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Isolation of phenolic constituents and characterization of antioxidant markers from sunflower (*Helianthus annuus*) seed extract

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ABSTRACT

A new compound, benzyl alcohol β-D-apiofuranosyl-(1→6)-β-D-(4-*O*-caffeoyl) glucopyranoside (**1**), was isolated from the seed of sunflower (*Helianthus annuus*), together with eight known phenolic compounds: caffeic acid (**2**), methyl caffeoate (**3**), chlorogenic acid (**4**), 4-*O*-caffeoylquinic acid (**5**), 3-*O*-caffeoylquinic acid (**6**), methyl chlorogenate (**7**), 3,5-di-*O*-caffeoylquinic acid (**8**), and eriodictyol 5-*O*-β-D-glucoside (**9**). Their structures were elucidated on the basis of spectroscopic methods and chemical evidence. The antioxidative effect of the phenolic constituents from the sunflower seeds was also evaluated based on the oxygen-radical absorbance capacity (ORAC), and the fraction containing caffeic acid derivatives showed a high antioxidant potency.

1. Introduction

Sunflower (*Helianthus annuus* L.) is an annual plant native to North America, and one of the most important oilseed crops, being the second largest oilseed crop as a global source of vegetable oil (Canella and Sodini, 1977). Sunflower seeds are widely used in the food and nutraceutical industries because of their high oil and protein contents and other valuable bioactive components. The most abundant fatty acids in sunflower oil are linoleic acid (*ca.* 65%), oleic acid (*ca.* 25%), and palmitic and stearic acids (each *ca.* 5%) (Canella et al., 1982; Economides, 1998; Pereira et al., 2003). Sunflower oil also contains high levels of tocopherols and phytosterols (Rashid et al., 2009). On the other hand, extracts with high antioxidant activity may also be obtained from sunflower seed shells and kernels (De Leonardis et al., 2003). In Japan, natural antioxidant "sunflower seed extract" is defined as an ethanol or hot water extract from the seeds of sunflower, and this additive is characterized as an antioxidant containing isochlorogenic and chlorogenic acids (Notice No. 210, 1996). Sunflower seeds are thus suggested to be rich in polyphenols. This paper describes the isolation and structural characterization of phenolics including a new glycoside from the seed of sunflower. The antioxidant activities of fractions partitioned with solvent and the isolated phenolics were also estimated by an oxygen radical absorbance capacity (ORAC) assay (Ou et al., 2001; Huang et al., 2002).

2. Results and discussion

A homogenate of sunflower seeds in 80% ethanol (EtOH) was extracted with *n*-hexane and ethyl acetate (EtOAc) to give the respective *n*-hexane, EtOAc, and water extracts. The antioxidative activity of each extract was evaluated by ORAC (Fig. 1A). The EtOAc extract, which exhibited significant antioxidant activity, was repeatedly chromatographed over MCI-GEL CHP-20P and/or YMC GEL ODS-AQ with aqueous methanol (MeOH) in a stepwise gradient mode to afford a new compound (**1**), together with caffeic acid (**2**), methyl caffeoate (**3**) (Fujioka et al., 1999), chlorogenic acid (**4**) (Iwai et al., 2004), methyl chlorogenate (**7**) (Deyama et al., 1987; Ge et al., 2007), isochlorogenic acid (3,5-di-*O*-caffeoylquinic acid (**8**)) (Dini et al., 2006), and eriodictyol 5-*O*-β-D-glucoside (**9**) (Gujer et al., 1986). Similar chromatographic separation of the water extract gave **4**, 4-*O*-caffeoylquinic acid (**5**), and 3-*O*-caffeoylquinic acid (**6**) (Iwai et al., 2004). The known compounds **2**–**9** were identified, respectively, by direct comparison with authentic specimens and by comparison of their spectral data with those reported in the literature.

Compound **1** was isolated as a brown amorphous powder. Its molecular formula was assigned as $C_{27}H_{32}O_{13}$ from its HR-ESI-MS (*m*/*z* 563.1741 $[M-H]^-$; calcd. for $C_{27}H_{32}O_{13}$-H: 563.1765) and ^{13}C-NMR (27 ^{13}C signals) spectra. The UV spectrum showed absorption maxima at 207, 248sh, 289, and 330 nm, respectively.

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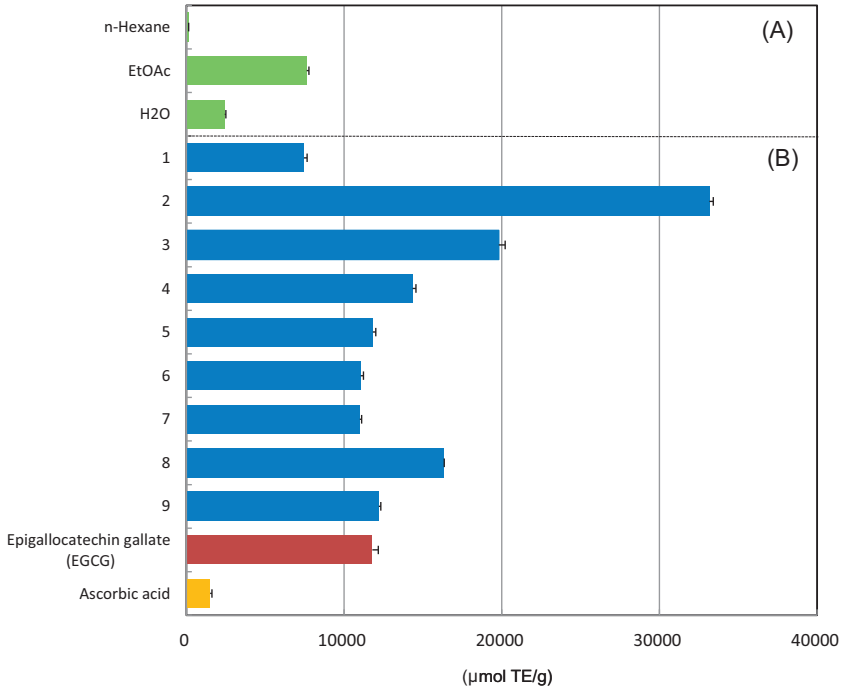

Fig. 1. ORAC values of each fraction (A) and isolated compounds **1**–**9** (B).

The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited the signals characteristic of *trans*-caffeoyl and benzyl moieties, as follows. The ^{1}H NMR spectrum exhibited AB-type proton signals due to a benzylic methylene group at δ 4.68 and 4.91 (each d, J = 12 Hz, H-7) and multiplets due to a phenyl group at δ 7.25–7.43 (5H) as well as those assignable to a caffeoyl group [δ 6.28 (d, J = 16 Hz, H-8′), 6.77 (d, J = 8.5 Hz, H-5′), 6.95 (dd, J = 2, 8.5 Hz, H-6′), 7.05 (d, J = 2 Hz, H-2′), and 7.68 (dd, J = 16 Hz, H-7′)]. These aromatic units were also supported by 16 carbon signals due to the caffeoyl [δ 114.8, 115.2, 116.5, 123.1, 127.7, 146.8, 147.6, 149.8, 168.4 (C-1′-9′)] and benzyl group [δ 71.9, 128.8, 129.3 (4C), 138.9 (C-1–7)] in the ^{13}C NMR spectrum. The presence of two sugar units in **1** was shown by two anomeric proton signals at δ 4.40 (d, J = 8 Hz, Glc H-1) and 4.95 (d, J = 2 Hz, Api H-1) and others assigned by the ^{1}H–^{1}H shift correlation spectrum (Table 1). The sugar residues were presumed to be hexose and branched pentose, as revealed by the ^{13}C NMR spectrum exhibiting 11 aliphatic carbon signals which included a quaternary (δ 80.6, Api C-3) and three methylene carbon resonances (δ 68.6, 65.7 and 75.1). D-Glucose and D-apiose as the sugar units in **1** were confirmed by acid hydrolysis followed by RP-HPLC analysis for derivatives prepared by reaction with L-cysteine methyl ester and *O*-tolyl isothyocyanate according to the previously reported method (Tanaka et al., 2007). The linking position of each unit was determined by cross-peaks among glucose H-1 (δ 4.40)/C-7 (δ 71.9) of the benzyl group, glucose H-4 (δ 4.87)/C-9′ (δ 168.4) of the caffeoyl group, and apiose H-1 (δ 4.95)/glucose C-6 (δ 68.6) in HMBC (Fig. 2). β-Glycosidic linkage in the glucose core was evidenced by large coupling constants (J = 8 Hz). The configuration at the anomeric center of the apiose was also determined as β on the basis of the comparison of the ^{13}C-NMR data for **1** with those for α- and β-D-apiofuranoside (Kitagawa et al., 1989), and the $J_{H\text{-}1,\ H\text{-}2}$ coupling constant consistent with reported data of β-D-apiofuranoside (Liu et al., 2010). Therefore, **1** was established as benzyl alcohol β-D-apiofranosyl-(1→6)-β-D-(4-*O*-caffeoyl) glucopyranoside.

To clarify the relationship between the polarity of the ingredients and antioxidative activity, the ORAC values were estimated for each fraction obtained by partitioning with organic solvent for the sunflower seed extracts (Fig. 1A). As a result, the EtOAc fraction exhibited a marked activity which is considered to be responsible for **4** and **8** as the main component in the extract (Fig. 3). The antioxidant activity of isolated compounds **1**–**9** was also evaluated (Fig. 1B). All of them showed potent antioxidative activity with ORAC values of *ca.* 10,000 μmol TE/g or above. It is particularly notable that the potency of **2** was about three times more potent than that of epigallocatechin gallate (EGCG), a typical tea catechin. These results suggest that the antioxidant activity of

Table 1
^{1}H-(500 MHz) and ^{13}C-NMR(126 MHz) data of compound **1** measured in MeOH-d_4.

Position	δ_C	δ_H (J in Hz)
1	138.9	–
2	129.3	7.25-7.43[a]
3	129.3	7.25-7.43[a]
4	128.8	7.25-7.43[a]
5	129.3	7.25-7.43[a]
6	129.3	7.25-7.43[a]
7	71.9	4.68 (d, J = 12), 4.91 (d, J = 12)
1′	127.7	–
2′	115.2	7.05 (d, J = 2)
3′	146.8	–
4′	149.8	–
5′	116.5	6.77 (d, J = 8.5)
6′	123.1	6.95 (dd, J = 2, 8.5)
7′	147.6	7.68 (d, J = 16)
8′	114.8	6.28 (d, J = 16)
9′	168.4	–
Glucose (Glc)-1	103.2	4.40 (d, J = 8)
2	75.3	3.35 (dd, J = 8, 9.5)
3	75.8	3.60 (t, J = 9.5
4	72.8	4.87 (t, J = 9.5)
5	74.9	3.66 (m)
6	68.6	3.53 (dd, J = 6.5, 11), 3.73 (dd, J = 2.5, 11)
Apiose (Api)-1	111.1	4.95 (d, J = 2)
2	78.1	3.89 (d, J = 2)
3	80.6	–
4	65.7	3.53 (d, J = 9.5), 3.74 (d, J = 9.5)
5	75.1	3.56 (2H, s)

[a] Overlapped signals.