α-Glucosidic hydroquinone derivatives from *Viburnum erosum*

Jinyoung Park,[†] Jiho Lee,[†] Hyeon S. Jang,[†] Birang Jeong,[†] Seong Y. Choi,[†] Juyeol Kim,[†] Yong S. Kwon,[†] Heejung Yang[†,‡,*]

[†]College of Pharmacy, Kangwon National University, Chuncheon 24341, Republic of Korea

[‡]Bionsight, Inc., Chuncheon 24341, Republic of Korea

ABSTRACT

Six new compounds (**1**−**6**) were isolated from the leaves of *Viburnum erosum* along with four known compounds **7**−**10**. The structures were determined by NMR and MS spectroscopic analyses, and their absolute configurations were established by chemical and spectroscopic methods. Compounds **1–6** were α-glucosidic hydroquinone derivatives with different linear monoterpenoid structures. Compounds **1**−**10** were also evaluated for their tyrosinase inhibitory activities, and **10** showed potent inhibition of tyrosinase enzyme with IC_{50} of 37.9 μM compared to 47.6 μM of the positive control (β-arbutin).

Keywords: *Viburnum erosum*, tyrosinase inhibition, β-arbutin

INTRODUCTION

The plants of genus *Viburnum* (Caprifoliaceae) are widely distributed worldwide and contain terpenes, iridoids, flavonoids, lignans, phenols, coumarins, lactones, and alkaloids.[1] Several species exhibit a wide range of biological activities including antitumor, antioxidant, antimicrobial, antihyperglycemic, and hepatoprotective activities.[1] Among the *Viburnum* species, *V. erosum* is a deciduous tree native to the East Asian countries including Korea, China, and Japan. Neuroprotective and anti-inflammatory lignans from the stems of *V. erosum* have been reported, but their constituents have not been extensively investigated.[2]

Tyrosinase plays a key role in the browning of fruits, fungi, and vegetables as well as melanogenesis in human skin.[3] Tyrosinase is involved in melanin production, which causes skin pigmentation and protects it from UV-induced damage. Tyrosinase inhibitors can be developed as skin-whitening agents in the cosmetic industry, and are considered as the most prominent targets for inhibiting hyperpigmentation in skin cancer.[4] Many plant-based natural products are used for the development of tyrosinase inhibitors for cosmetic products as medical agents, but only a few compounds are known to show inhibitory activities against tyrosinase.[3]

Phenolic compounds, hydroquinones, resveratrol, and tannins act as substrates for the production of reactive quinones.[5] *β*-arbutin ((2*R*,3*S*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-(4-hydroxyphenoxy)oxane-3,4,5-triol)), a glycosylated hydroquinone, is found in many species of the Ericaceae and Caprifoliaceae families, and is a natural product with potent anti-tyrosinase activity.[5,6] In the present study, potent tyrosinase inhibitory constituents from the leaves of *V. erosum* are identified.

RESULTS AND DISCUSSION

In the pursuit of bioactive compounds with tyrosinase inhibitory activities, compounds **1**–**10** were isolated from the EtOAc fraction of *V. erosum*. The EtOAc sub-fractions, VEE1–8, showed different bioactive profiles (Figure 1).

VEE1 and VEE2 showed potent tyrosinase inhibitory activities with weak cytotoxic activities against the B16F10 and HEp-2 cells, but fractions VEE4–7 showed contrasting results. Therefore, VEE2 fraction with sufficient volume (compared to VEE1) was subjected to further separation to isolate tyrosinase inhibitory compounds with weak cytotoxic activities. Consequently, seven *α*-glucosidic hydroquinones with linear monoterpenoids (**1**–**7**), one monoterpenoid ((*R*)-menthiafolic acid; **8**), one biflavonoid (amentoflavone; **9**), and one lignan ((-)-epipinoresinol; **10**) were isolated.

Compound **1** was obtained as a white powder and had the molecular formula, $C_{22}H_{28}O_8$, based on HRESIMS analysis (*m/z* 419.1690 $[M-H]^-$ calcd for 419.1706). The 1D and HSQC NMR data of **1** exhibited characteristic signals at δ_H/δ_C 4.71 (1H, d, *J* = 7.4 Hz, H-1)/103.6 (C-1), 4.51 (1H, dd, *J* = 2.1 and 11.8 Hz, H-6)/65.1 (C-6), 4.24 (1H, dd, *J* = 7.2 and 11.8 Hz, H-6)/65.1 (C-6), 3.62 (1H, ddd, *J* = 2.1, 7.2 and 9.5 Hz, H-5)/75.4 (C-5), 3.43 (H, m, H-3)/77.9 (C-3), 3.43 (H, m, H-2)/74.9 (C-2), and 3.36 (1H, dd, *J* = 6.5 and 11.7 Hz, H-4)/71.9 (C-4) for the *β*-D-glucosyl moiety, as well as 153.9 (C-6′), 152.3 (C-1′), 6.93 (2H, d, *J* = 8.9 Hz, H-2′ and 6′)/119.5 (C-4′, 6′) and 6.66 (2H, d, *J* = 8.9 Hz, H-3′ and 5′)/116.6 (C-3′ and 5′) for the hydroquinone group, with two hydroxyl groups at the para position. The HMBC NMR data showed a correlation between δ_H 4.71 (1H, d, *J* = 7.4 Hz, H-1) and δ_C 152.3 (C-1′), establishing the linkage of *β*-D-glucosyl and hydroquinone groups, and confirmed that the compound **1** was a derivative of *β*-arbutin.[7] The 1D and HSQC NMR data also exhibited the presence of two olefinic groups (δ_H/δ_C 6.82

(1H, td, *J* = 1.4, 7.3 and 7.3 Hz, H-3″)/143.7 (C-3″) and 6.40 (1H, dd, *J* = 10.9 and 17.7 Hz, H-7″)/139.7 (C-7″)), two exomethylenes (5.28 (1H, d, *J* = 14.3 Hz, H-8″)/5.09 (1H, d, *J* = 14.3 Hz, H-8″)/113.9 (C-8″) and 5.03 (2H, d, *J* = 16.2 Hz, H-7″)/116.9 (C-7″)), two methylenes (2.43 (2H, dd, *J* = 7.3 and 14.5 Hz, H-4″)/28.4 (C-4″) and 2.37 (2H, dd, *J* = 6.8 and 7.9 Hz, H-5″)/31.1 (C-5″)), and one methyl (1.84 (3H, s, H-9″)/12.6 (C-9″)). The ^{13}C NMR signals at δ_C 169.4 (C-1″), 146.76 (C-6″), and 128.9 (C-2″) suggested the presence of one carboxylic and two olefinic quaternary carbons, respectively. The COSY NMR data showed correlations between H-3″/H-4″, H-4″/H-5″ and H-7″/H-8″, and the HMBC correlations from H-3″ to C-1″ and C-9″, H-4″ to C-2″, C-3″, and C-6″, H-5″ to C-3″, C-6″, C-7″, and C-10″, H-7″ to C-5″, C-6″, and C-10″, H-8″ to C-6″ and C-7″, H-9″ to C-1″, C-2″, and C-3″, and H-10″ to C-5″ and C-7″ suggested the presence of 2-methyl-6-methylene-2,7-octadienoate. Finally, the structure of compound **1** was completed based on the HMBC correlation between H-6 of the *β*-arbutin moiety and C-1″ of the 2-methyl-6-methylene-2,7-octadienoyl group. Consequently, the structure of compound **1** was identified as 6-*O*-(2-methyl-6-methylene-2,7-octadienoyl)-arbutin.

Compound **2** was obtained as a white powder, and its molecular formula was determined to be $C_{22}H_{30}O_9$ by HRESIMS analysis (*m/z* 437.1834 $[M-H]^-$ calcd for 437.1812). The 1D and 2D NMR data of **2** showed similar patterns to those of **1,** except for the signals of the linear monoterpenoid moiety that was attached to C-6 of the glucopyranosyl group. The 1D and HSQC NMR data showed the presence of two olefinic groups (δ_H/δ_C 6.79 (1H, td, *J* = 1.4, and 7.3 Hz, H-3″)/143.7 (C-3″) and 5.39 (1H, t, *J* = 6.1 Hz, H-7″)/125.7 (C-7″)), one oxymethylene (4.08 (2H, d, *J* = 6.7 Hz, H-8″)/59.4 (C-8″)), two methylenes (2.36 (2H, dd, *J* = 7.4 and 14.9 Hz, H-4″)/28.0 (C-4″) and 2.16 (2H, t, *J* = 7.6 and 7.6 Hz, H-5″)/39.1 (C-5″)), and two methyl groups (1.85 (3H, s, H-9″)/12.5 (C-9″) and 1.70 (3H, s, H-10″)/16.2 (C-10″)). The ^{13}C NMR signals at

δ_C 169.4 (C-1″), 138.5 (C-6″), and 128.9 (C-2″) indicated the presence of one carboxylic and two olefinic quaternary carbons, respectively. The COSY NMR correlations between H-3″/H-4″, H-4″/H-5″, and H-7″/H-8″, and the HMBC correlations from H-3″ to C-1″ and C-9″, H-4″ to C-2″ and C-6″, H-5″ to C-3″, C-6″, C-7″, and C-10″, H-7″ to C-5″ and C-10″, H-8″ to C-6″ and C-7″, H-9″ to C-1″, C-2″, and C-3″, and H-10″ to C-6″ and C-7″ suggested the presence of a 2,6-dimethyl-8-hydroxy-2,6-octadienoyl group. The location of this group was confirmed by the HMBC correlation from H-6 to C-1″, and **2** was identified as 6-*O*-(2,6-dimethyl-8-hydroxy-2,6-octadienoyl)-arbutin.

Compound **3** was obtained as a white powder with a molecular formula of $C_{23}H_{32}O_9$, determined based on the HRESIMS data (*m/z* 451.1975 $[M-H]^-$ calcd for 451.1968). The 1D and 2D NMR data showed similar patterns to those of **2**, except for the presence of a methoxy group at C-8″ instead of a hydroxyl group. Significant changes in the chemical shifts of C-6″ (δ_C 140.8, +2.3 ppm), C-7″ (δ_C 122.6, -3.1 ppm), and C-8″ (δ_C 69.7, +10.3 ppm) were observed compared to those of **2**. The position of the methoxy group at C-8″ was confirmed by the HMBC correlation between δ_H 3.28 (3H, s, H-11″) and δ_C 69.7 (C-8″). As a result, the substituent attached to the aglycone was determined to be 2,6-dimethyl-8-methoxy-2,6-octadienoate. Finally, the structure of compound **3** was identified as 6-*O*-(2,6-dimethyl-8-methoxy-2,6-octadienoyl)-arbutin.

Compound **4** was isolated as a white powder and had the molecular formula of $C_{23}H_{32}O_9$ (HRESIMS; *m/z* 451.1975 $[M-H]^-$ calcd for 451.1968). Compound **4** was identified as a derivative of **7** with the substitution of a methoxy group at C-6″, which was confirmed by 1D and 2D NMR data. The chemical shifts of C-5″ (δ_C 39.2, -2.5 ppm), C-6″ (δ_C 78.5, +4.8 ppm), C-7″ (δ_C 144.3, -1.6 ppm), C-8″ (δ_C 115.8, +3.3 ppm), and C-10″ (δ_C 21.9, -6.0 ppm) confirmed

the modification of C-6″. The HMBC correlation between δ_H 3.17 (3H, s, H-11″) and δ_C 78.5 (C-6″) verified the location of the methoxy group. The absolute configuration of C-6″ in compound **4** was confirmed by the acid hydrolysis of **4** and **7**. The hydrolytic products of **4** and **7** showed the same Rf values in the thin layer chromatography (TLC) analysis (Figure S37). The substituent attached to the aglycone, *β*-arbutin, was determined to be (6*S*)-2,6-dimethyl-6-methoxy-2,7-octadienoate. Consequently, **4** was identified as 6-*O*-[(6*S*)-2,6-dimethyl-6-methoxy-2,7-octadienoyl]-arbutin.

Compound **5** was obtained as a white powder with a molecular formula of $C_{22}H_{32}O_{11}$, based on HRESIMS analysis (*m/z* 471.1868 $[M-H]^-$ calcd for 471.1866). The 1D and 2D NMR data showed similar patterns to those of **7**, except for the signals of the linear monoterpenoid moiety attached to C-6 of the glucopyranosyl group. The 1D and HSQC NMR data indicated the presence of one olefinic group (δ_H/δ_C 6.83 (1H, td, *J* = 1.1, and 7.4 Hz, H-3″)/144.7 (C-3″)), one oxymethine (3.50 (H, dd, *J* = 3.2, and 7.9 Hz, H-7″)/78.2 (C-7″)), one oxymethylene (3.80 (H, m, H-8″) and 3.55 (H, m, H-8″)/64.0 (C-8″)), two methylenes (2.34 (2H, m, H-4″)/23.7 (C-4″) and 1.70 (H, td, *J* = 4.5, 13.6 and 11.7 Hz, H-5″)/1.54 (H, m, H-5″)/38.6 (C-5″)), and two methyl groups (1.87 (3H, s, H-9″)/12.5 (C-9″) and 1.16 (3H, s, H-10″)/22.1 (C-10″)). The ^{13}C NMR signals at δ_C 169.5 (C-1″), 128.5 (C-2″), and 74.6 (C-6″) suggested the presence of one carboxylic and two olefinic quaternary carbons, respectively. The HMBC correlations from H-7″ to C-6″, C-8″, and C-10″ and H-10″ to C-5″, C-6″, and C-7″ suggested the presence of the 2,6-methyl-6,7,8-trihydroxy-2-octaenoyl group. The location of the derivative was confirmed by the HMBC correlation from H-6 to C-1″. Subsequently, compound **5** was identified as 6-*O*-(2,6-methyl-6,7,8-trihydroxy-2-octaenoyl)-arbutin.

Compound **6** had the same molecular formula as **5** ($C_{22}H_{32}O_{11}$ (*m/z* 471.1868 $[M-H]^-$ calcd for 471.1866)) as well as similar 1D and 2D NMR data, except for the splitting pattern of H-4″, H-5″, and H-8″ of the linear monoterpenoid moiety. Small changes in the chemical shifts of C-4″ (δ_C 24.0, +0.3 ppm), C-5″ (δ_C 37.9, -0.7 ppm), and C-7″ (δ_C 78.5, +0.3 ppm) in the ^{13}C NMR data as well as H-5″ (δ_H 1.57, +0.03 ppm), H-5″ (δ_H 1.67, -0.03 ppm), and H-8″ (δ_H 3.75, -0.05 ppm) in the ^{1}H NMR data suggested that **5** and **6** were stereoisomers with different stereochemical configurations of the two hydroxyl groups at C-7 and C-8.

The absolute configuration of the hydroxyl moiety at C-7 of compounds **5** and **6** was determined by the modified Mosher ester method.[8] The $\Delta\delta^{SR}$ ($\delta_S - \delta_R$) values of H-4, H-5, and H-7 in **5** and **6** showed similar values, indicating that the hydroxyl moiety at C-7 in both compounds had the *S*-configuration (Figure 2). The absolute configuration of the hydroxyl moiety at C-6 was determined based on the helicity rule using the electronic circular dichroism (ECD) data after derivatization with $[Mo_2(OAc)_4]$ because of the difference in the bulkiness of the two substituents around the hydroxyl moiety at C-6.[9] The ECD measurements after complexation with $[Mo_2(OAc)_4]$ showed that compound **5** contained an erythro-2,3-diol based on the negative Cotton effect in the band II region (418 nm, -0.35 mdeg), but **6** did not show significant changes (Figure 2). These results suggested that the absolute configurations of the hydroxyl moiety at C-6 in **5** and **6** were opposite to those of 6-*O*-[(6*S*, 7*R*)-2,6-methyl-6,7,8-tri hydroxy-2-octaenoyl]-arbutin and 6-*O*-[(6*S*, 7*S*)-2,6-methyl-6,7,8-trihydroxy-2-octaenoyl]-arbutin, respectively.

Compounds **1**–**7**, derivatives of *β*-arbutin, were expected to show tyrosinase inhibitory activity owing to the presence of a glycosylated hydroquinone moiety, but these did not show potent inhibition of tyrosinase (Figure 3). It was speculated that the long chain of the monoterpenoid

moiety could hinder the binding of tyrosinase enzymes to the compounds. Interestingly, compound **10**, a lignin that was unambiguously identified as (-)-epipinoresinol based on the chemical shifts of the furofuran ring and negative optical rotation, was isolated from the VEE2-3 fraction that showed the highest tyrosinase inhibition activity.[10] These results are consistent with those reported in prior literature.[11]

EXPERIMENTAL SECTION

General Experimental Procedures. 1D and 2D NMR data were obtained at 600 MHz using a Bruker Avance Neo 600 (Brucker, Germany) spectrometer located at the Central Laboratory of the Kangwon National University (Chuncheon, Korea). Normal-phase silica gel Kieselgel 60 (40–60 μm, 230–400 mesh, Merck, Germany) and Sephadex LH-20 gel (18-111 μm, GE Healthcare, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC using precoated silica gel plates (Kieselgel 60 F_{254} Merck, Germany) and reversed phase (RP)-C_{18} plates (Kieselgel 60 F_{254s}, Merck, Germany). The TLC spots were detected using 254- and 356-nm UV light or H_2SO_4-EtOH (v/v) spray followed by heating. Preparative high-performance liquid chromatography (HPLC) was performed using an Empower Pro system equipped with a Waters 996 photodiode array detector (Waters, Germany) using a HECTOR C_{18} column (5 μm, 21.2 × 250 mm) and YMC-Actus Triart C_{18} column (s-5 μm, 12 nm, 20 × 250 mm) at a flow rate of 50 mL/min with monitoring at 210 nm. High-purity solvents for the extraction, fractionation, and separation were purchased from DaeJung (Sicheung, Korea).

Plant Material. *V. erosum* leaves were collected from the Medicinal Plant Garden, Seoul National University, Korea, in September 2004. (N37°71'23.2", E126°81'88.8") The herbarium

(KNUVE-01) was deposited at the College of Pharmacy, Kangwon National University, and identified by Prof. Yong Soo Kwon at the College of Pharmacy, Kangwon National University.

Extraction and Isolation. *V. erosum* leaves (2 kg) were extracted three times using 80% MeOH with ultrasonic extraction for three hours. The crude extract (365 g) was dissolved in H_2O and partitioned successively using *n*-hexane (0.5 g), EtOAc (66 g), and *n*-BuOH (123 g). A portion of EtOAc fraction (20 g) was subjected to RP C_{18} medium-pressure liquid chromatography (MPLC) using MeOH:H_2O (1:1 to 1:0) solvent system to afford eight sub-fractions (VEE1–VEE8). The fraction VEE2 (9.13 g) was separated by silica gel MPLC using CH_2Cl_2:MeOH (9:1 to 3:7) to yield nine fractions. Sub-fraction VEE2-2 (268 mg) was separated into seven sub-fractions (VEE2-2-1–VEE2-2-7) by RP C_{18} MPLC using MeOH:H_2O (1:1 to 1:0). Compound **10** (10.3 mg) was purified from VEE2-2-5 (42 mg) by preparative HPLC using a gradient solvent system of MeOH:H_2O with 0.1% FA (1:1, 5.0 mL/min). Fraction VEE2-4 (836 mg) was further separated into four sub-fractions (VEE2-4-1–VEE2-6-4) by Sephadex LH-20 CC using MeOH. Compound **8** (153 mg) was purified from VEE2-4-1 (600 mg) with RP C_{18} MPLC using a gradient solvent system of MeOH:H_2O (1:1 to 1:0). Fraction VEE2-6 (2.82 g) was further separated into 14 sub-fractions (VEE2-6-1–VEE2-6-14) via Sephadex LH-20 CC using MeOH. VEE2-6-4 (1.36 g) was subjected to RP C_{18} MPLC using MeOH:H_2O (1:1 to 1:0) to afford 12 sub-fractions (VEE2-6-4-1–VEE2-6-4-12). Compounds **5** (6.3 mg) and **6** (5.2 mg) were purified from VEE2-6-4-2 (19.4 mg) by preparative HPLC using a gradient solvent system of MeOH:H_2O with 0.1% FA (35:65, 5.0 mL/min). Compound **7** (114 mg) was purified from VEE2-6-4-4 (340.6 mg) by preparative HPLC using a gradient solvent system of MeOH:H_2O with 0.1% FA (60:40, 5.0 mL/min). Compounds **1** (11.7 mg), **3** (19.4 mg), and **4** (11.1 mg) were purified from VEE2-6-4-7 (267 mg) by preparative HPLC using a gradient solvent system of

MeOH:H_2O with 0.1% FA (65:35, 10.0 mL/min). Sub-fraction VEE2-6-4-9 (89.9 mg) was further separated with silica gel CC using CH_2Cl_2:MeOH (9:1 to 1:1). Compound **2** (0.9 mg) was purified from fraction VEE2-6-4-9-5 (3.2 mg) by preparative HPLC using MeOH-H_2O with 0.1% FA (72:28, 5.0 mL/min). Compound **9** (198 mg) was recrystallized from VEE3.

*6-O-(2-methyl-6-methylene-2,7-octadienoyl)-arbutin (**1**):* white, amorphous powder; $[\alpha]^{25}_D$ −5.5 (c 0.001 MeOH); ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 419.1690 $[M - H]^-$ (calcd for $C_{22}H_{28}O_8$ 419.1706).

*6-O-(2,6-dimethyl-8-hydroxy-2,6-octadienoyl)-arbutin (**2**):* white, amorphous powder; $[\alpha]^{25}_D$ −14.3 (c 0.001 MeOH); ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 437.1834 $[M - H]^-$ (calcd for $C_{22}H_{30}O_9$ 437.1812).

*6-O-(2,6-dimethyl-8-methoxy-2,6-octadienoyl)-arbutin (**3**):* white, amorphous powder; $[\alpha]^{25}_D$ −6.4 (c 0.001 MeOH); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 451.1975 $[M - H]^-$ (calcd for $C_{23}H_{32}O_9$ 451.1968).

*6-O-[(6S)-2,6-dimethyl-6-methoxy-2,7-octadienoyl]-arbutin (**4**):* white, amorphous powder; $[\alpha]^{25}_D$ −13.0 (c 0.001 MeOH); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 451.1975 $[M - H]^-$ (calcd for $C_{23}H_{32}O_9$ 451.1968).

*6-O-[(6S, 7R)-2,6-methyl-6,7,8-trihydroxy-2-octaenoyl]-arbutin (**5**):* white, amorphous powder; $[\alpha]^{25}_D$ −17.8 (c 0.001 MeOH); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 471.1868 $[M - H]^-$ (calcd for $C_{22}H_{32}O_{11}$ 471.1866).

*6-O-[(6S, 7S)-2,6-methyl-6,7,8-trihydroxy-2-octaenoyl]-arbutin (**6**):* white, amorphous powder; $[\alpha]^{25}_D$ −9.4 (c 0.001 MeOH); ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 471.1868 $[M - H]^-$ (calcd for $C_{22}H_{32}O_{11}$ 471.1866).

Tyrosinase Inhibition Assay. Tyrosinase inhibitory activities of the fractions of *V. erosum* and compounds **1**–**10** were determined using previously described methods with slight modifications.[12] First, 220 μL of phosphate buffer (0.1 M, pH 6.5), 20 μL of inhibitors with different concentrations (dissolved in ethanol), and 20 μL of mushroom tyrosinase solution (2000 U/mL) were placed in the wells of a 96-well microplate. After incubation for 10 min at 37 °C, 40 μL of L-tyrosine (1.5 mM) was added to the reaction mixture (300 μL). Immediately, the absorbance was measured at a wavelength of 490 nm using a microplate reader, and after reacting for 17 min at 37 °C, the absorbance was measured again. The blank group, i.e., the same volume of ethanol without the inhibitor was used, and β-arbutin was used as the positive control. All experiments were performed in triplicate for each compound. The relative tyrosinase activity was calculated using the following expression: tyrosinase activity (%): [(A1-A0)/(B1-B0)] ×100, where A0 is the absorbance of the sample before the reaction and A1 is the absorbance of the sample after the reaction. B0 and B1 are the absorbances before and after the reaction of the blank group, respectively. The inhibitory effects of these compounds on mushroom tyrosinase were expressed by the activity of the remaining enzyme.

Cell Culture. B16F10 murine melanoma, HEp-2 laryngeal carcinoma, and PC3 prostate cancer cell lines were purchased from the American Type Culture Collection (ATCC). The cells were cultured in RPMI 1640 and DMEM containing 10% fetal bovine serum and 1% penicillin/streptomycin under 5% CO_2 at 37 °C.

Cell Viability Assay. The cytotoxic effects of VEE1-8 on three types of cells were determined using the MTT assay. Briefly, after incubating overnight in 96-well plates at a concentration of 5×10^3 cells/100 μL, the cells were treated with 100 μg/mL of VEE1-8 for 48 h. DMSO was used as the negative control and etoposide (100 μM) was used as the positive

control. The MTT solution with a concentration of 0.5 mg/mL was added to each well and maintained at 37 °C for four hours. The absorbance was measured at 490 nm using a SpectraMax i3 multimode microplate reader (Molecular Devices).

Acid Hydrolysis. The absolute configuration of the monoterpenoid moiety in **4** was determined using a slightly modified literature procedure. Compounds **4** and **7** (1.0 mg each) were dissolved in 1 mL of 2 M HCl (H_2O/1,4-dioxane, 1:1, v/v), and each solution was heated at 90 °C for four hours. After cooling the solution, it was evaporated under N_2, re-dissolved in water, and extracted with *n*-hexane. The monoterpenoids from which the monosaccharides were removed were extracted in *n*-hexane, and chromatographed on TLC plate.

Preparation of MTPA esters of compounds 5 and 6. (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl) (3.3 μL) and dried pyridine (3.3 μL) were dissolved in a solution of **5** (or **6**) (0.5 mg) in anhydrous $CDCl_3$ (100 μL). The mixtures were sealed and kept overnight at room temperature. A capillary column was used to purify the product with *n*-hexane:EtOAc (50:50, v/v) solution to obtain the pure (*S*)-MTPA ester of **5** (or **6**). (*R*)-MTPA esters were prepared in a similar manner using (*S*)-MTPACl.

Preparation of Mo_2-complexes of compounds 5 and 6 The ECD spectra were recorded at room temperature in DMSO with 1.0 nm/step scans using a 10-mm cell at 200–600 nm according to Snatzke's method. To form complexes, compound **5** (or **6**) was dissolved in a solution of $[Mo_2(OAc)_4]$ in DMSO at a 1:1 ratio of the molybdenum complex to diol.

FIGURES

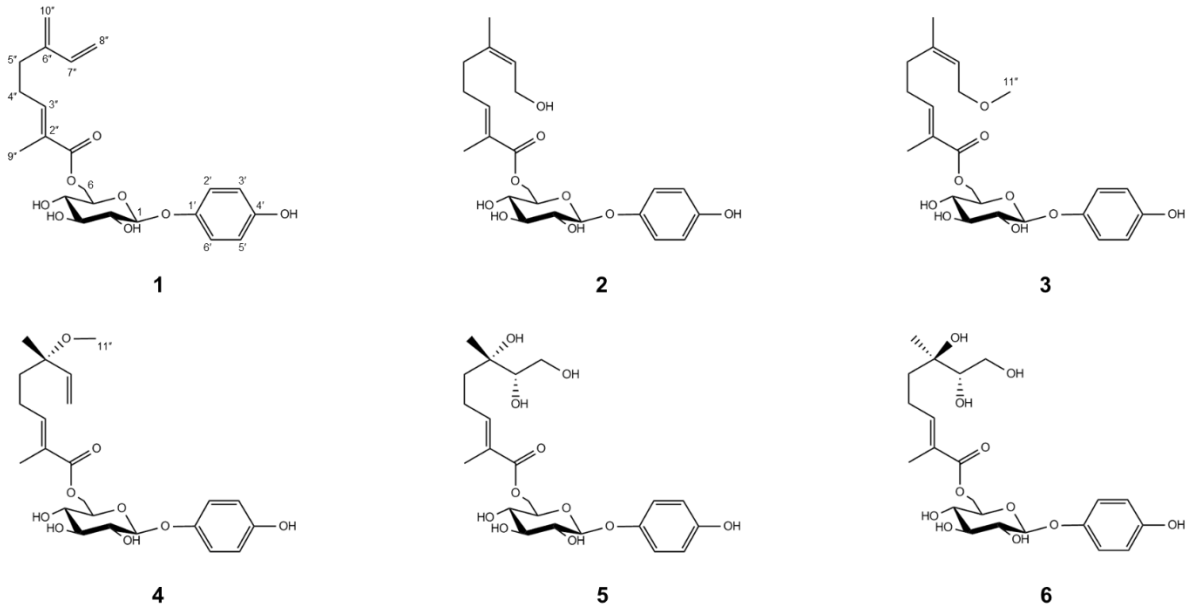


Chart 1.

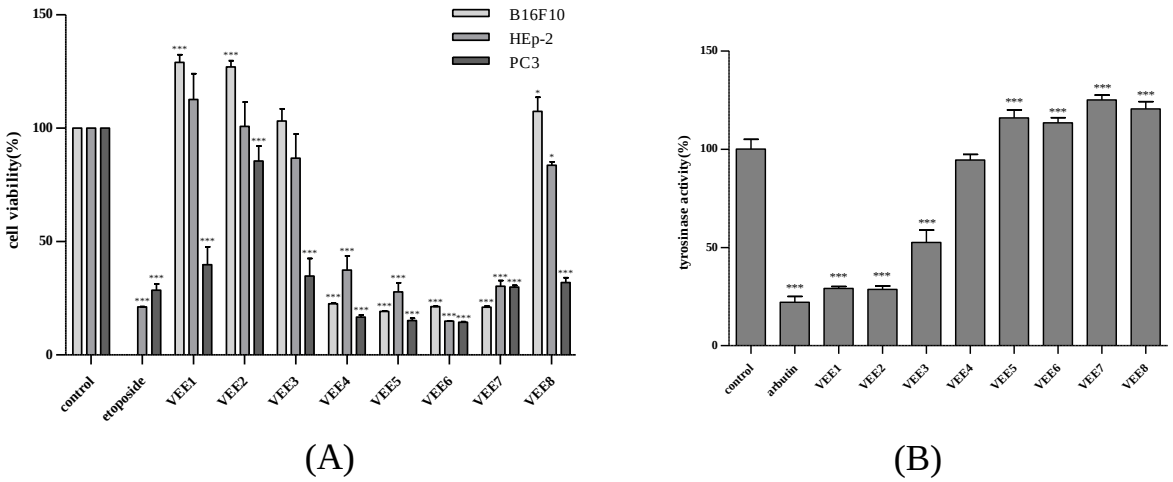
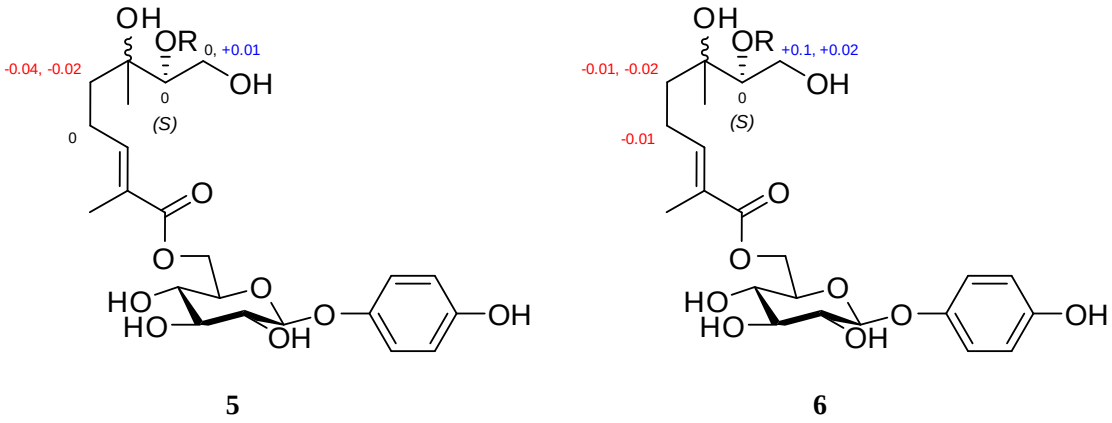


Figure 1. Cytotoxic (A) and tyrosinase inhibition activities (B) of eight EtOAc sub-fractions; VEE1–8 (100 mg/ml).

(A)



(B)

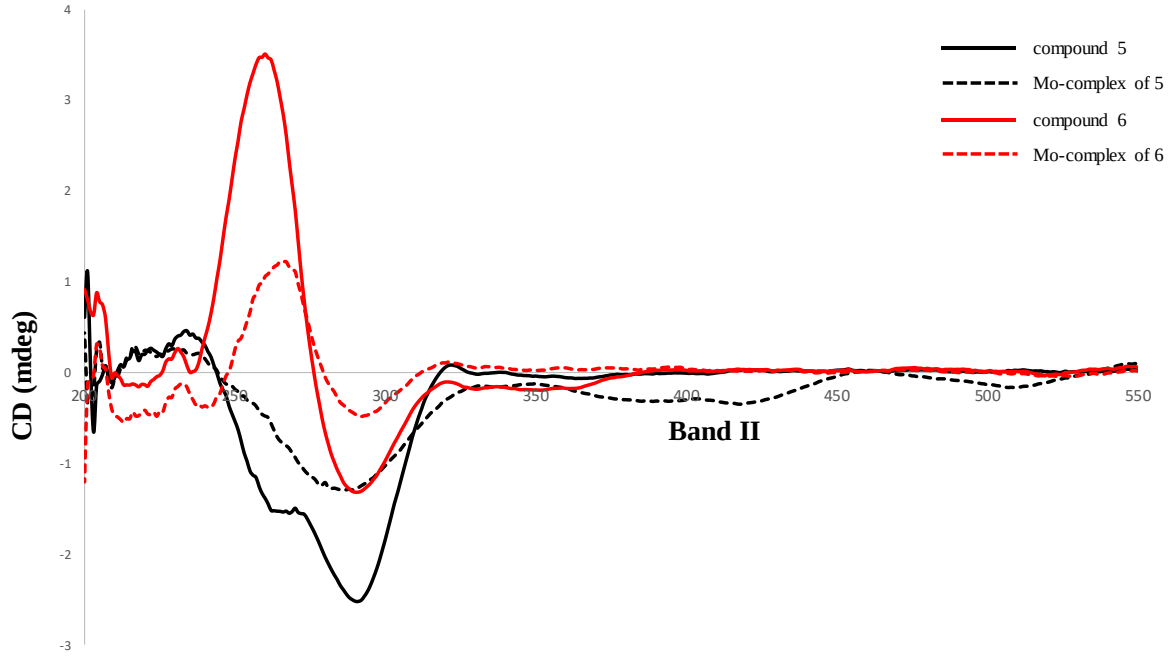


Figure 2. $\Delta\delta^{SR}$ ($\delta_S - \delta_R$) values (ppm, $CDCl_3$) of the MTPA esters of **5** and **6** (A), and comparison of the ECD spectra of **5**, **6**, and their molybdenum derivatives in DMSO (B).

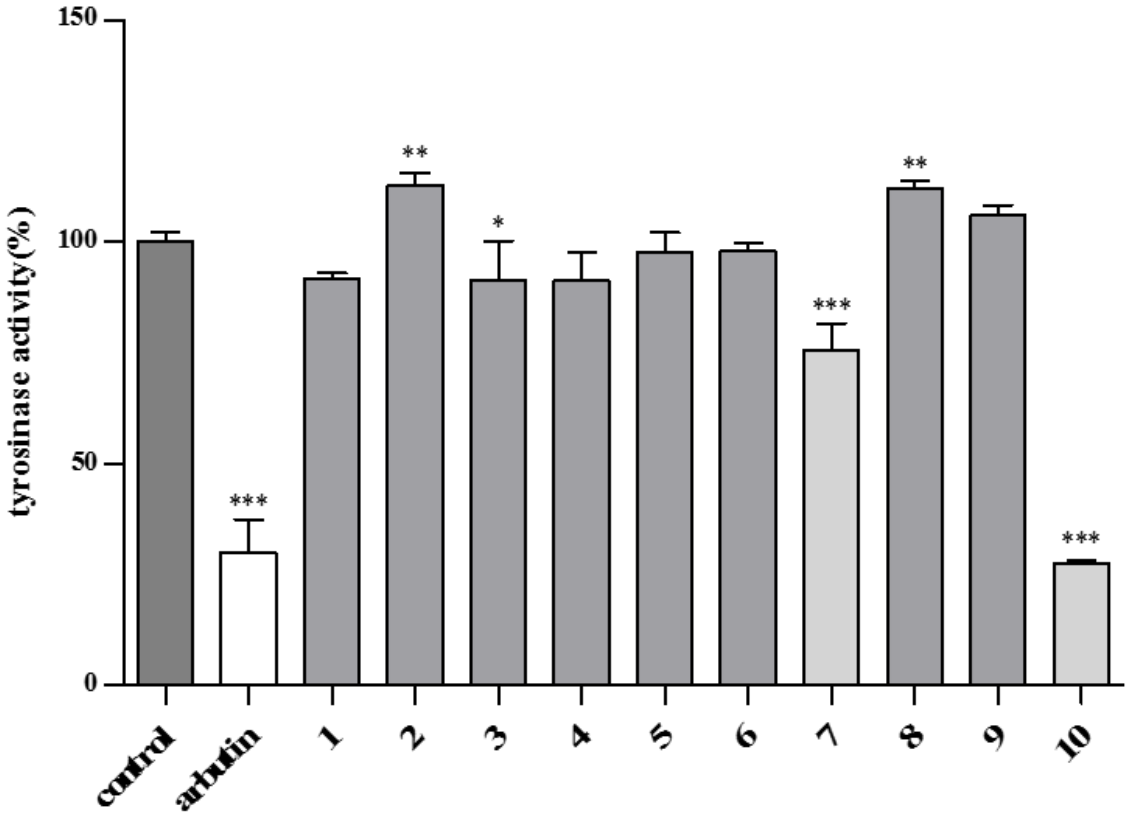


Figure 3. Tyrosinase inhibitory activities of compounds **1**–**10**, isolated from *V. erosum*. *β*-arbutin is used as the positive control.

Table 1. NMR Spectroscopic Data (methanol-d_4, 150 MHz for ^{13}C and 600 MHz for ^{1}H NMR) for Compounds **1**−**6**.

	1		**2**		**3**	
position	δC, type	δH (*J* in Hz)	δC, type	δH (*J* in Hz)	δC, type	δH (*J* in Hz)
1	103.6, CH	4.71 d (7.4)	103.6, CH	4.71 d (7.4)	103.5, CH	4.71 d (7.4)
2	74.9, CH	3.43 m	74.9, CH	3.43 m	74.9, CH	3.42 m
3	77.9, CH	3.43 m	77.9, CH	3.43 m	77.9, CH	3.42 m
4	71.9, CH	3.36 dd (6.5, 11.7)	71.9, CH	3.36 dd (6.5, 11.7)	71.9, CH	3.35 t (8.7)
5	75.4, CH	3.62 ddd (2.1, 7.2, 9.5)	75.4, CH	3.62 m	75.4, CH	3.62 ddd (2.1, 5.8, 7.3)
6	65.1, CH2	4.24 dd (7.2, 11.8), 4.51 dd (2.1, 11.8)	65.0, CH2	4.23 dd (7.2, 11.8) 4.51 dd (1.9, 11.8)	65.1, CH2	4.22 dd (7.3, 11.8), 4.51 dd (2.2, 11.8)
1'	152.3, C		152.2, C		152.2, C	
2'	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)
3'	116.6, CH	6.66 d (8.9)	116.6, CH	6.66 d (8.9)	116.6, CH	6.66 d (8.9)
4'	153.9, C		153.9, C		153.9, C	
5'	116.6, CH	6.66 d (8.9)	116.6, CH	6.66 d (8.9)	116.6, CH	6.66 d (8.9)
6'	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)
1"	169.4, C		169.4, C		169.3, C	
2"	128.9, C		128.9, C		128.8, C	
3"	143.7, CH	6.82 td (1.4, 7.3, 7.3)	143.7, CH	6.79 td	143.6, CH	6.78 td (1.3, 7.3, 7.2)
4"	28.4, CH2	2.43 dd (7.3, 14.5)	28.0, CH2	2.36 dd (7.4, 14.9)	27.9, CH2	2.37 m
5"	31.1, CH2	2.37 dd (6.8, 7.9)	39.1, CH2	2.16 t (7.6, 7.6)	39.1, CH2	2.21 dt (7.4, 7.4, 29.0)
6"	146.8, C		138.5, C		140.8, C	
7"	139.7, CH	6.4 dd (10.9, 17.7)	125.7, CH	5.39 t (6.1, 6.1)	122.6, CH	5.34 m
8"	113.9, CH2	5.09 d (14.3), 5.28 d (14.3)	59.4, CH2	4.08 d (6.7)	69.7, CH2	3.94 d (6.7)
9"	12.6, CH3	1.84 s	12.5, CH3	1.85 s	12.5, CH3	1.85 s
10"	116.9, CH2	5.03 d (16.2)	16.2, CH3	1.70 s	16.4, CH3	1.71 s
11"					57.8, CH3	3.28 s

	4		5		6	
position	δC, type	δH (*J* in Hz)	δC, type	δH (*J* in Hz)	δC, type	δH (*J* in Hz)
1	103.5, CH	4.71 d (7.4)	103.5, CH	4.71 d (7.4)	103.5, CH	4.71 d (7.4)
2	74.9, CH	3.43 m	74.9, CH	3.43 m	74.9, CH	3.43 m
3	77.9, CH	3.43 m	77.9, CH	3.43 m	77.9, CH	3.43 m
4	71.9, CH	3.34 m	71.9, CH	3.35 dd (6.5, 11.8)	71.9, CH	3.36 m
5	75.4, CH	3.62 ddd (2.1, 7.3, 9.6)	75.4, CH	3.63 ddd (1.9, 7.5, 9.6)	75.4, CH	3.63 ddd
6	65.1, CH2	4.22 dd (7.3, 11.8), 4.51 dd (2.0, 11.8)	65.1, CH2	4.21 dd (7.4, 11.8) 4.52 dd (2.1, 11.8)	65.1, CH2	4.22 dd (7.4, 11.8) 4.52 dd (1.1, 11.8)
1'	152.3, C		152.3, C		152.3, C	
2'	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)
3'	116.6, CH	6.66 d (8.9)	116.6, CH	6.68 d (8.9)	116.6, CH	6.68 d (8.9)
4'	153.9, C		153.9, C		153.9, C	
5'	116.6, CH	6.66 d (8.9)	116.6, CH	6.68 d (8.9)	116.6, CH	6.68 d (8.9)
6'	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)	119.5, CH	6.93 d (8.9)
1"	169.4, C		169.5, C		169.5, C	
2"	128.5, C		128.5, C		128.5, C	
3"	143.5, CH	6.80 td (1.3, 7.6, 7.6)	144.7, CH	6.83 td (1.1, 7.4, 7.5)	144.5, CH	6.82 td (1.3, 7.4, 7.5)
4"	24.2, CH2	2.22 dt (10.4, 10.4, 20.9)	23.7, CH2	2.34 m	24.0, CH2	2.33 dd (7.9, 16.4)
5"	39.2, CH2	1.63 m	38.6, CH2	1.54 m 1.7 td (4.5, 13.6, 11.7)	37.9, CH2	1.57 m 1.67 ddd (6.92, 10.41, 18.67)
6"	78.5, C		74.6, C		74.7, C	
7"	144.3, CH	5.80 ddd (1.3, 11.0, 17.6)	78.2, CH	3.50 dd (3.2, 7.9)	78.5, CH	3.49 dd (3.3, 7.7)
8"	115.8, CH2	5.21 m	64.0, CH2	3.55 m 3.80 m	63.9, CH2	3.55 dd (7.8, 11.2) 3.75 ddd (1.8, 3.3, 11.9)
9"	12.4, CH3	1.83 s	12.5, CH3	1.87 s	12.5, CH3	1.87 s
10"	21.9, CH3	1.28 s	22.1, CH3	1.16 s	22.8, CH3	1.19 s
11"	50.4, CH3	3.17 s				

ASSOCIATED CONTENT

The Supporting Information is available free of charge at xxx. 1D and 2D NMR spectra and HRESIMS of compounds 1−6 (PDF)

AUTHOR INFORMATION

Corresponding Author

***Heejung Yang** − Laboratory of Natural Products Chemistry, College of Pharmacy, Kangwon National University, Gangwondaehak-gil 1, Chuncheon, Gangwon 24341, Republic of Korea**;** orcid.org/0000-0001-5986-9024; Email: heejyang@hust.edu.cn

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (NRF-2018R1C1B6002574).

REFERENCES

(1) Wang, X.-Y.; Shi, H.-M.; Li, X.-B. *Chem. Biodivers.* **2010**, *7*, 567–593.

(2) In, S.-J.; Seo, K.-H.; Song, N.-Y.; Lee, D.-S.; Kim, Y.-C.; Baek, N.-I. *Arch. Pharm. Res.* **2015**, *38*, 26–34.

(3) Zolghadri, S.; Bahrami, A.; Hassan Khan, M. T.; Munoz-Munoz, J.; Garcia-Molina, F.; Garcia-Canovas, F.; Saboury, A. A. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 279–309.

(4) Kim, Y.-J.; Uyama, H. *Cell. Mol. Life Sci.* **2005**, *62*, 1707–1723.

(5) Migas, P.; Krauze-Baranowska, M. *Phytochem. Lett.* **2015**, *13*, 35–40.

(6) Avonto, C.; Wang, Y.; Avula, B.; Wang, M.; Rua, D.; Khan, I. *Planta Med.* **2014**, *80*, s-0034-1382659.

(7) Pawlowska, A. M.; De Leo, M.; Braca, A. *J. Agric. Food Chem.* **2006**, *54* (26), 10234–10238.

(8) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451–2458.

(9) Snatzke, G.; Wagner, U.; Wolff, H. P. *Tetrahedron* **1981**, *37*, 349–361.

(10) Shao, S.-Y.; Yang, Y.-N.; Feng, Z.-M.; Jiang, J.-S.; Zhang, P.-C. *J. Nat. Prod.* **2018**, *81*, 1023–1028.

(11) Azhar-ul-Haq; Malik, A.; Khan, M. T. H.; Anwar-ul-Haq; Khan, S. B.; Ahmad, A.; Choudhary, M. I. *Phytomedicine* **2006**, *13*, 255–260.

(12) Zhai, Y.-J.; Huo, G.-M.; Zhang, Q.; Li, D.; Wang, D.-C.; Qi, J.-Z.; Han, W.-B.; Gao, J.-M. *J. Nat. Prod.* **2020**, *83*, 1592–1597.

Graphical abstract

Leaves of *Viburnum erosum* Thunb.