New Phenylpropanoids from *Sasa quelpaertensis* Nakai with Tyrosinase Inhibition Activities

Nasim Sultana[†] and Nam Ho Lee[*]

Department of Chemistry, Cheju National University, Ara-1, Jeju 690-756, Korea. [*]E-mail: namho@cheju.ac.kr
[†]Analytical Research Division, Bangladesh Council of Scientific & Industrial Research (BCSIR) Laboratories Dhaka, Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh
Received April 21, 2009, Accepted June 12, 2009

Bioactivity-guided fractionation led to the isolation of two new phenylpropanoids, 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (**1**) and 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-*O*-β-gulcopyranosylpropanol (**2**), together with three known compounds, *N*-*p*-coumaroylserotonin (**3**), *N*-feruloylserotonin (**4**) and *p*-coumaric acid (**5**) from the leaves of *Sasa quelpaertensis* Nakai. Their structures were elucidated by spectroscopic methods including 1D and 2D-NMR. Compared to arbutin (IC_{50} 0.048 mM) as a control, compounds **3** and **4** exhibited stronger tyrosinase inhibition activities with an IC_{50} values of 0.027 mM and 0.026 mM, respectively. Compounds **1** (IC_{50} 0.055 mM) and **2** (IC_{50} 0.053 mM) also showed strong activities.

Key Words: *Sasa quelpaertensis*, Isolation, Phenylpropanoids, Tyrosinase inhibition

Introduction

Jeju Island is the largest island located in the southernmost part of Korea, and has diversity of a plant community.[1] In continuation of our studies on plants in Jeju island,[2] we have now investigated the leaves of *Sasa quelpaertensis* Nakai. Among the plant varieties in the mountain area of Jeju Island, *S. quelpaertensis* is one of the most abundant plant species. *S. quelpaertensis* (Gramineae) is an edible bamboo grass, and its dried leaves have been used to make a leaf tea for the treatment of diabetes and gastritis. In terms of chemical constituents of this plant, only *p*-coumaric acid has been previously identified.[3]

Tyrosinase (EC 1.14.18.1) is the copper-containing oxygenase enzyme widely distributed in plants and animals. L-tyrosine is oxidized to 3,4-dihydroxyphenylalanine (DOPA) and subsequently to DOPA quinone by tyrosinase enzyme, which is the key step in the biosynthesis of melanin. Tyrosinase is responsible for melanization in animals as well as browning in plants.[4] Therefore, tyrosinase inhibitors have applications not only for skin melanin control in cosmetics but also in keeping fruits and vegetables fresh in food industry.[5,6]

From methanol extracts of this plant, strong tyrosinase inhibition activities were observed. Bioactivity-guided fractionation of *S. quelpaertensis* leaves have led to the isolation of two new phenylpropanoids, 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxy-phenyl)-1-propanone (**1**) and 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-*O*-β-D-gulcopyranosylpropanol (**2**) as well as three known compounds, *N*-*p*-coumaroylserotonin (**3**), *N*-feruloylserotonin (**4**) and *p*-coumaric acid (**5**). The subject of this paper is the isolation and identification of the compounds as well as evaluation of their tyrosinase inhibition activities.

Results and Discussion

The methanol extract (55.8 g) of the leaves of *S. quelpaertensis* was suspended in water and partitioned successively into *n*-hexane, ethyl acetate, and *n*-butanol. All fractions were tested for their tyrosinase inhibition activities. The ethyl acetate fraction showed significant dose dependent inhibitory effect. The fraction was purified by vacuum liquid chromatography, column chromatography over silica gel, Sephadex LH-20, and led to the isolation of two new coumaroyl compounds **1** and **2**, together with three known compounds, *N*-*p*-coumaroylserotonin (**3**),[7] *N*-feruloylserotonin (**4**)[8] and *p*-coumaric acid (**5**) (Figure 1).

Compound **1**, obtained as yellow solid, showed a $[M+Na]^-$ peak at *m/z* 395.1104 in the HRFAB mass spectrum corresponding to the molecular formula $C_{20}H_{20}O_7$. The IR spectrum displayed absorptions for hydroxyl (3320 cm^{-1}) and carbonyl (1658 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Table 1) of **1** showed signals for a 3-hydroxy-1-(4-hydroxy-3,5-dimethoxy-phenyl)-1-propanone nucleus.[9] Its ^1H NMR spectrum (Table 1) showed signals for two equivalent methoxy groups resonating at δ 3.90 (s, 6H), an aliphatic methylene at δ 3.39 (t, J = 6.4 Hz), an oxymethylene at δ 4.55 (t, J = 6.4 Hz), and equivalent two aromatic methines at δ 7.34 (s). A pair of

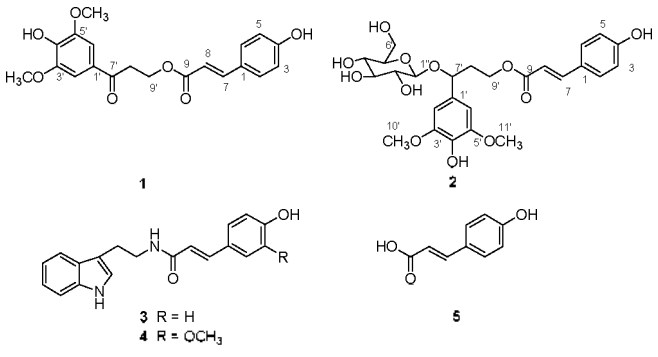

Figure 1. Structures of compounds **1-5** isolated from the leaves of *S. quelpaertensis*.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data (δ in ppm, J in Hz) of **1** (in CD_3OD)

Position	δ_H	δ_C	HMBC (H to C)
1		127.3	
2 & 6	7.41 (d, J = 8.5)	131.3	C-3, C-7, C-4
3 & 5	6.78 (d, J = 8.5)	116.9	C-1, C-4
4		161.5	
7	7.56 (d, J = 16.1)	146.8	C-2, C-9
8	6.28 (d, J = 16.1)	115.1	C-1, C-9
9		169.3	
1'		129.1	
2' & 6'	7.34 (s)	107.4	C-2', C-4', C-7', C-3'
3' & 5'		149.2	
4'		142.9	
7'		198.3	
8'	3.39 (t, J = 6.4)	38.2	C-9', C-7'
9'	4.55 (t, J = 6.4)	61.6	C-8', C-9, C-7'
3' & 5'-OMe	3.90 (s)	57.0	C-3'

ortho-coupled doublets at δ 7.41 and 6.78 (2H, J = 8.5 Hz) and two *trans* olefinic doublet protons at δ 7.56 and 6.28 (d, J = 16.1 Hz), indicated that compound **1** should consist of an additional *p*-substituted aromatic ring with a *trans*-side chain. The ^{13}C NMR spectrum (Table 1) of **1** showed the presence of 20 signals including nine carbons corresponding to *p*-coumaroyl moiety. The another 11 carbon signals were for two equivalent methoxy carbons (δ 57.0), two equivalent methines (δ 107.4), two methylenes (δ 38.2, 61.6), one carbonyl (δ 198.3), and four quaternary carbons (δ 149.2 for two equivalent carbons, 129.1 and 142.9). The degree of protonation of each atom was determined by DEPT experiments, while the HMQC spectrum allowed the complete correlation of the protonated carbon resonances with those of the ^{1}H spectrum. The ^{1}H-^{1}H COSY also confirmed the assignments of the protons.

The positions of the groups were confirmed by long-range heteronuclear correlation studies using HMBC. A singlet signal at δ 3.90, accounted for two methoxy groups, was placed at C-3' and C-5', respectively, as they showed ^{3}J correlation with oxygen bearing quaternary carbons at δ 149.2 in the HMBC spectrum (Table 1). The esterification site at C-9' was confirmed through HMBC correlation of oxymethylene proton (δ_H 4.55, H-9') with *p*-coumaric acid carbonyl carbon (δ_C 169.3, C-9). On the basis of these data, compound **1** was identified as a novel compound, 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone.

Compound **2** was obtained as a yellowish syrup. The HRFAB mass spectrum showed molecular ion at *m*/*z* 537.1928 $[M+H]^+$ consistent with the molecular formula $C_{26}H_{32}O_{12}$. It gave a fragment ion at *m*/*z* 357 due to the loss of a hexose sugar moiety. Compound **2** has similar structure as of **1**, except the disappearance of carbonyl signal and additional signals corresponding to an oxymethine and a sugar moiety (Table 2). The presence of a sugar was evident by the appearance of six oxygen-bearing sp^3 carbons at δ 62.2-104.3 in combination with proton signals at δ 5.20 and 3.93-4.49. As all the sugar at H-2", H-3" and H-3" showed axial-axial coupling constants,

Table 2. ^{1}H and ^{13}C NMR spectroscopic data (δ in ppm, J in Hz) of **2** (in C_5D_5N)

Position	δ_H	δ_C	HMBC (H to C)
1		126.6	
2 & 6	7.62 (d, J = 8.5)	131.2	C-7, C-4
3 & 5	7.16 (d, J = 8.5)	117.3	C-1, C-4
4		161.9	
7	7.98 (d, J = 16.1)	145.7	C-2, C-9, C-8
8	6.62 (d, J = 16.1)	115.7	C-1, C-9
9		167.9	
1'		133.3	
2'& 6'	7.10 (s)	105.7	C-2', C-4', C-7',C-3' & 5'
3'& 5'		149.5	
4'		137.5	
7'	5.46 (t, J = 6.3)	79.6	C-9', C-3', C-1', C-8', C-1"
8'	2.68 (ddd, J = 2.6, 6.8, 13.4)	37.4	C-9', C-1', C-7'
	2.53 (ddd, J = 2.6, 6.8, 13.4)		
9'	4.80 (dd, J = 6.3, 13.2)	63.2	C-7', C-9
	4.70 (dd, J = 5.9, 13.2)		
3'& 5'-OMe	3.77 (s)	56.8	C-3'
1"	5.20 (d, J = 7.8)	104.3	C-7'
2"	4.30 (t, J = 7.8)	76.3	C-4", C-3"
3"	4.19 (t, J = 7.8)	79.1	
4"	4.29 (t, J = 7.8)	72.1	
5"	3.93 (m)	79.2	
6"	4.49 (dd, J = 4.9, 12.2)	62.2	
	4.25 (dd, J = 7.8, 12.2)		

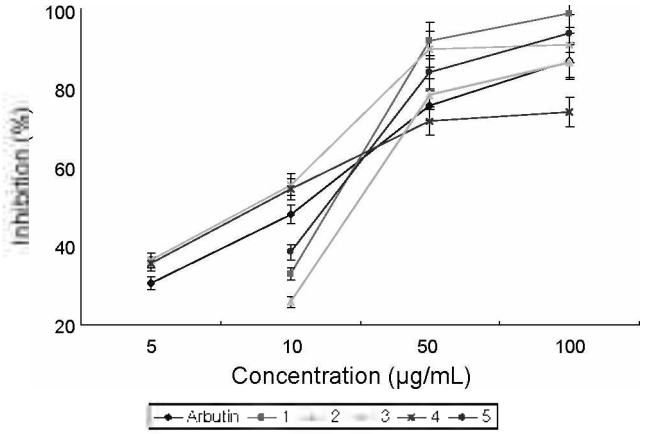

Figure 2. Tyrosinase inhibitory activities of compounds **1-5** isolated from *S. quelpaertensis*.

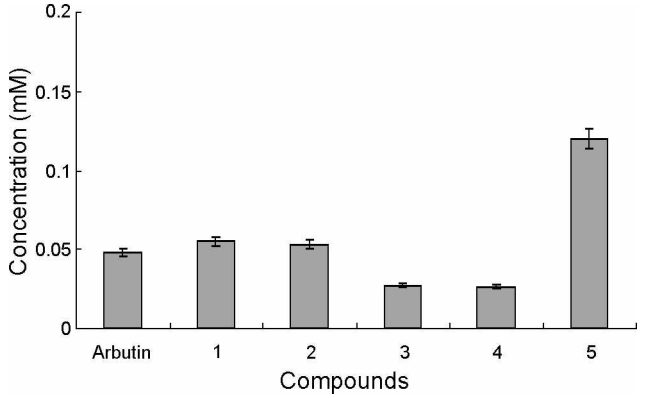

Figure 3. Relative representation of 50% inhibition activities (IC_{50}) for the compounds **1-5** and arbutin on mushroom tyrosinase.

the attached sugar was confirmed to be a glucose. The observed chemical shift values for C-1"-C6" also supported the glucose moiety.[10] The large coupling constant (J = 7.8 Hz) of the anomeric doublet at δ 5.20 (H-1") accounted for a β-glucopyranose moiety in **2**. The location of β-glucopyranose residue in **2** was confirmed by HMBC experiment (Table 2). The anomeric proton of the glucose (δ_H 5.20) showed a ^3J correlation with C-7' (δ_C 79.6) of the aglycone moiety and also oxymethine proton (δ_H 5.46) showed ^3J correlation with C-1" (δ_C 104.3) of the sugar moiety confirmed the sugar position at C-7'. The ^{1}H and ^{13}C chemical shift values were unambiguously assigned based on HMQC, HMBC and ^{1}H-^{1}H COSY studies. Thus, **2** was identified as a novel compound, 3-*O*-*p*-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-*O*-β-glucopyranosylpropanol.

The compounds, **1-5** were investigated for their anti-tyrosinase activities using L-tyrosine as the substrate. The test solutions for each compound were prepared at varying concentrations (5.0 to 100 μg/mL) and tested for their inhibitory effects on mushroom tyrosinase. The increase in absorbance at 480 nm due to the formation of dopachrome was monitored with a spectrophotometer using arbutin as a positive control. The results are summarized in Figure 2. Among the compounds tested, serotonin derivatives **3** (IC_{50} 0.027 mM) and **4** (IC_{50} 0.026 mM) showed stronger inhibition activity than arbutin (IC_{50} 0.048 mM). The new compounds **1** (IC_{50} 0.055 mM) and **2** (IC_{50} 0.053 mM) also exhibited strong inhibitory activities, whereas *p*-coumaric acid (**5**, 0.12 mM) showed relatively weak activity. Inhibition potency (IC_{50}) for compounds **1-5** as well as arbutin is compared in Figure 3. It is of interest to note that the isolated serotonin alkaloids, **3** and **4** have been described as strong melanogenesis inhibition components elsewhere.[11]

In conclusion, bio-assay guided isolation led to the isolation of two new phenylpropanoids, **1** and **2**, besides three known compounds, **3-5** from methanol extract of *S. quelpaertensis*. The isolated compounds showed strong inhibitory activities on mushroom tyrosinase. These compounds may prove to have considerable value as cosmetic and food ingredients in the future.

Experimental Section

General procedures. All solvents used for extraction and open column chromatography were of reagent grade and used without further purification. Melting points were determined on Fisher Scientific 2555 melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1030 automatic digital polarimeter. UV spectra were recorded on a Biochrom Libra S22 UV-Visible spectrophotometer. IR spectra were obtained using a Shimadzu FT-IR 8400S spectrometer. ^{1}H (400 MHz) and ^{13}C (100.60 MHz) NMR spectra were recorded on a JEOL, JNM-LA400 instrument with chemical shift data reported in ppm (δ) relative to the solvent used. 2D NMR spectra were recorded on the same instrument using field gradient FG2 (inverse) probe. FABMS were obtained with *m*-nitrobenzyl alcohol matrix on JEOL, JMS-700 spectrometer. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed on Merck silica gel 60H (15 μm) and silica gel (0.063 ~ 0.2 mm), respectively. Silica gel 60 F_{254} coated aluminium plates for thin layer chromatography (TLC) was supplied by Merck. Sephadex LH-20 (25 ~ 100 μm) for gel filtration chromatography (GFC) was obtained from Fluka. L-tyrosine was purchased from Sigma Chemical Co. Arbutin is donated by Bioland Ltd. Republic of Korea.

Plant material. The culms and leaves of *S. quelpaertensis* (Korean name, Jeju joritdae) were harvested from the Halla Mountain, Jeju Island in February 2007. A voucher specimen (J-010) has been deposited in the Laboratory of Natural Products, Department of Chemistry, Cheju National University.

Extraction and isolation. Air-dried and ground plant material (670.6 g) was extracted by stirring with magnetic stirrer in methanol at room temperature for three days. The extract was filtered and concentrated using a rotary evaporator at 40 °C. The resulting gummy mass (55.8 g) was suspended in water and partitioned successively with *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol.

Among all the fractions tested for tyrosinase inhibition activity, the EtOAc fraction showed significant activity and hence was subjected to further purification. The EtOAc fraction (4.89 g) was fractionated by VLC over silica gel eluting with *n*-hexane-EtOAc (0 ~ 100%) and then EtOAc-MeOH (0 ~ 50%). A total of 26 fractions (200 mL each) were collected, of

which 20 fractions showed inhibition activities. The fractions obtained from 55 ~ 60% EtOAc in *n*-hexane were combined (185.9 mg) and chromatographed over a sephadex LH-20 column using $CHCl_3$-MeOH to give compound **1** (8.7 mg). and a subfraction was further purified by CC using $CHCl_3$-MeOH (95:5 v/v) to give *p*-coumaric acid (**5**. 45.7 mg). The fractions from 80 ~ 100% EtOAc in *n*-hexane were combined (471.8 mg) and further treated by gel filtration followed by CC on silica gel using $CHCl_3$-MeOH (90:10 v/v) to yield more **5** (30 mg) and two indole alkaloids. *N*-*p*-coumaroylserotonin (**3**, 7.6 mg) and *N*-feruloylserotonin (**4**. 18.9 mg). The fraction eluted with 2 ~ 7% MeOH in EtOAc were combined (1.10 g) and subsequently subjected to GFC, eluted with $CHCl_3$-MeOH (95:5 v/v) and finally CC using $CHCl_3$ -MeOH (90:10 v/v) to yield compound **2** (55.6 mg). The fractions (250.8 mg) from 10% MeOH in EtOAc showed precipitation and was purified by cleaning with $CHCl_3$ to afford more **2** (162.8 mg).

***3-O-p*-Coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (1):** Yellowish syrup; UV (MeOH) λ_{max} 297 nm; IR ν_{max} 3323, 1658, 1643, 1448, 1115, 1022, 650 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HRFABMS *m/z* 395.1104 ($[M+Na]^+$, calcd. For $C_{20}H_{20}O_7Na$, 395.1107).

***3-O-p*-Coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-*O*-β-D-gulcopyranosyl propanol (2):** Yellow amorphous solid; Mp 180-182 °C; $[\alpha]_D^{25}$ - 30.75 (c 0.16, MeOH); UV (MeOH) λ_{max} 312 nm; IR ν_{max} 3320, 1643, 1448, 1415, 1115, 1022, 850 cm^{-1}; ^1H and ^{13}C NMR see Table 2; HRFABMS *m/z* 537.1927 ($[M+H]^+$, calcd. For $C_{26}H_{33}O_{12}$, 537.1972).

Tyrosinase inhibition assay. Tyrosinase activity was determined as previously described with some modifications.[12] The test reaction mixture was prepared by adding total 2.0 mL sample solution [with variable amount of 0.1 M phosphate buffer (pH 6.8) for different concentrations], to which 2100 unit/mL of mushroom tyrosinase had been added, to 667 μL of 0.1 M L-tyrosine. The test mixture (2.0 mL) was incubated for 10 min at 37 °C and the absorbance was measured at 480 nm. The same mixture except the plant extract was used as the control. The extent of inhibition by the addition of samples is expressed as the percentage necessary for 50% inhibition (ID_{50}). Arbutin was used as a positive control.

Acknowledgments. This research work was supported by grants from the Ministry of Knowledge and Economy. Nasim acknowledges the Korean Science and Engineering Foundation (KOSEF) for the fellowship.

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