-epoxy-caryopyll-8(14)-one (**32**),[37] caryophyllenol I (**33**),[38] isoegomaketon (**34**),[39] and perillaketone (**35**)[40] by comparison of their spectroscopic data with those in the

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **36** in CD_3OD[a]

Position	36	
	^{1}H (J = Hz)	^{13}C
2	8.41 brs	150.3
3	-	129.0
4	6.80 brs	109.4
5	7.60 brs	145.9
6	-	198.7
7a	3.06 m	36.2
7b	2.99 m	
8	1.94 m	36.9
9	-	78.3
10	1.31 s	27.0
11	1.32 s	27.2
1′	4.87 d (8.0)	98.8
2′	3.16 dd (9.0, 8.0)	75.4
3′	3.38 m	78.2
4′	3.33 m	71.8
5′	3.32 m	77.8
6a′	3.82 dd (12.0, 2.0)	62.9
6b′	3.65 dd (12.0, 5.0)	

[a]NMR data were obtained at 700 MHz for ^{1}H and 175 MHz for ^{13}C.

literatures (Figure 1).

Experimental Section

Plant Material. The leaves of *P. frutescens* var. *acuta* (25 kg) were collected from Namwon in Jeollanam-do, Korea, in April 2012. The plants were authenticated by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL-1207) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The dried leaves of *P. frutescens* var. *acuta* (25 kg) were extracted with petroleum ether, and methanol, successively and evaporated under reduced pressure to give residues (264 g and 2 kg, respectively). The MeOH extracts (1 kg) were suspended in distilled water (2.4 L) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and hydrated *n*-BuOH, 190 g, 144 g, 60 g, and 95 g, respectively. The purification of 36 compounds (**1-36**) is described in Supplementary material.

Perillascens (1). Colorless gum. $[\alpha]_D^{25}$ + 1.4 (c = 0.30 MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.33), 284 (4.27), 314 (4.44) nm; IR (KBr) ν_{max} 3359. 2938, 2844, 1728, 1652, 1598, 1516, 1454, 1372, 1273, 1125, 1032, 872 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1. Positive-ion HR-FAB-MS m/z = 344.1497 $[M + H]^+$ (calcd. for $C_{19}H_{22}NO_5$: 344.1498).

Perillaside (36). Colorless gum. IR (KBr) ν_{max} 3307, 2916, 1991, 1671, 1561, 1509, 1371, 1155, 1074, 1035, 698 cm^{-1}; ^{1}H and ^{13}C NMR: see table 2. Positive-ion HR-FAB-MS m/z = 345.1548 $[M + H]^+$ (calcd. for $C_{16}H_{25}O_8$: 345.1549).

Acidic Hydrolysis and Sugar Identification. Compound **36** (1.0 mg) was heated at 60 °C and stirred with 1 mL of 7% HCL for 2 h. After cooling, the reaction mixture was neutralized with an Amberlite IRA400 column, and the eluate was extracted. The sugars residue obtained from the hydrolysis were dissolved in anhydrous pyridine (0.5 mL) and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between hexane and H_2O (1 mL each), and organic layer (1 μL) was analyzed by GC-MS. Identification of D-glucopyranoside for **36** was detected in each case by co-injecion of hydrolysate with standard silylated samples, giving single peaks at D-glucopyranose (10.12 min) for **36**. Retention times of authentic samples treated in the same way with 1-trimethylsilylimidazole in pyridine, were D-glucopyranose (10.14 min).

D-Glucopyranose was identified by co-TLC with standard samples and comparison of R_f values with the literature; D-glucopyranose ($CHCl_3$-MeOH-H_2O = 6:4:1. R_f value : 0.31).

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Supporting Information. Spectral data of compounds **1** and **36**, general experimental procedures, and the isolation details are available upon request from the corresponding author.

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