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Constituents of the Leaves of *Peucedanum japonicum* Thunb. and Their Biological Activity

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In the course of our study on the isolation and structure determination of constituents in tropical plants, we focused on *Peucedanum japonicum* Thunb., belonging to the family Umbelliferae. In this study, a new C_{13} norisoprenoid glucoside, (3*S*)-*O*-*β*-D-glucopyranosyl-6-[3-oxo-(2*S*)-butenylidenyl]-1,1,5-trimethylcyclohexan-(5*R*)-ol (**1**), and two new phenylpropanoid glucosides, 3-(2-*O*-*β*-D-glucopyranosyl-4-hydroxyphenyl)propanoic acid (**3**) and methyl 3-(2-*O*-*β*-D-glucopyranosyl-4-hydroxyphenyl)-propanoate (**4**), were isolated from the *n*-butanol soluble fraction of this plant's leaves, together with five known compounds. The structures of these compounds were determined on the basis of spectroscopic evidence. In addition, all isolated compounds were examined for scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical and inhibitory activity against mushroom tyrosinase. These results suggested that 2-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**7**) and 3-*O*-*β*-D-glucopyranosyl-2-(4-hydroxy-3-methoxyphenyl)propanol (**8**) showed an appreciable activity in both assay systems.

KEYWORDS: ***Peucedanum japonicum*** **Thunb.; food preservation; DPPH radical scavenging activity; tyrosinase inhibitory activity**

INTRODUCTION

Peucedanum japonicum Thunb. (Umbelliferae), which is commonly known as Botanbofu in Japan, is widely distributed in Japan, the Philippines, China, and Taiwan. The leaves are frequently served as a vegetable or a garnish for raw fish in Okinawa. It has been reported that some coumarins isolated from *P. japonicum* were found to have antiplatelet (*1*−*3*), antiallergic (*4*), antagonistic, and spasmolytic (*5*) effects. We have been interested in the subtropical plant leaves, which are traditionally used for food preservation. Our earlier investigations reported that the polar fraction of *P. japonicum* leaves showed strong antioxidant activities (*6*). Furthermore, 17 compounds have been isolated from *P. japonicum* leaves. Rutin and caffeoylquinic acid isomers were found to be the major antioxidative constituents and play important roles in the strong antioxidant activity of *P. japonicum* leaves (*7*). Also, tyrosinase is responsible for the browning of some fruits and vegetables (*8*), and the investigation of the antioxidants and tyrosinase inhibitors from *P. japonicum* leaves may have important implications in food preservation.

In the course of research on the novel biologically active constituents from *P. japonicum* leaves, we now report the isolation and structure elucidation of compounds (**1**−**8**) from the *n*-butanol soluble fraction of *P. japonicum* leaves as well as their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and tyrosinase inhibitory activities.

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MATERIALS AND METHODS

General Procedures. ^{1}H (500 MHz), ^{13}C (125 MHz), and two-dimensional NMR spectra were recorded with a Varian Unity 500 instrument (Varian Inc., Palo Alto, CA). MS were measured on a Hitachi M-1200AP mass spectrometer (Hitachi Ltd., Tokyo, Japan) with an atmospheric pressure chemical ionization (APCI) interface, and high-resolution (HR)-MS analysis was carried out on a JMS 700T mass spectrometer (JEOL Ltd., Tokyo, Japan) with a fast atom bombardment (FAB) ionization interface. Optical rotations were measured using a Jasco P-1030 automatic digital polarimeter (Jasco Co., Tokyo, Japan). Analytical high-performance liquid chromatography (HPLC) was performed on a Jasco PU-1580 Intelligent Pump equipped with a Jasco MD 910 multiwavelength detector using a 250 mm × 4.6 mm i.d. Capcell Pak UG80 C_{18} column (Shiseido Ltd., Tokyo, Japan). Preparative HPLC was carried out on a Hitachi HPLC system (L-6250 pump; L-4200 detector) using a 250 mm × 20 mm i.d. Capcell Pak UG80 C_{18} column (Shiseido). The DPPH radical scavenging activity was measured using an Arvo 1420sx multilavel counter (PerkinElmer Life Sciences Inc., Boston, MA). Column chromatography (CC) was performed using 70−230 mesh silica gel 60 (Merck, Darmstadt, Germany) and 100−200 mesh Chromatorex ODS DM1020T (Fuji Sylisia Chemical Ltd., Tokyo, Japan). Thin-layer chromatography was run using silica gel 60 F_{254} and PR-18 F_{254S} (Merck).

Plant Material. Dried leaves of *P. japonicum* were kindly provided from the Yonaguni Commerce and Industry Association (Okinawa, Japan) in April 1998 and identified by Professor Shigetomo Yonemori (Tropical Biosphere Research Center, Iriomote Station, University of the Ryukyus). A voucher specimen has been deposited at the Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan.

Chemicals. DPPH, AAPH, α-tocopherol, and L-ascorbic acid were bought from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tyrosinase (EC 1.14.18.1; PPO) from mushroom was purchased from

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** in MeOH-d_4

	1		**2**	
position	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C
1		36.9		37.0
2ax	1.40 (1H, dd, 12.0, 12.8)	48.1	1.46 (1H, dd, 12.0, 12.1)	48.1
2eq	2.05 (1H, ddd, 2.0, 4.1, 12.8)		2.08 (1H, ddd, 2.1, 4.0, 12.1)	
3ax	4.34 (1H, dddd, 4.0, 4.1, 11.5. 12.0)	72.6	4.34 (1H, dddd, 3.9, 4.0, 11.3, 12.0)	72.5
4ax	1.39 (1H, dd, 11.5, 13.0)	46.8	1.45 (1H, dd, 11.3, 13.1)	46.6
4eq	2.32 (1H, ddd, 2.0, 4.0, 13.0)		2.36 (1H, ddd, 2.1, 3.9, 13.1)	
5		72.5		72.4
6		120.2		120.0
7		211.1		211.5
8	5.90 (1H, s)	101.4	5.82 (1H, s)	101.2
9		202.2		200.9
10	2.27 (3H, s)	27.1	2.18 (3H, s)	26.5
11	1.12 (3H, s)	32.5	1.15 (3H, s)	32.2
12	1.44 (3H, s)	29.3	1.38 (3H, s)	29.4
13	1.37 (3H, s)	30.8	1.39 (3H, s)	30.8
Glc-1	4.42 (1H, d, 7.8)	102.7	4.43 (1H, d, 7.8)	102.6
Glc-2	3.13 (1H, dd, 7.8, 9.0)	75.1	3.14 (1H, dd, 7.8, 9.0)	75.1
Glc-3	3.35 (1H, dd, 9.0, 9.0)	78.0	3.36 (1H, dd, 9.0, 9.0)	78.1
Glc-4	3.30 (overlapped)	71.6	3.29 (overlapped)	71.6
Glc-5	3.30 (overlapped)	77.9	3.30 (overlapped)	77.9
Glc-6a	3.87 (1H, dd, 2.0, 12.0)	62.7	3.86 (1H, dd, 1.6, 11.9)	62.7
Glc-6b	3.68 (1H, dd, 5.1, 12.0)		3.68 (1H, dd, 5.1, 11.9)	

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **3** and **4**

	3[a]		**4**[b]	
position	δ ^{1}H (*J* in Hz)	δ ^{13}C	δ ^{1}H (*J* in Hz)	δ ^{13}C
1		185.9		176.1
2	2.41 (2H, m)	41.0	2.59 (2H, m)	35.7
3	2.83 (2H, m)	28.8	2.86 (2H, m)	26.4
1′		125.6		121.9
2′		158.0		157.7
3′	6.69 (1H, d, 2.4)	105.4	6.63 (1H, d, 2.2)	104.0
4′		157.7		158.2
5′	6.57 (1H, dd, 8.3, 2.4)	112.3	6.36 (1H, dd, 8.3, 2.2)	110.0
6′	7.12 (1H, d, 8.3)	133.6	6.92 (1H, d, 8.3)	131.4
OMe			3.62 (3H, s)	52.0
Glc-1	5.05 (1H, d, 7.3)	103.0	4.84 (1H, d, 7.8)	102.6
Glc-2	3.62 (1H, dd, 9.3, 7.3)	75.6	3.46 (1H, dd, 9.3, 7.8)	74.3
Glc-3	3.61 (1H, dd, 9.3, 9.3)	78.3	3.43 (1H, dd, 9.4, 9.3)	78.2
Glc-4	3.50 (1H, dd, 9.8, 9.3)	72.2	3.37 (1H, dd, 9.4, 9.2)	71.3
Glc-5	3.65 (1H, m)	78.8	3.40 (1H, m)	78.1
Glc-6a	3.94 (1H, dd, 12.5, 2.2)	63.4	3.90 (1H, dd, 12.1, 1.6)	62.5
Glc-6b	3.75 (1H, dd, 12.5, 5.9)		3.71 (1H, dd, 12.1, 5.0)	

[a] D_2O. [b] MeOH-d_4.

Sigma Chemical Co. (St. Louis, MO). Kojic acid was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). All solvents and other chemicals used were of the highest analytical grade.

Extraction, Isolation, and Determination of the Constituents of *P. japonicum* Leaves. As a preliminary step, the dried powdered leaves of *P. japonicum* (2650 g) were extracted exhaustively with *n*-hexane (6 × 1.3 L), dichloromethane (6 × 1.3 L), and 70% aqueous acetone (6 × 1.3 L) successively at room temperature. The organic solvent in the 70% aqueous acetone extract was evaporated in vacuo to give the aqueous part, which was partitioned into the ethyl acetate soluble, *n*-butanol soluble, and water soluble fractions. These extracts were concentrated in vacuo to give the *n*-hexane (74.8 g), dichloromethane extracts (70.6 g), ethyl acetate soluble (41.8 g), *n*-butanol soluble (79.0 g), and water soluble fractions (428.2 g). The *n*-butanol soluble fraction (37.3 g) was separated by silica gel CC using EtOAc/MeOH/H_2O (from 10:2:1 to 3:2:1) stepwise to give seven fractions (fractions 1−7). Fraction 3 (8.8 g) was further purified by ODS CC with H_2O/MeOH (2:1) to furnish seven fractions (fractions 3-1 to 3-7). Fraction 3-2 was further subjected to the ODS column and preparative HPLC using H_2O/MeOH (3:1) to afford compounds **1** (30 mg), **2** (2 mg), **3** (3 mg), **4** (8 mg), **5** (1 mg), **6** (2 mg), **7** (1 mg), and **8** (1 mg), respectively.

(3*S*)-*O*-*β*-D-Glucopyranosyl-6-[3-oxo-(2*S*)-butenylidenyl]-1,1,5-trimethylcyclohexan-(5*R*)-ol (1). Colorless powder; $[\alpha]^{20}_D$ −45.5° (*c* 0.1, MeOH). UV (MeOH) λ_{max} (log ϵ): 232 (4.19) nm. IR (Nujol): ν_{max} 3382, 1941, 1667, 1308, 1079 cm^{-1}. ^{1}H and ^{13}C NMR, see **Table 1**. APCI-MS (negative): *m*/*z* 385 [M − H]$^-$. HR-FAB-MS: *m*/*z* 385.1032 [M − H]$^-$ [calcd for $C_{19}H_{29}O_8$, 385.1029].

(3*S*)-*O*-*β*-D-Glucopyranosyl-6-[3-oxo-(2*R*)-butenylidenyl]-1,1,5-trimethylcyclohexan-(5*R*)-ol (Icariside B$_1$) (2) (*9*). Colorless powder; $[\alpha]^{21}_D$ −71.8° (*c* 0.1, MeOH). ^{1}H and ^{13}C NMR, see **Table 1**. APCI-MS (negative): *m*/*z* 385 [M − H]$^-$.

3-(2-*O*-*β*-D-Glucopyranosyl-4-hydroxyphenyl)propanoic Acid (3). Colorless powder; $[\alpha]^{21}_D$ −22.3° (*c* 0.1, EtOH). UV (EtOH) λ_{max} (log ϵ): 227 (2.94), 280 (2.62) nm. IR (Nujol): ν_{max} 3400−3200, 1708, 1599 cm^{-1}. ^{1}H and ^{13}C NMR, see **Table 2**. APCI-MS (negative): *m*/*z* 343 [M − H]$^-$. HR-FAB-MS: *m*/*z* 343.1032 [M − H]$^-$ [calcd for $C_{15}H_{19}O_9$, 343.1029].

Methyl 3-(2-*O*-*β*-D-Glucopyranosyl-4-hydroxyphenyl)propanoate (4). Colorless powder; $[\alpha]^{25}_D$ −44.2° (*c* 0.1, EtOH). UV (EtOH) λ_{max} (log ϵ): 212 (3.25), 292 (2.77) nm. IR (Nujol): ν_{max} 3400−3200, 1723, 1600, 1512 cm^{-1}. ^{1}H and ^{13}C NMR, see **Table 2**. APCI-MS (nega-

tive): *m/z* 357 $[M - H]^-$. HR-FAB-MS: *m/z* 357.1179 $[M - H]^-$ [calcd for $C_{16}H_{21}O_9$, 357.1185].

1-(4-Hydroxyphenyl)ethane-1,2-diol (5). Colorless powder. ^{1}H NMR (MeOH-d_4, 500 MHz): δ 7.17 (2H, d, J = 8.3 Hz, H-2′, 6′), 6.74 (2H, d, J = 8.3 Hz, H-3′, 5′), 4.58 (1H, dd, J = 6.1, 6.1 Hz, H-1), 3.56 (2H, brd, J = 6.1 Hz, H-2). ^{13}C NMR (MeOH-d_4, 125 MHz): δ 158.0 (C-4′), 134.0 (C-1′), 128.7 (C-2′, 6′), 116.0 (C-3′, 5′), 75.7 (C-1), 68.7 (C-2). APCI-MS (positive): *m/z* 155 $[M + H]^+$.

β-D-Glucopyranosyloxy-2-(4-hydroxyphenyl)ethane (Salidroside) (6) (*10*). Colorless powder. APCI-MS (positive): *m/z* 301 $[M + H]^+$.

2-(4-Hydroxy-3-methoxyphenyl)propane-1,3-diol (7) (*11*). Colorless powder. APCI-MS (positive): *m/z* 199 $[M + H]^+$.

3-*O*-β-D-Glucopyranosyl-2-(4-hydroxy-3-methoxyphenyl)propanol (8) (*11*). Colorless powder. APCI-MS (positive): *m/z* 361 $[M + H]^+$.

Photoepimerization of Compounds 1 and 2. According to the method of Isoe et al. (*12*) with some modifications, the ethanol solution of compound **1** or **2** (each 100 μg/mL) was irradiated with a UV lamp through a glass vial in a dark box for 6 h. Aliquots (2 μL) of each irradiated solution were analyzed by HPLC using a 250 mm × 4.6 mm i.d. Mightysil RP-18 GP column (Kanto Kagaku). The mobile phase was H_2O/MeOH (2:1). The flow rate was 0.5 mL/min at room temperature. The detection was carried out by photodiode array (PDA) (200−400 nm). Retention times were 15.1 min for **2** and 20.5 min for **1**.

Analytical HPLC. Compound **4** was prepared at 10 μg/mL in ethanol. The *n*-butanol soluble fraction of *P. japonicum* was dissolved in ethanol (1 mg/mL). Each solution was filtered through a cellulose acetate membrane filter (0.45 μm) (Advantec, Japan), and aliquots of the filtrate (2 μL) were injected to HPLC. HPLC analysis was carried out according to the previous report (*13*) with slight modifications to the mobile phase: A, 50 mM $NH_4H_2PO_4$ (pH 2.6); B, 80% acetonitrile and 20% A; and C, 50 mM phosphoric acid (pH 2.0). Elution was performed with the linear gradient as follows: 0.0 min, 100/0/0 (A/B/C, %/%/%); 4.0 min, 92/8/0; 10.0 min, 0/14/86; 22.5 min, 2/16/82; 27.5 min, 0/22/78; 45.0 min, 0/50/50; 47.5 min, 100/0/0; 75.0 min, 100/0/0. The flow rate was 1.0 mL/min. The column temperature was set at 40 °C. The detection was carried out by PDA (200−600 nm). All of the above experiments were replicated three times each.

Evaluation of DPPH Radical Scavenging Activity. The DPPH radical scavenging activity was performed as previously described (*14*). Two hundred millimolar DPPH in ethanol (75 μL) was added to the ethanolic sample solution (75 μL) in a 96 well flat microtiter plate. The absorbance at 520 nm was determined after 30 min of incubation at room temperature in a multilabel counter. The scavenging activity was calculated by comparing the absorbance with that of a blank containing only DPPH and solvent. The activity of the isolated compounds and positive references (α-tocopherol and L-ascorbic acid) was evaluated until the reaction reached the steady state at room temperature. Measurements were performed in triplicate.

Evaluation of Tyrosinase Inhibitory Activity. The assay was performed according to the procedure of Mason et al. (*15*) with slight modifications. Seventy microliters of mushroom tyrosinase solution (135 units/mL) in 0.07 M phosphate buffer (pH 6.8) and 70 μL of test substance in 10% DMSO solution were added to a 96 well microplate and preincubated at 25 °C for 10 min. Then, the reaction was carried out by adding 70 μL of 0.5 mM l-3-(3,4-dihydroxyphenyl)alanine (L-dopa) (Wako Pure Chemical Industries, Ltd.) as the substrate in phosphate buffer (pH 6.8). The reaction mixture was incubated at 37 °C for 5 min. The amount of dopachrome produced in the reaction mixture was measured at 475 nm in a microplate reader. The kinetic analysis of the tyrosinase inhibitory activity was obtained by the method previously described (*16*, *17*). Kojic acid was used as the positive reference. All analyses were run in triplicate. The percentage of inhibition of tyrosinase activity was calculated as follows:

$$\% \text{ inhibition} = \{[(A - B) - (C - D)]/(A - B)\} \times 100$$

where A is the absorbance at 475 nm with tyrosinase but without test substance, B is the absorbance at 475 nm without test substance and tyrosinase, C is the absorbance at 475 nm with test substance and tyrosinase, and D is the absorbance at 475 nm with test substance but without tyrosinase.

Statistical Analysis. Multiple comparisons were by one way analysis of variance, followed by Scheffe test using StatView (SAS Institute Inc., Cary, NC). Significance was established at $p < 0.01$.

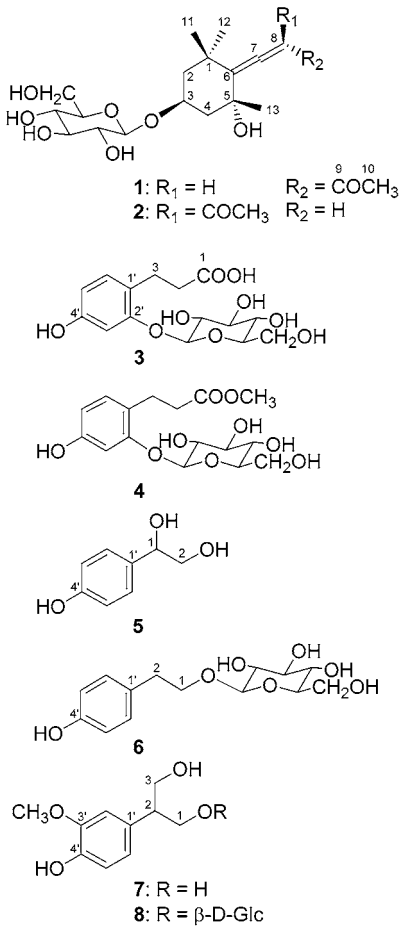

Figure 1. Structure of chemical components isolated from the *n*-butanol soluble fraction of *P. japonicum* leaves.

RESULTS AND DISCUSSION

The *n*-butanol soluble fraction of the leaves of *P. japonicum* was successively chromatographed on silica gel, ODS, and preparative HPLC to afford compounds **1**−**8** (**Figure 1**).

Compound **1** was obtained as a colorless powder, $[\alpha]^{20}_D$ −45.5° (*c* 0.1, MeOH). Its molecular formula was assigned as $C_{19}H_{30}O_8$ based on its HR-FAB-MS data. The UV spectrum showed an absorption maximum at 232 nm. The IR spectrum indicated the presence of a hydroxyl (3382 cm^{-1}), an allenic (1941 cm^{-1}), and a conjugated carbonyl (1667 cm^{-1}) group. The ^{13}C NMR spectrum (**Table 1**) showed the presence of three methyls [δ_C 29.3, 30.8, and 32.5], two methylenes [δ_C 46.8 and 48.1], an oxygenated methine [δ_C 72.6], a methyl ketone group [δ_C 27.1 and 202.2], a quaternary carbon [δ_C 36.9], and an oxygenated quaternary carbon [δ_C 72.5], which were determined by combination of the results of ^{1}H NMR (**Table 1**) and heteronuclear multiple quantum coherence (HMQC) measurements. In the heteronuclear multiple bond coherence (HMBC) spectrum (**Figure 2**), an olefinic proton signal at δ_H 5.90 (1H, s, H-8) correlating with signals at δ_C 120.2 and 211.1, characteristically indicated the presence of an allenic group in the molecule. The signals of six carbons (δ_C 62.7, 71.6, 75.1, 77.9, 78.0, and 102.7) in the ^{13}C NMR spectrum and complex proton signals in the region of δ_H 3.30−3.87 and a doublet signal

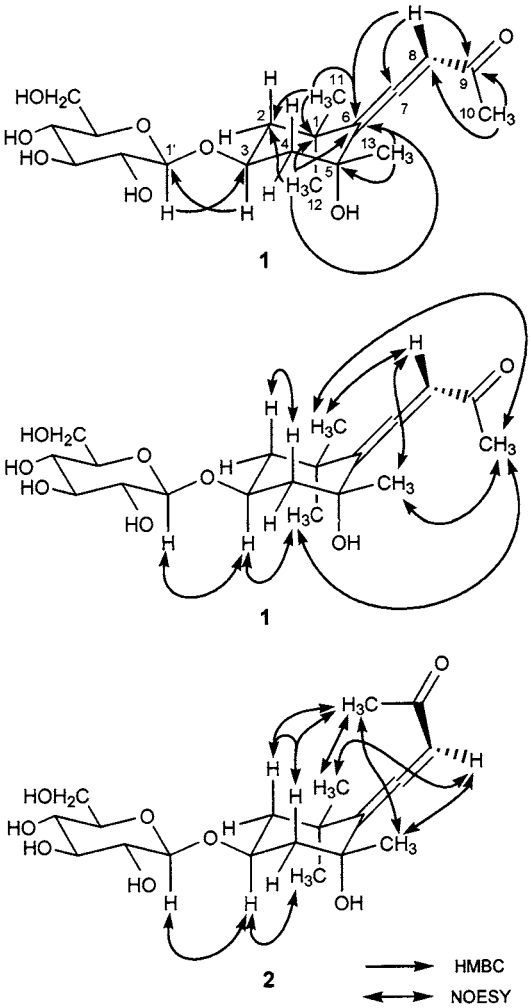

Figure 2. Pertinent correlations in the HMBC and NOESY spectra of compounds **1** and **2**.

at δ_H 4.42 with a large coupling constant (J = 7.8 Hz) in the ^{1}H NMR spectrum suggested the presence of a β-glucopyranosyl residue in the molecule. In the HMBC spectrum, there was a correlation between the anomeric proton of the β-glucopyranosyl moiety and the C-3, indicating that C-1 of the β-glucopyranosyl moiety was linked to the hydroxy group of C-3. These spectroscopic data indicated that **1** was similar to **2**, icariside B_1, whose absolute structure was already confirmed (*9*, *18*). However, in the ^{1}H NMR spectrum of **1**, the olefinic proton signal at δ_H 5.90 (1H, s, H-8) was shifted downfield by 0.08 ppm and the acetyl methyl signal at δ_H 2.27 (3H, s, H-10) was shifted downfield by 0.09 ppm in comparison with that of **2**. From the analysis of nuclear Overhauser effect correlated spectroscopy (NOESY) spectrum of **1** (**Figure 2**), the signal at δ_H 4.34 (1H, dddd, 4.0, 4.1, 11.5, 12.0 Hz, H-3ax) exhibited an NOE correlation of the signal at δ_H 1.44 (3H, s, H-12), which suggested the configuration of the 12-methyl group to be axial, and those of the 11- and 13-methyl groups to be equatorial. These correlations were also observed among the signals of **2**. Furthermore, the NOESY correlation was observed for the signals between H-10 and H-12 in the structure of **1**, while in compound **2** the cross-peaks were observed for the signals between H-2ax/H-10 and H-4ax/H-10 instead of H-10/H-12 in **1**. These data suggested that **1** was an isomer of **2** at the C-8 position. To confirm the absolute structure of **1**, we investigated the photoisomerization of the allenic double bond (*12*). Ethanol solutions of compounds **1** and **2** were separately irradiated with

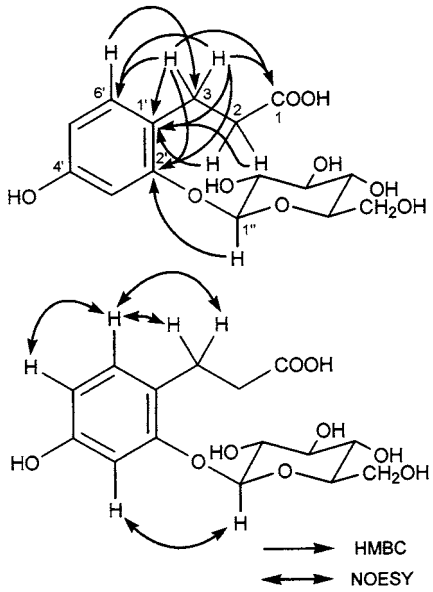

Figure 3. Pertinent correlations in the HMBC and NOESY spectra of compound **3**.

UV light through a glass vial in a dark case, and aliquots of each irradiated solution were analyzed immediately by HPLC. After 6 h of reaction, two peaks corresponding to **1** (R_t 20.5 min) and **2** (R_t 15.1 min) in a 1:1 ratio were observed, which indicated photoequilibrium between **1** and **2**. On the basis of these spectroscopic data, the stereochemistry of **1** at the C-8 position was assigned as the *S* configuration. Thus, the structure of **1** was elucidated to be (3*S*)-*O*-β-D-glucopyranosyl-6-[3-oxo-(2*S*)-butenylidenyl]-1,1,5-trimethylcyclohexan-(5*R*)-ol.

Compound **3**, obtained as a white powder, displayed the molecular formula $C_{15}H_{20}O_9$ from its HR-FAB-MS. In the ^{1}H NMR spectrum (**Table 2**), the signals corresponding to a 1,2,4-trisubstituted phenyl group were observed at δ_H 6.57 (1H, dd, J = 8.3, 2.4 Hz, H-5′), 6.69 (1H, d, J = 2.4 Hz, H-3′), and 7.12 (1H, d, J = 8.3 Hz, H-6′). The ^{13}C NMR (**Table 2**) and HMQC spectra also showed the signals for three unsubstituted aromatic carbons [δ_C 105.4 (C-3′), 112.3 (C-5′), and 133.6 (C-6′)] and three substituted aromatic carbons [δ_C 125.6 (C-1′), 157.7 (C-4′), and 158.0 (C-2′)]. In addition, two methylene carbons [δ_C 28.8 (C-3) and 41.0 (C-2)] and a carboxyl carbon [δ_C 185.9 (C-1)], together with two methylene proton signals at δ_H 2.41 (2H, m, H-2) and 2.83 (2H, m, H-3), demonstrated the presence of a carboxyethyl moiety. In the HMBC spectrum (**Figure 3**), the correlations were noted for H-2/C-1′; H-3/C-1, C-1′, C-2′, and C-6′; and H-6′/C-3, respectively. These data proved that C-3 of the carboxyethyl moiety was linked to C-1′ of the aromatic ring. The ^{13}C NMR spectrum further revealed the signals of six glucopyranosyl carbons (δ_C 63.4, 72.2, 75.6, 78.3, 78.8, and 103.0). In the ^{1}H NMR spectrum, the coupling constant (J = 7.3 Hz) of the anomeric proton signal at δ 5.05 (1H, d) indicated the β-glucopyranosyl moiety. The attachment of β-glucopyranosyl moiety to the oxygen at C-2′ resulted from the HMBC and NOESY correlations (**Figure 3**). Because all proton and carbon signals have been assigned, compound **3** was determined to be 3-(2-*O*-β-D-glucopyranosyl-4-hydroxyphenyl)-propanoic acid.

Compound **4** was assigned the molecular formula $C_{16}H_{22}O_9$ on the HR-FAB-MS measurement. The ^{1}H and ^{13}C NMR data of **4** were very similar to those of **3** except for the presence of the proton signal at δ_H 3.62 (3H, s) and the carbon signal at δ_C 52.0 due to a methoxyl group in **4** (**Table 2**). The location of

Table 3. DPPH Radical Scavenging Activity at the Steady State of Reaction and Time Needed To Reach the Steady State of the Isolated Compounds from *P. japonicum* Leaves, α-Tocopherol, and L-Ascorbic Acid at 100 μM

compound	DPPH radical scavenging activity[a,b] (% ± SD)	time (h)
1	3.0 ± 0.9	1.0
2	2.2 ± 0.4	1.0
3	1.1 ± 0.7	1.0
4	8.0 ± 0.8	1.0
5	5.2 ± 1.2	3.0
6	20.4 ± 0.9	3.0
7	77.1 ± 3.2	24.0
8	84.2 ± 3.7	24.0
α-tocopherol	93.7 ± 1.3	0.5
L-ascorbic acid	91.7 ± 0.6	0.3

[a] The final concentration of DPPH ethanol solution was 100 μM. [b] DPPH radical scavenging activity (%) was the mean of three replications.

Table 4. IC_{50} Values of Tyrosinase Inhibitory Activity of the Isolated Compounds from *P. japonicum* Leaves and Kojic Acid

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	>100	**6**	>100
2	>100	**7**	80.9 ± 2.5
3	78.2 ± 1.8	**8**	78.0 ± 2.8
4	75.8 ± 0.7	kojic acid	47.6 ± 1.4
5	83.1 ± 1.6		

the methoxyl group was considered to be at C-1 from both the HMBC and the NOESY correlations. Thus, compound **4** was elucidated to be methyl 3-(2-*O*-β-D-glucopyranosyl-4-hydroxyphenyl)propanoate.

In this study, to confirm whether compound **4** was a naturally occurring product or an artifact, identification of **4** in the *n*-butanol soluble fraction of *P. japonicum* leaves was carried out by HPLC analysis. In the HPLC chromatogram, the peak that appeared at 25.7 min was identical to the retention time of **4** and the UV absorption pattern was identical to the purified standard. Consequently, compound **4** was proved to be a naturally occurring product.

Compounds **5**−**8** were determined as 1-(4-hydroxyphenyl)-ethane-1,2-diol (**5**), β-D-glucopyranosyloxy-2-(4-hydroxyphenyl)ethane (salidroside) (**6**) (*10*), 2-(4-hydroxy-3-methoxyphenyl)-propane-1,3-diol (**7**) (*11*), and 3-*O*-β-D-glucopyranosyl-2-(4-hydroxy-3-methoxyphenyl)propanol (**8**) (*11*), respectively. All compounds were isolated for the first time from *P. japonicum*.

The antioxidant properties of the isolated compounds were estimated by the DPPH radical scavenging activity (*14*). Recent studies indicated that the analysis of kinetics of free radical scavenging of test compounds is important for a better understanding of their hydrogen-donating capacity (*19*). We evaluated the DPPH radical scavenging activity including kinetic behaviors of the compounds isolated from *P. japonicum* together with positive references, L-ascorbic acid and α-tocopherol. The DPPH radical scavenging activity at the steady state of reaction and time needed to reach the steady state of test compounds at 100 μM are shown in **Table 3**. As a result, L-ascorbic acid and α-tocopherol reacted rapidly with DPPH radical. Compounds **1**−**6** showed little radical scavenging activity, but **7** and **8** showed a recognizable activity at 24 h of the reaction. It is suggested that **7** and **8** are slow-reacting scavengers and that the 4-hydroxyl-3-methoxyphenyl moiety in the molecule contributes to the radical scavenging activity.

Compounds **1**−**8** were subjected to the evaluation of the inhibitory activity against mushroom tyrosinase. It is known that tyrosinase has two separate binding sites in its active center (*17*). Generally, 4-substituted resorcinols have been reported as potent tyrosinase inhibitors of the competitive inhibitory type (*20*). The resorcinol moiety may bind to the binuclear active site of the enzyme, and its side chain may be associated with the hydrophobic protein pocket close to the active site (*21*). 3-(2,4-Dihydroxyphenyl)propanoic acid, possessing a 4-subsutituted resorcinol skeleton, showed stronger inhibitory activity than that of kojic acid, which is a potent tyrosinase inhibitor, as previously reported (*22*). Compound **3** (2′-*O*-β-D-glucoside of dihydroxyphenylpropanoic acid) also showed the tyrosinase inhibitory activity, but its activity was lower than that of kojic acid (**Table 4**). The inhibition kinetics was analyzed by a Lineweaver−Burk plot, which indicated that **3** and **4** behaved as competitive type inhibitors of L-dopa oxidation. Less inhibitory activity of **3** might be due to a steric hindrance by the glucosyl moiety at C-2′. On the other hand, **4** showed stronger inhibitory activity than **3**, which suggested that the side chain of **4** (methyl ester group) has a higher affinity for the hydrophobic protein pocket of the enzyme than that of **3** (carboxylic acid group). Therefore, hydrophobic and less bulky substituents are important for controlling the tyrosinase inhibitory activity in 4-subsutituted resorcinols as previously mentioned (*20*).

Compounds **7** and **8** also showed tyrosinase inhibitory activity, which was lower than that of kojic acid. 4-Hydroxy-3-methoxycinnamic acid was reported as a noncompetitive tyrosinase inhibitor (*23*). Thus, the tyrosinase inhibitory activity of **7** and **8** may be caused by the 4-hydroxy-3-methoxyphenyl moiety.

In conclusion, we isolated a new C_{13} norisoprenoid glucoside, (3*S*)-*O*-β-D-glucopyranosyl-6-[3-oxo-(2*S*)-butenylidenyl]-1,1,5-trimethylcyclohexan-(5*R*)-ol (**1**), and two new phenylpropanoid glucosides, 3-(2-*O*-β-D-glucopyranosyl-4-hydroxyphenyl)propanoic acid (**3**) and methyl 3-(2-*O*-β-D-glucopyranosyl-4-hydroxyphenyl)propanoate (**4**), together with five known compounds (**5**−**8**) from the *n*-butanol soluble fraction of the leaves of *P. japonicum*. Compounds **7** and **8** exhibited an appreciable activity in a DPPH radical scavenging activity and an inhibitory activity on tyrosinase.

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