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ARTICLE

Flavonoids from the Buds of *Rosa damascena* Inhibit the Activity of 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase and Angiotensin I-Converting Enzyme

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Rosa damascena has been manufactured as various food products, including tea, in Korea. A new flavonoid glycoside, kaempferol-3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-xylopyranoside, named roxyloside A was isolated from the buds of this plant, along with four known compounds, isoquercitrin, afzelin, cyanidin-3-*O*-*β*-glucoside, and quercetin gentiobioside. The chemical structures of these compounds were determined by spectroscopic analyses, including FAB-MS, UV, IR, ^{1}H and ^{13}C NMR, DEPT, and 2D NMR (COSY, HSQC, and HMBC). All the isolated compounds except cyanidin-3-*O*-*β*-glucoside exhibited high levels of inhibitory activity against 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase with IC_{50} values ranging from 47.1 to 80.6 μM. Cyanidin-3-*O*-*β*-glucoside significantly suppressed angiotensin I-converting enzyme (ACE) activity, with an IC_{50} value of 138.8 μM, while the other four compounds were ineffective. These results indicate that *R. damascena* and its flavonoids may be effective to improve the cardiovascular system.

KEYWORDS: *Rosa damascena*; flavonoids; roxyloside A; HMG-CoA reductase; angiotensin I-converting enzyme; cardiovascular system

INTRODUCTION

Atherogenesis is a complex, multifactorial process involving hypercholesterolemia, LDL oxidation, hypertension, and platelet aggregation (*1*). Increased blood cholesterol level is one of the most critical risk factors in the pathogenesis of coronary heart disease (*1*). Many cholesterol-lowering agents have been reported and introduced into clinical use, including nicotinic acid, cholestyramine, plant sterols, mevastatin, and lovastatin (*2*).

Statins such as mevastatin and lovastatin decrease the serum cholesterol level through inhibition of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (*2*). There have been several reports on the inhibitory activities of natural compounds to HMG-CoA reductase. Qureshi et al. (*3*) reported that novel tocotrienols isolated from rice bran had hypocholesterolemic, antioxidant, and antitumor properties. In the report of Jung et al. (*4*), two flavonoids (hesperidin and naringin) decreased the activities of hepatic HMG-CoA reductase and acyl CoA:cholesterol acyltransferase (ACAT). Sung et al. (*5*) investigated the inhibitory effects of genistein, daidzein, and glycitein from soy proteins against HMG-CoA reductase.

Hypertension, one of the risk factors of cardiovascular disease (CVD), is induced by an imbalance in the renin-angiotensin system, where angiotensin-I conversion is the rate limiting step in angiotensin II formation (*6*). Angiotensin II accelerates the contraction of the vascular smooth muscles and increases extracellular fluid volumes, which leads to an increase in blood pressure (*7*). Captopril is an antihypertensive drug which inhibits the activity of angiotensin-I-converting enzyme (ACE) (*8*). Flavonoids, procyanidins, and peptides isolated from natural products have been found to show potential ACE inhibitory activity (*9*). Chen et al. (*10*) reported antihypertensive tetrahydroxyxanthones isolated from *Tripterospermum lanceolatum* (*Gentianaceae*).

The members of the *Rosaceae* family have long been used not only in gardening but also for food and medicinal purposes (*11*). A rose (*Rosa* spp.) refers to the shrub and flowers of the genus *Rosa*. The fruits, petals, and buds of the roses are processed and manufactured as herbal tea, drinks, juice, and jam in Korea. Many reports are available on the characterization of flavonoids and anthocyanins from *Rosa* spp. by chromatography techniques (*12*−*14*). In particular, GC-MS has been applied to determine the volatile compound profiles from rose petals (*15*−*17*). Moreover, the phenolic antioxidants from *Rosa* spp. have been extensively studied using LC-MS (*18*). The physiological functions of Rosaceae may be partly attributed to their abundance of phenolics, which possess a wide spectrum of biochemical activities, such as antioxidant, free-radical scavenging, anticancer, anti-inflammatory, antimutagenic, and antidepressant activities (*19*−*26*). However, the effects of these bioactive compounds from *Rosa* spp. on the cardiovascular system still remain the main topic of investigation.

In a previous study (*27*), we found that water extracts of rose bud showed the highest effect on the mobilization of blood

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circulation among the nine herbs studied. In this study, five flavonoids showing strong inhibitory activity against HMG-CoA reductase or ACE were isolated from the ethyl acetate (EtOAc) fraction of the buds of *Rosa damascena*. We identified the compounds by spectroscopic analyses including FAB-MS, UV, IR, ^{1}H and ^{13}C NMR, DEPT, and 2D NMR (COSY, HSQC, HMBC). The cardiovascular protective activities of the isolated compounds, including a new compound, were evaluated using both HMG-CoA reductase and ACE inhibition assays.

MATERIALS AND METHODS

Plant Material. Air-dried whole buds of *R. damascena* were obtained from a local market at Kyung-Dong, Seoul, South Korea, in 2004. They were identified by Professor Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, South Korea. A voucher specimen (04004) is reserved at the Korea Food Research Institute.

Instruments. Optical rotation was recorded on a JASCO P-1010 digital polarimeter (Tokyo, Japan). UV spectra were measured on a Shimadzu UV-1601 (Kyoto, Japan). IR spectra were obtained with a Perkin-Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). EI-MS and FAB-MS data were recorded on a JEOL JMS-700 (Tokyo, Japan). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were recorded on a Varian Unity Inova AS-400 FT-NMR spectrometer (CA, USA).

Chemicals and Reagents. Rabbit acetone lung powder, hippuryl-his-leu (HHL), dithiothreitol (DTT), EDTA, NADPH, and HMG-CoA were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals were of analytical grade.

Extraction and Isolation. Air-dried whole buds of *R. damascena* (1700 g) were coarsely powdered and exhaustively extracted with distilled water at 100 °C for 2 h. Upon removal of the solvent under vacuum, the extract yielded 720 g of material. The extracts were suspended in distilled water and then partitioned repeatedly with *n*-hexane, chloroform, EtOAc, and *n*-butanol, sequentially. A concentrated *n*-hexane fraction (0.5 g), chloroform fraction (2.8 g), EtOAc fraction (124.7 g), *n*-butanol fraction (274.4 g), and water fraction (317.6 g) were obtained. The EtOAc extract (RE; 124.7 g) was loaded onto a silica gel column (10 cm × 60 cm) and eluted with $CHCl_3/MeOH/H_2O$ (7:3:1, v/v), monitored by thin layer chromatography (TLC). According to the TLC results, the fractions from the column were pooled, resulting in a total of 21 fractions (RE1 to RE21).

Fraction RE9 [264 mg, Ve/Vt (elution volume/total volume) 0.41−0.48] was subjected to octadecyl silane (ODS) column chromatography (3.5 cm × 35 cm) and eluted with $MeOH/H_2O$ (1:1.5, v/v), giving 12 subfractions (RE9-1 to RE9-12), which ultimately resulted in compound **1** [fraction No. 7: 11 mg, Ve/Vt 0.60−0.65, TLC (RP-18 F_{254}) R_f 0.60, $MeOH/H_2O$ (2:1)] and compound **2** [fraction No. 9: 16 mg, Ve/Vt 0.78−0.89, TLC (RP-18 F_{254}) R_f 0.55, $MeOH/H_2O$ (2:1)]. Subfraction RE9-1 (124 mg, Ve/Vt 0.01−0.15) was applied onto a Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) column (2 cm × 50 cm, 70% MeOH, 500 mL), resulting in 11 fractions where compound **3** was eluted [fraction No. 6: 46 mg, Ve/Vt 0.60−0.80, TLC (RP-18 F_{254}) R_f 0.5, $MeOH/H_2O$ (1:2)]. Fraction RE12 [160 mg, Ve/Vt 0.62−0.68] was subjected to ODS column chromatography (3.0 cm × 35 cm) and eluted with $MeOH/H_2O$ (1:2, v/v), resulting in 18 subfractions (RE12-1 to RE12-18), which yield compound **4** [fraction No. 13: 35 mg, Ve/Vt 0.70−0.80, TLC (RP-18 F_{254}) R_f 0.40, $MeOH/H_2O$ (1:1)]. Fraction RE15 (380 mg, Ve/Vt 0.78−0.85) was subjected to silica gel column chromatography (4.0 cm × 40 cm) and eluted with $CHCl_3/MeOH/H_2O$ (7:3:1, v/v) to give 11 fractions (RE15-1 to RE15-11) that yielded compound **5** [fraction No. 5: 55 mg, Ve/Vt 0.50−0.60, TLC (SiO_2F_{254}), R_f 0.65, $CHCl_3/MeOH/H_2O$ (5:4:1)].

1. Isoquercitrin. Yellow powder (MeOH); $[\alpha]^{27}_D = +58.0°$ (c = 0.10, MeOH); UV (MeOH) λ_{max}: 256, 207 nm; neg. FAB-MS *m/z* 463 $[M - H]^-$, 447, 423, 389, 297, 204; IR (KBr) ν_{max} 3400, 2919, 1656, 1606, 1508 cm^{-1}; ^{1}H and ^{13}C NMR: refer to the previous report (*28*).

2. Afzelin. Brown oil (MeOH); $[\alpha]^{25}_D = -184.0°$ (c = 0.10, MeOH); UV (MeOH) λ_{max} 328, 288 nm; EIMS *m/z*: 432 $[M]^+$; IR$_\nu$ (CaF_2 window, cm^{-1}) 3450, 2944, 1702; ^{1}H and ^{13}C NMR: refer to the previous report (*29*).

3. Cyanidin-3-O-β-glucoside. Dark pink oil (MeOH); $[\alpha]^{27}_D = +58.0°$ (c = 0.10, MeOH); UV (MeOH) λ_{max} 520 nm; EI-MS *m/z*: 449 $[M]^+$; IR$_\nu$ (KBr, cm^{-1}) 3450, 2944, 1702; ^{1}H and ^{13}C NMR: refer to the previous report (*30*).

4. Roxyloside A. Yellow amorphous powder (MeOH); $[\alpha]^{25}_D$ = −54.0° (c = 0.10, MeOH); UV (MeOH) λ_{max} 348, 266 nm; pos. FAB-MS *m/z*: 581 $[M + H]^+$, 419, 287, 185, 149, 115; pos. HRFAB-MS *m/z*: 581.1536 $[M + H]^+$ (calcd 581.1506 for $C_{26}H_{29}O_{15}$); IR$_\nu$ (CaF_2 window, cm^{-1}) 3401, 2966, 1680, 1608; ^{1}H NMR (400 MHz, CD_3OD, δ) 7.99 (2H, d, J = 8.8 Hz, H-2′/6′), 6.90 (2H, d, J = 8.8 Hz, H-3′/5′), 6.39 (1H, d, J = 2.4 Hz, H-8), 6.19 (1H, d, J = 2.4 Hz, H-6), 5.47 (1H, d, J = 5.6 Hz, H-1″), 4.67 (1H, d, J = 7.6 Hz, H-1‴), 3.85 (1H, m, H-4″), 3.82 (1H, dd, J = 11.2, 3.2 Hz, H-6a‴), 3.73 (1H, dd, J = 12.0, 7.2 Hz, H-5a″), 3.70 (1H, dd, J = 11.2, 4.8 Hz, H-6b‴), 3.65 (1H, dd, J = 7.6, 7.2 Hz, H-2″), 3.50 (1H, dd, J = 7.6, 7.8 Hz, H-3″), 3.43−3.34 (4H, overlapped, H-2‴, 3‴, 4‴, 5‴), 3.09 (1H, dd, J = 12.0, 6.8 Hz, H-5b″); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 179.4 (C-4), 165.9 (C-7), 162.9 (C-5), 161.4 (C-4′), 158.8 (C-9), 158.2 (C-2), 134.8 (C-3), 132.1 (C-2′, 6′), 122.4 (C-1′), 116.2 (C-3′, 5′), 105.5 (C-10), 104.8 (C-1‴), 101.5 (C-1″), 99.8 (C-6), 94.6 (C-8), 81.0 (C-4″), 78.1 (C-3‴), 77.8 (C-5‴), 75.2 (C-2‴), 74.8 (C-2″), 71.3 (C-4‴), 70.3 (C-3″), 65.9 (C-5″), 62.6 (C-6‴).

5. Quercetin Gentiobioside. Yellow amorphous powder (MeOH); $[\alpha]^{25}_D = -34.0°$ (c = 0.50, MeOH); UV (MeOH) λ_{max} 348, 301, 267 nm; FAB-MS *m/z* 627 $[M + H]^+$; IR$_\nu$ (CaF_2 window, cm^{-1}) 3450, 2944, 1702; ^{1}H and ^{13}C NMR: refer to the previous report (*31*).

Inhibition of HMG-CoA Reductase. The inhibition of HMG-CoA reductase was evaluated using a modified protocol of Kleinsek et al. (*32*). Microsomes were obtained from livers of male Sprague−Dawley rats (JoongAng Lab Animal Inc., Seoul, Korea) that weighed 200−300 g and had been fed *ad libitum* on an AIN-76A diet for a week. The rats were sacrificed in the middle of the 12 h dark period. Excised livers were placed in ice-cold homogenization medium containing buffer A [50 mM potassium phosphate buffer (pH 7.0), 0.2 M sucrose, and 2 mM dithiothreitol]. All of the following operations were carried out at 4 °C if not specified. The livers were homogenized in Buffer A (2 mL/g wet liver) for 15 s in a blender (Waring, CT, USA), followed by three strokes with a motor-driven Teflon pestle in a Potter-Elvehjem type glass homogenizer (ACE Homogenizer, Tokyo, Japan). The homogenate solution was centrifuged at 100,000*g* for 10 min. The supernatant was decanted and then centrifuged at 100,000*g* for 75 min. The microsomal pellet was resuspended in Buffer A containing 50 mM EDTA and recentrifuged at 100,000*g* for 60 min. The pellet was slowly frozen in the centrifuge tube and stored at −20 °C until use. The microsomal pellet was kept frozen at −20 °C for at least 2 h before being thawed at room temperature. Microsomes from an average of 1.5 rat livers occupied each ultracentrifuge tube. The thawed pellet was homogenized in 3 mL of solubilization buffer (Buffer B), composed of 50 mM potassium phosphate (pH 7.0), 0.1 M sucrose, 2 mM dithiothreitol, 50 mM KCl, and 30 mM EDTA. The homogenization was performed in a Potter-Elvehjem homogenizer with a tight fitting Teflon pestle. Another 7 mL of Buffer B was added, and the sample was homogenized again with three strokes of the pestle. After being left at room temperature for at least 15 min, the suspension was centrifuged at 100,000*g* for 60 min at 20 °C. The collected supernatant was used for the enzyme assay. All further operations were carried out at room temperature unless otherwise specified. Preincubations for the assay mixtures were carried out at 37 °C for 10 min. The assay mixtures contained 20 μL of the prepared sample (or 20 μL of distilled water for the control), 600 μL of 0.5 M potassium phosphate buffer (pH 7.0), 100 μL of DTT (20 mM), 100 μL of NADPH (3 mM), and 100 μL of enzyme source in a 1 mL test tube. The enzyme reaction was started by adding 100 μL of HMG-CoA (3 mM), and the absorbance at 340 nm was measured for 5 min. The HMG-CoA reductase inhibition rate was calculated as follows:

$$\text{HMG-CoA reductase inhibition rate } (\%) = (1 - \Delta A_s/\Delta A_c) \times 100$$

where ΔA_s is the difference of absorbance in the presence of sample for 5 min and ΔA_c is the difference of absorbance of the control reaction.

Inhibition of ACE. All the experiments on the activity of ACE were performed using an extract of rabbit lung acetone powder. The extract was prepared by blending 10 g of the powder in 100 mL of 50 mM potassium phosphate buffer (pH 8.3) and centrifuging at 40,000*g* for 40 min (L 7-55

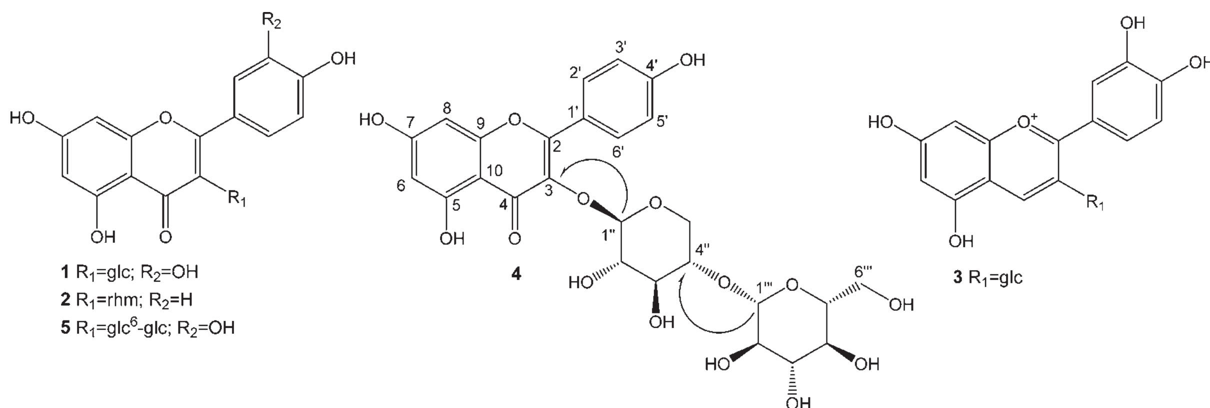

Figure 1. Chemical structures of the five compounds isolated from the EtOAc fraction of *Rosa damascena*: 1, isoquercitrin; 2, afzelin; 3, cyanidin-3-*O*-β-glucoside; 4, roxyloside A; 5, quercetin gentiobioside. The one-way arrows indicate the long-range correlations between proton and carbon signals in the HMBC spectrum.

Ultracentrifuge, Beckman, CA, USA); the supernatant is clear, highly active, and stable for 1 month at 5 °C. Prior to the assay, the supernatant was diluted 10 times in 50 mM potassium phosphate buffer (pH 8.3).

The spectrophotometric assay for ACE inhibition was performed using a modified method of Cushman and Cheung (*33*). Incubation for hippuryl-his-leu (HHL) hydrolysis by ACE was carried out at 37 °C for 30 min. Each assay mixture (0.25 mL) contained the following components at the indicated final concentrations: 100 μL of the reaction buffer [100 mM potassium phosphate buffer (pH 8.3) containing 300 mM NaCl and 5 mM HHL] and 150 μL of the enzyme. An aliquot of 60 μL of diluted sample or distilled water was added to the sample or control mixture, respectively. The enzymatic reactions were terminated by addition of 0.25 mL of 1 N HCl. In the case of all the blanks, HCl was added at zero-time before the addition of the enzyme. The hippuric acid formed by reaction of the ACE on HHL was extracted from the acidified solution into 1.5 mL of ethyl acetate using vortex for 15 s. After a brief centrifugation, a 1.0 mL aliquot of each ethyl acetate layer was transferred to a clean tube. The ethyl acetate aliquots were evaporated by heating at 120 °C for 30 min in a Temp-Blok module heater. The hippuric acid was redissolved in 1.0 mL of water, and the amount of hippuric acid was determined from its absorbance at 228 nm. The ACE inhibition rate was calculated as follows:

$$\text{ACE inhibition rate } (\%) = [1 - (S - \text{SB})/(C - \text{CB})] \times 100$$

where S is the absorbance in the presence of sample, C is the absorbance of the control reaction, and SB and CB are the absorbance of the sample- and control-blank, respectively.

RESULTS AND DISCUSSION

Elucidation of the Structure of the New Flavonoid Glycoside. The rose bud extract was fractionated using five different solvents, such as *n*-hexane, chloroform, EtOAc, *n*-butanol, and water. Among the solvent fractions, the EtOAc fraction showed a high level of activity, determined by the inhibition of the two enzymes, HMG-CoA reductase and ACE (data not shown). Repeated silica gel, ODS, and Sephadex LH-20 column chromatography of the EtOAc fraction revealed five flavonoids, as described in the Materials and Methods.

A new flavonoid glycoside, roxyloside A, was isolated to be a yellow amorphous powder from methanol fraction. It exhibited the maximal UV absorption at 348 and 266 nm and resulted in peaks due to $[M + H]^+$ at m/z 581, $[M - \text{hexose} + H]^+$ at m/z 419, and $[M - \text{hexosylpentose} + H]^+$ at m/z 287 in the positive FAB-MS, which suggest the existence of one hexose and one pentose. The molecular formula, $C_{26}H_{28}O_{15}$, was deduced from the pseudomolecular ion at m/z 581.1536 $[M + H]^+$ by positive HRFAB-MS. The IR spectral data revealed the presence of a hydroxyl group (3401), a CH stretching vibration (2966), a conjugated carbonyl group (1680), and an aromatic ring system (1608). The ^{1}H NMR spectrum clearly indicates signals due to a *para*-disubstituted B-ring [δ 7.99 (2H, d, J = 8.8 Hz, H-2′/6′), 6.90 (2H, d, J = 8.8 Hz, H-3′/5′)] and a 5,7-disubstituted A-ring [δ 6.39 (1H, d, J = 2.4 Hz, H-8), 6.19 (1H, d, J = 2.4 Hz, H-6)] on the flavonol skeleton. The ^{13}C NMR and DEPT spectra of roxyloside A showed resonances for 26 carbons and the presence of a kaempferol moiety with hexose and pentose moieties (see the NMR data). At the sugar moieties, an anomeric proton signal at δ 5.47 (d, J = 5.6 Hz) and a carbon signal at δ_C 101.5 (C-1″), including oxygenated methine and methylene carbons such as δ_C 81.0 (C-4″), 74.8 (C-2″), 70.3 (C-3″), and 65.9 (C-5″), suggest the presence of a β-xylopyranosyl group. The connection between the xylopyranosyl unit (C-1″) and the C-3 of the aglycone was verified by the cross-peak between δ 5.47 (H-1″) and δ_C 134.8.0 (C-3) in the HMBC spectrum. A further anomeric proton signal at δ 4.67 (d, J = 7.6 Hz) and a carbon signal at δ_C 104.8 (C-1‴) were also observed. The chemical shifts of other glycosidic carbon signals at δ_C 78.1 (C-3‴), 77.8 (C-5‴), 75.2 (C-2‴), 71.3 (C-4‴), and 62.6 (C-6‴) suggest the presence of a β-glucopyranosyl unit. Moreover, the cross peak between the anomer proton signal of glucopyranose (δ 4.67, H-1‴) and the oxygenated methine carbon signal (δ_C 81.0, C-4″) of the xylopyranose suggest that the β-glucopyranosyl group is linked to the hydroxyl of C-4″ of xylopyranose. The observation was confirmed by the downfield shifts of the carbon (δ_C 81.0) and proton signals (δ 3.85, H-4″), owing to the glycosidation effect. This confirmed that C-1‴−OH of D-glucose is linked to C-4″−OH of D-xylose and C-1″−OH of D-xylose is attached to C-3-OH of the aglycone, kaempferol. The peaks at m/z 581 $[M + H]^+$, 419 $[M - \text{glucose} + H]^+$, and 287 $[M - \text{glucose} - \text{xylose} + H]^+$ in the positive FAB-MS spectrum of roxyloside A were also in good agreement with the above deduced structure. Using these spectroscopic analyses, the structure of roxyloside A was determined to be kaempferol-3-*O*-β-D-glucopyranosyl (1→4)-β-D-xylopyranoside (**Figure 1**). To our knowledge, this is the first report on the isolation of roxyloside A from a natural source.

The other four compounds isolated from the EtOAc fraction were identified as isoquercitrin (*28*), afzelin (*29*), cyanidin-3-*O*-β-glucoside (*30*), and quercetin gentiobioside (*31*), respectively, using EI-MS, FAB-MS, and ^{1}H and ^{13}C NMR.

Inhibition of HMG-CoA Reductase. The four isolated compounds, except cyanidin-3-*O*-β-glucoside, completely inhibited HMG-CoA reductase activity at a concentration of 0.12 mg/mL, while an anthocyanin, cyanidin-3-*O*-β-glucoside, exhibited no inhibitory effect on the enzyme (data not shown). Lovastatin, a cholesterol lowering agent, was used as a positive control in this

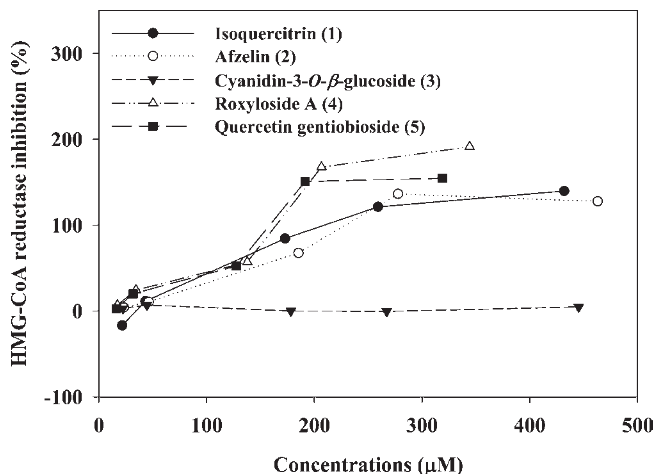

Figure 2. HMG-CoA reductase inhibition rates of the five compounds isolated from the EtOAc fraction of *Rosa damascena*.

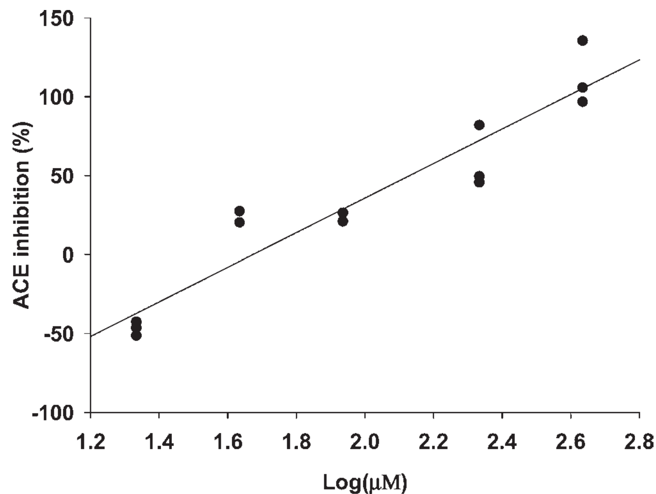

Figure 3. ACE inhibition rate of cyanidin-3-*O*-β-glucoside (**3**) isolated from the EtOAc fraction of *Rosa damascena*.

Table 1. IC_{50} Values of the Five Compounds Isolated from the EtOAc Fraction of *Rosa damascena* for HMG-CoA Reductase and ACE

	IC_{50} value[a] (μM)	
	HMG-CoA reductase	ACE
isoquercitrin (**1**)	80.6	
afzelin (**2**)	80.1	
cyanidin-3-*O*-β-glucoside (**3**)		138.8
roxyloside A (**4**)	47.1	
quercetin gentiobioside (**5**)	50.6	

[a] The IC_{50} value represents the concentration of each compound that inhibits HMG-CoA reductase or ACE activity by 50%.

study, due to its structural similarity to mevastatin and similar biochemical and pharmacological activities. Lovastatin completely inhibited HMG-CoA reductase at a concentration of 0.01 mg/mL (data not shown). To further characterize the inhibitory action of these compounds, the five compounds at different concentrations, ranging from 0.01 to 0.20 mg/mL, were tested. As shown in **Figure 2**, the four isolated compounds, except cyanidin-3-*O*-β-glucoside, suppressed HMG-CoA reductase activity in concentration-dependent manners. The calculated IC_{50} values, corresponding to the concentration that inhibits the enzyme activity by 50% at a given substrate value, were 80.6, 80.1, 47.1, and 50.6 μM, respectively (**Table 1**), similar to the values reported by Park et al. (*34*). In the report, luteolin-7-*O*-β-D-glucoside and hyperoside isolated from the EtOAc fraction of *Angelica keiskei* inhibited HMG-CoA reductase activity by 65.5% and 14.8%, respectively, at a concentration of 30 μM. Among the five isolated compounds, the new compound, roxyloside A, was the most potential inhibitor to HMG-CoA reductase.

Inhibition of ACE. It was elucidated that only cyanidin-3-*O*-β-glucoside from the EtOAc fraction of *R. damascena* significantly suppressed ACE activity at a concentration of 0.2 mg/mL, whereas the other four compounds did not inhibit ACE (data not shown). The IC_{50} value of cyanidin-3-*O*-β-glucoside (**Figure 3**) against ACE was determined to be 138.8 μM (**Table 1**). Captopril was used as a reference inhibitor, and its IC_{50} value was determined to be 9.12 nM (data not shown), similar to the reference values in the literature for captopril, which are in the range of 8.91−23 nM (*8*). Through the interactions between flavonoids and iron copper ions, flavonoids can form complexes with the ions and subsequently reduce metal ions. It was reported that the ability of flavonoids to chelate iron ions depends on the molecular structure containing a hydroxyl group at the 3, 5, and 3,4′ positions (*35*). Therefore, it was assumed that the strong affinity of the 3,4′-dihydroxy group of cyanidin-3-*O*-β-glucoside to metal ions may be a key factor for enhancing ACE inhibitory activity.

In conclusion, we isolated five flavonoids, including a new compound, roxyloside A, showing a high level of cardiovascular protective activity from buds of *R. damascena*. It was elucidated that HMG-CoA reductase is suppressed by the four isolated compounds isoquercitrin, afzelin, roxyloside A, and quercetin gentiobioside, whereas cyanidin-3-*O*-β-glucoside inhibits ACE activity. Consequently, the bioactive phenolics from the buds of *R. damascena* may improve the cardiovascular condition, in part, by inhibition of HMG-CoA reductase or ACE.

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