Short Communication

Radical-Scavenging Activities of Four Quinochalcones of Safflower

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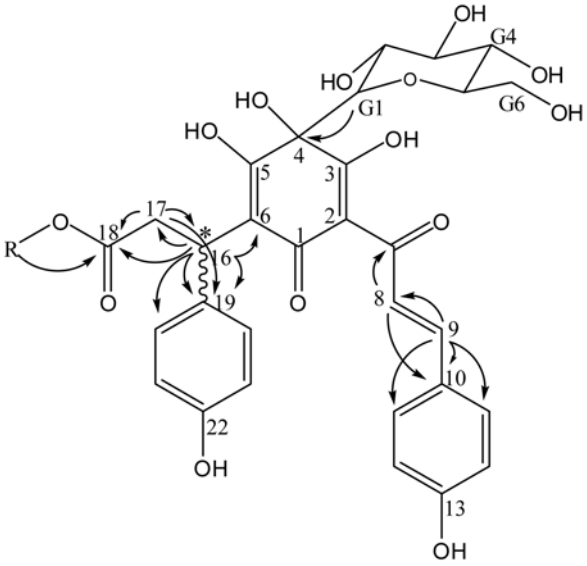
Safflower florets (*Carthamus tinctorius* L., Compositae) have been used as natural red and yellow color additives for cosmetics, foods and soft drinks, and also as a source of folk medicine for the treatment of cardiovascular and hematological diseases by removing the blood stasis [Tang and Eisenbrand, 1992]. The structures of these red and yellow colorants, carthamin, hydroxysafflor yellow A, safflor yellow B, safflomin A, safflor yellow A, safflomin C, isosafflomin C, tinctormin, precarthamin, anhydrosafflor yellow B, and cartormin, in the petals of safflower are classified as members of the *C*-glucosylquinochalcone family of the flavonoids that occurs only in *C. tinctorius* [Kazuma *et al*., 2000]. As a part of our ongoing search for the chemical constituents from *C. tinctorius*, we isolated seven *C*-glucosyl-quinochalcones (carthamin, hydroxysafflor yellow A, safflor yellow B, precarthamin, cartormin, safflomin C, and isosafflomin C) and examined their pH and thermal stabilities, enzymatic conversions, and antioxidant activities [Kim and Paik, 1997; Cho *et al*., 2000; Yoon *et al*., 2003; Lim *et al*., 2007; Yoon *et al*., 2007; Yoon and Paik, 2008]. Our continuing work on the BuOH–soluble fraction of the safflower florets by HPLC led to the detection of two new minor peaks, along with the peaks of safflomin C [Onodera *et al*., 1989] and isosafflomin C [Yoon and Paik, 2008]. This paper describes the radical-scavenging activities of safflomin C, isosafflomin C, and two new related quinochalcones, methylsafflomin C and methylisosafflomin C (**1-4**, Fig. 1), against the ABTS radical.



	R	C-16 (stereochemistry)
1	H	R
2	H	S
3	CH_3	R
4	CH_3	S

Fig. 1. Structures of 3 (methyl ester of safflomin C, 1) and 4 (methyl ester of isosafflomin C, 2) with opposite configuration relationship at C-16 and selected HMBC (H→C) correlation of 3.

The dried petals of *C. tinctorius* (3 kg) were extracted at room temperature with 80% aqueous MeOH (10 L×2). After filtration and evaporation of the solvent under reduced pressure, the combined crude extract was suspended in water and then successfully partitioned with EtOAc and *n*-BuOH to afford EtOAc- and BuOH-soluble fractions. The BuOH-soluble fraction was subjected to Sephadex LH-20 (5.5×15 cm) using 20~70% CH_3CN/H_2O gradient to yield five fractions. The fourth fraction (1.0 g) was subjected to reversed phase HPLC [20~40% CH_3CN/0.1% TFA-H_2O; ODS column, 250×10 mm; flow rate, 2 mL/min; MD2010 UV PDA detector (JASCO, Tokyo, Japan)] to afford safflomin C (**1**, R_t 28.107 min) and isosafflomin C (**2**, R_t 30.093 min) [Yoon and Paik, 2008]. Two new minor constituents, **3** (R_t 40.573 min, 8 mg) and **4** (R_t 39.773 min, 4.5 mg) accompanied **1** and **2**.

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Abbreviations: ABTS, 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TFA, trifluoroacetic acid

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Compound **3:** $[\alpha]_D$ +22.4 (*c* 0.03, MeOH); UV (MeOH) 404, 340 (sh), 227 nm; ESI-LCMS (quadrupole) *m/z* 627.4 $[M-H]^+$; ^1H NMR (400 MHz, CD_3OD); δ 7.71 (1H, d, *J*=15.6 Hz, H-9), 7.50 (2H, d, *J*=8.4 Hz, H-11, 15), 7.43 (1H, d, *J*=15.6 Hz, H-8), 7.19 (2H, d, *J*=8.4 Hz, H-20, 24), 6.79 (2H, d, *J*=8.4 Hz, H-12, 14), 6.64 (2H, d, *J* =8.4 Hz, H-21, 23), 4.71 (1H, dd, *J*=8.0, 8.0 Hz, H-16), 3.70 (1H, br d, *J*=12.0 Hz H-G6), 3.58 (3H, s, OMe), 3.51 (1H, dd, *J*=12.0, 2.0 Hz H-G6), 3.47 (1H, d, overlapped, H-G1), 3.46 (1H, overlapped, H-G2), 3.39 (1H, dd, *J*=9.2, 9.2 Hz, H-G4), 3.22 (1H, dd, *J*=9.2, 9.2 Hz, H-G3), 3.21 (1H, overlapped, H-17), 3.09 (1H, dd, *J* =16.0, 8.0 Hz, H-17), 2.92 (1H, br d, *J*=9.2 Hz, H-G5); ^{13}C NMR (100 MHz, CD_3OD); δ 195.50 (C-3), 192.53 (C-1), 180.22 (C-7), 174.98 (C-18), 172.50 (C-5), 161.14 (C-13), 156.39 (C-22), 143.41 (C-9), 135.43 (C-19), 131.51 (C-11, 15), 129.47 (C-20, 24), 128.22 (C-10), 119.14 (C-8), 116.68 (C-12, 14), 115.49 (C-21, 23), 112.50 (C-6), 108.42 (C-2), 88.19 (C-G1), 80.54 (C-G5), 78.41 (C-G3), 78.41 (C-4), 70.39 (C-G2), 69.54 (C-G4), 61.02 (C-G6), 51.98 (C-OMe), 38.57 (C-17), 36.51 (C-16).

Compound **4:** $[\alpha]_D$ −16.0 (*c* 0.03, MeOH); UV (MeOH) 408, 337 (sh), 231 nm; ESI-LCMS (quadrupole) *m/z* 627.3 $[M-H]^+$; ^1H NMR (400 MHz, CD_3OD); δ 7.70 (1H, d, *J*=16.0 Hz, H-9), 7.49 (2H, d, *J*=8.4 Hz, H-11, 15), 7.42 (1H, d, *J*=16.0 Hz, H-8), 7.16 (2H, d, *J*=8.8 Hz, H-20, 24), 6.78 (2H, d, *J*=8.4 Hz, H-12, 14), 6.63 (2H, d, *J* =8.8 Hz, H-21, 23), 4.77 (1H, dd, *J*=9.2, 7.2 Hz, H-16), 3.77 (1H, br d, *J*=11.6 Hz, H-G6), 3.69 (1H, dd, *J*=11.6, 2.4 Hz, H-G6), 3.59 (3H, s, OMe), 3.52 (1H, overlapped, H-G1), 3.52 (1H, overlapped, H-G2), 3.41 (1H, dd, *J*= 9.6, 9.6 Hz, H-G4), 3.26 (1H, overlapped, H-G3), 3.25 (1H, overlapped, H-17), 3.08 (1H, dd, *J*=15.6, 7.2 Hz, H-17), 3.05 (1H, m, H-G5).

During the isolation of safflomin C (**1**, R_t 28.107 min) by HPLC, a minor peak of compound **3** (R_t 40.573 min) was detected (Fig. 2A). A minor peak of compound **4** (R_t 39.773 min) was also accompanied with the peak of isosafflomin C (**2**, R_t 30.093 min; Fig. 2B). The UV spectrum patterns of compounds **3** (404, 340 sh, 227 nm) and **4** (408, 337 sh, 231 nm) were very similar with those of **1** (406, 346 sh, 230 nm) and **2** (407, 348 sh, 230 nm), suggesting that both minor components **3** and **4** were also *C*-glucosylquinochalcones. The ESI-LCMS (ESI-; quadrupole) of **3** and **4** showed the peaks at *m/z* 627.4 $[M-H]^+$ and 627.3 $[M-H]^+$, respectively. The molecular weight difference of **1** and **2** (both 614) and that of **3** and **4** (both 628) was 14, suggesting the presence of a methyl group in **3** and **4**.

The ^1H and ^{13}C NMR spectra of **3** (R_t 40.573 min) were very similar to those of **1** (R_t 28.107 min), suggesting that **3** (^1H, δ 3.58; ^{13}C, δ 51.98) was a methyl ester of **1**. In the

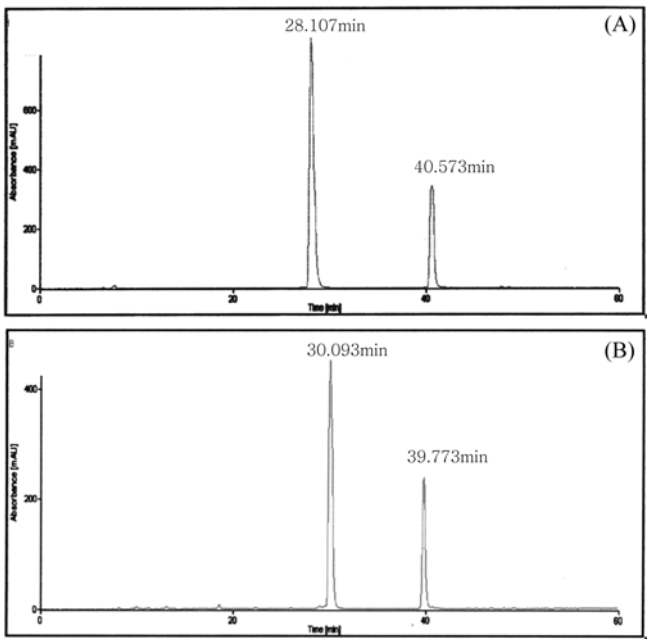

Fig. 2. Characteristic HPLC profiles of (A) 1 and 3 and (B) 2 and 4 at 400 nm.

^1H NMR spectrum of **3**, two trans-olefinic protons at δ 7.71 (1H, d, *J*=15.6 Hz, H-9) and 7.43 (1H, d, *J*=15.6 Hz, H-8), and aromatic protons of two AA'BB' spin systems at δ 7.50 (2H, d, *J*=8.4 Hz, H-11, 15) and 6.79 (2H, d, *J*= 8.4 Hz, H-12, 14), and at δ 7.19 (2H, d, *J*=8.4 Hz, H-20, 24) and 6.64 (2H, d, *J*=8.4 Hz, H-21, 23) were observed. One *C*-glucosyl anomeric proton resonated at δ 3.47 (1H, overlapped with H-G2) was connected with six sugar protons of the glucosyl moiety at δ 3.46 (H-G2), 3.22 (H-G3), 3.39 (H-G4), 2.92 (H-G5), and 3.70 and 3.51 (2H, H-G6). In the HMBC spectrum of **3**, the presence of the following cross peaks were observed: H-16 (δ 4.71) with C-18 (δ 174.98), C-19 (δ 135.43), C-20, 24 (δ 129.47), C-6 (δ 112.50), and C-17 (δ 38.57); H-17 (δ 3.21 and 3.09) with C-18 (δ 174.98), C-19 (δ 135.43), and C-16 (δ 36.51). The HMBC spectrum of **3** also showed that the methoxy group (δ 3.58) was connected with C-18 (δ 174.98), indicating that **3** was a methyl ester of **1**. These ^1H and ^{13}C NMR and HMBC data of **3**, with the characteristic UV absorption maxima at 404 and 227 nm and mass data (molecular weights of **1** and **3**, 614 and 628, respectively) were consistent with the structure of the methyl ester of **1**. Although the amount of compound **4** (R_t 39.773 min) was not sufficient to take ^{13}C NMR spectrum, its ^1H NMR spectrum, together with its UV absorption maxima at 408 and 231 nm and mass data (molecular weights of **2** and **4**, 614 and 628, respectively), were very similar to those of **2** (R_t 28.107 min), indicating that **4** (^1H; δ 3.59) was a methyl ester of **2**. These findings confirmed the structures of **3** and **4** were methylsafflomin

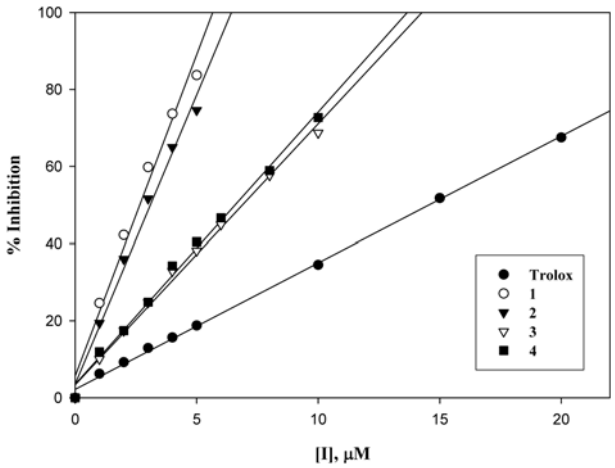

Fig. 3. ABTS radical-scavenging activities of quinochalcones 1-4 and Trolox.

C and methylisosafflomin with opposite configuration relationship at C-16 as in **1** and **2** (Fig. 1), respectively.

Measurement of the radical-scavenging activities of compounds **1-4** was carried out using the decolorization of the ABTS radical at 734 nm [van den Berg *et al*., 1999; Huang *et al*., 2005]. The radical-scavenging activities of these compounds and Trolox, the standard reference compound, showed dose-dependent effects on the ABTS radical (Fig. 3). Trolox suppressed the absorbance of the ABTS radical with the EC_{50} value of 14.4±0.22 μM [Yoon *et al*., 2007], whereas those of **1-4** were 2.24±0.06, 2.74±0.01, 6.50±0.02, and 6.15±0.05 μM, respectively (Fig. 3), indicating these *C*-glucosylquinochalcones have strong free radical-scavenging activities. Although the EC_{50} values of **1-4** did not show significant differences, **1** and **2** (2.24 and 2.74 μM) had better antioxidant activities than **3** and **4** (6.50 and 6.15 μM), suggesting carboxylic acid group could play some role. These EC_{50} values (2.24-6.50 μM) are comparable to the values of caffeic acid (5.49 μM) [Lim *et al*., 2007] and flavonol glycosides (kaempferol 3-*O*-sophoroside, 4.07 μM; quercetin 3-*O*-rutinoside, 5.26 μM; kaempferol 3-*O*-rutinoside, 6.08 μM) from the BuOH-soluble fraction of this plant [Yoon *et al*., 2007].

In conclusion, four *C*-glucosylquinochalcones (safflomin C, isosafflomin C, methylsafflomin C, and methylisosafflomin C) were isolated from the BuOH-soluble fraction of safflower using reversed phase HPLC. These four isolated quinochalcones exhibited EC_{50} values similar with those of caffeic acid and ascorbic acid, with Trolox Equivalent Antioxidant Capacity value of 1.34 as determined by ABAP/ABTS assay [Floris *et al*., 2000], and showed strong antioxidant activities against the ABTS radical system.

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