Structural Identification of Two Antioxidant Quinic Acid Derivatives from Garland (*Chrysanthemum coronarium* L.)

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The structures of a novel compound, 3,5-dicaffeoyl-4-succinylquinic acid, and the related compound 3,5-dicaffeoylquinic acid were determined by analysis of spectral data. These caffeoylquinic acid derivatives, with antioxidative activity, were isolated from the leaves of garland (*Chrysanthemum coronarium* L.).

Keywords: *Antioxidants, dicaffeoylquinic acids, Chrysanthemum coronarium*

INTRODUCTION

The garland (*Chrysanthemum coronarium* L.) has been regarded as a health food in East Asia because the edible portions, such as leaf and stem, contain abundant β-carotene, iron, potassium, calcium, and dietary fiber (Wills et al., 1984). In addition to these common nutrients, some compounds responsible for the chemoprevention of cancers and other diseases are thought to be contained in garland. Two compounds, SP-1 and SP-2, were isolated during a screening test for antioxidative activity (Tsushida et al., 1994). Based on infrared spectra (IR), mass spectra (MS), nuclear magnetic resonance spectra (NMR), and circular dichroism spectra (CD), we report here the stereochemical structures of SP-1 and SP-2.

MATERIALS AND METHODS

Reagents. Acetone-d_6 was obtained from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Preparation. The garland was purchased from a retail source. Garland leaves and stems (800 g) were minced and extracted twice with 2 L of 80% methanol in water. The methanolic extract was filtered through no. 5A filter paper and then concentrated to dryness with a rotary evaporator.

Isolation. The crude extract, dissolved in 60 mL of 30% methanol in water containing 0.2% formic acid, was subjected to gel permeation chromatography (GPC) on a 40 × 450-mm column (Toyopearl HW − 40; TOSOH, Tokyo) each fraction was 10 mL, and gradient elution started from fraction no. 18 and finished at fraction 104, with the linear gradient of 30−100% methanol in water containing 0.2% formic acid. The ultraviolet (UV) detector was set at 330 nm. The active fractions (80−110) were combined and further purified by high-performance liquid chromatography (HPLC; JASCO Company Ltd., Hachioji, Japan) on a 20 × 250-mm column (Inertsil ODS; GL Sciences Inc., Tokyo). The samples were eluted with the linear gradient of 12−40% acetonitrile in 10 mM phosphate buffer (pH 2.5) at a flow rate of 7.0 mL/min. The UV detector was set at 280 nm. Two major antioxidants, SP-1 (retention time, 19 min; 310 mg) and SP-2 (retention time, 28 min; 185 mg) were isolated (Tsushida et al., 1994).

Antioxidant Assay. The antioxidant activity was assayed by the β-carotene/linoleate method (Miller, 1971) with a modification. The assay reagent was prepared as follows. After the chloroform solutions of β-carotene (0.5 mg/0.5 mL), linoleate (20 mg/0.2 mL), and Tween 40 (polyoxyethylene (20) sorbitan monopalmitate, 200 mg/mL) were mixed, chloroform was removed under a stream of nitrogen. The mixture was dissolved in distilled water (100 mL) with gentle shaking. To maintain the pH, 0.2 M phosphate buffer (pH 6.8, 4 mL) was added to the solution (45 mL). The aliquots (4.9 mL) of this reagent were transferred into a tube containing test sample (100 μL, finally 1 ppm). The absorbance at 470 nm was recorded after 0, 5, 10, 15, 20, 30, 40, 50, and 60 min on a UV−vis spectrometer (UV-3100, Shimadzu Company, Kyoto, Japan).

Structural Identification. The UV absorption spectra were recorded on a UV-3100 spectrometer. The MS of the compounds were obtained on an SX-102 spectrometer (JEOL Ltd., Akishima, Japan) by fast-atom bombardment (FAB) ionization. The IR spectra were analyzed on an FTIR-4200 spectrometer (Shimadzu Company, Kyoto, Japan) in KBr. The CD spectra were measured at room temperature on a J-720W spectrometer (JASCO Company Ltd., Hachioji, Japan). The ^{1}H NMR data at 600.05 MHz and the ^{13}C NMR data at 150.80 MHz were recorded on a JNM-A 600 spectrometer (JEOL Ltd., Akishima, Japan) at room temperature in deuterated acetone. Chemical shifts are reported relative to tetramethylsilane as the internal standard. Specific rotations ($[\alpha]_D$) were measured at room temperature (23 °C) on a DIP-181 spectrometer (JASCO Company, Ltd. Hachioji, Japan).

RESULTS AND DISCUSSION

Structural Determination. Physicochemical properties of the two isolates were as follows. *SP-1:* colorless plates; mp 150−153 °C; UV (ethanol) λ_{max} 331 (ϵ 3.34 × 10^4), 246 (1.89 × 10^4), 220 (2.56 × 10^4) nm; CD (ethanol) λ_{ext} 344 ($\Delta\epsilon$ − 16.23), 288 (+6.21), 249 (+1.06), 227 (−1.04), 212 (+3.56) nm; FAB-MS, *m*/*z* 517 ($[M + H]^+$, relative intensity 20.5%), 631 ($[M + Na + glycerol]^+$, 2.2), 539 ($[M + Na]^+$, 8.9), 499 (7.6), 337 (9.9), 163 (100); high-resolution FAB-MS calcd for $C_{25}H_{25}O_{12}$ ($[M + H]^+$) 517.1346 found 517.1380. NMR assignments are shown in Tables 1 and 2.

SP-2 (Figure 1): colorless plates; mp 128−130 °C; UV (ethanol) λ_{max} 333 nm (ϵ 3.16 × 10^4), 247 (1.79 × 10^4), 220 (2.43 × 10^4); $[\alpha]^{23}_D$ −204° (methanol, *c* 1.53); CD (ethanol) λ_{ext} 345 ($\Delta\epsilon$ − 12.1), 289 (+3.68), 243 (−0.33), 224 (−1.90), 207 (+2.18) nm; IR ν_{max} 3435, 1714, 1630, 1604, 1516, 1445, 1365, 1277, 1178, 1130, 980, 814 cm^{-1}; FAB-MS, *m*/*z* 617 ($[M + H]^+$, relative intensity 17.0%), 709 ($[M + H + glycerol]^+$, 3.8), 455 (2.5), 437 (5.8), 337 (2.5), 163 (100); high-resolution FAB-MS calcd for $C_{29}H_{29}O_{15}$, 617.1506, found 617.1506. NMR assignments are shown in Tables 1 and 2.

SP-1 and SP-2 are thought to be dicaffeoylquinic acid and a derivative, respectively, according to mass spectra

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Table 1. ^{1}H NMR Spectral Data of SP-1 and SP-2

	^{1}H NMR, ppm		J, Hz		
	SP-1	SP-2		SP-1	SP-2
2_{eq}	[a]2.245 br d	2.274 dd	$J_{2ax,\,2eq}$		13.3
2_{ax}	[a]2.245 br d	2.337 dd	$J_{2_{eq},3}$	5.6	3.2
3	5.417 t d	5.538 ddd	$J_{2_{ax},3}$	5.6	8.3
4	4.047 dd	5.266 dd	$J_{3,4}$	7.4	8.0
5	5.464 ddd	5.605 ddd	$J_{4,5}$	3.2	3.4
6_{eq}	2.223 dd	2.199 dd	$J_{5,6_{eq}}$	7.1	6.6
6_{ax}	2.346 dd	2.463 dd	$J_{5,6_{ax}}$	3.9	4.0
2′	7.138 d	7.139 d	$J_{6_{eq},6_{ax}}$	13.7	14.2
2″	7.148 d	7.163 d	$J_{2',6'}$	2.1	2.1
5′	6.863 d	6.859 d	$J_{2'',6''}$	2.1	2.1
5″	6.865 d	6.863 d	$J_{5',6'}$	8.2	8.2
6′	[b]7.023 d	7.032 dd	$J_{5'',6''}$	8.2	8.2
6″	[b]7.023 d	7.050 dd	$J_{7',8'}$	16.0	16.0
7′	7.544 d	7.530 d	$J_{7'',8''}$	16.9	15.9
7″	7.571 d	7.591 d			
8′	6.234 d	6.219 d			
8″	6.306 d	6.305 d			
2‴		[c]2.601 m			
3‴		[c]2.601 m			

[a–c] Overlapped each other.

Table 2. ^{13}C NMR Spectral Data of SP-1 and SP-2

	^{13}C NMR, ppm		DEPT[e]
	SP-1	SP-2	3/4π
1	74.3	74.1	*
2	37.6	38.1	−
3	71.6	68.4	+
4	70.2	71.8	+
5	71.9	69.0	+
6	35.8	36.3	−
7	175.8	175.5	*
1′	127.5	127.5	*
1″	127.6	127.6	*
2′	115.1	115.3	+
2″	115.2	115.2	+
3′	[a]146.1	[b]146.2	*
3″	[a]146.1	[b]146.2	*
4′	148.7	148.8	*
4″	148.6	148.7	*
5′	[c]116.3	[d]116.3	+
5″	[c]116.3	[d]116.3	+
6′	122.5	122.7	+
6″	122.4	122.6	+
7′	145.9	146.3	+
7″	145.6	146.1	+
8′	115.5	115.1	+
8″	116.1	115.6	+
9′	166.7	166.3	*
9″	167.0	166.8	*
1‴		172.0	*
2‴		29.1	−
3‴		29.7	−
4‴		173.5	*

[a–d] Overlapped each other. [e] (+) CH or CH_3; (−) CH_2; (*) C.

fragmentation patterns and the products from hydrolytic experiments (Tsushida et al., 1994). Furthermore, the ^{1}H NMR spectrum of SP-1 revealed the signals of two caffeoyl groups and a quinic acid moiety; that is, H-3, H-4, and H-5 protons in the quinic acid moiety were observed between 4 and 5.5 ppm. Of the double doublet signals, the signal that appeared at the lowest frequency region was assigned to H-4 (4.047 ppm), which was bonded to a sp^3 carbon that was also connected to a hydroxyl group. The other two signals appeared at an analogous frequency region because H-3 and H-5 occupied symmetrical positions in the planar structure of the SP-1 molecule. However, because H-3 and H-5 had different stereochemical configurations (ax or eq given by the chirality of SP-1), they were able to be distinguished by their coupling patterns. The broad signal due to 1,2-axial coupling was assigned to H-3 (5.417

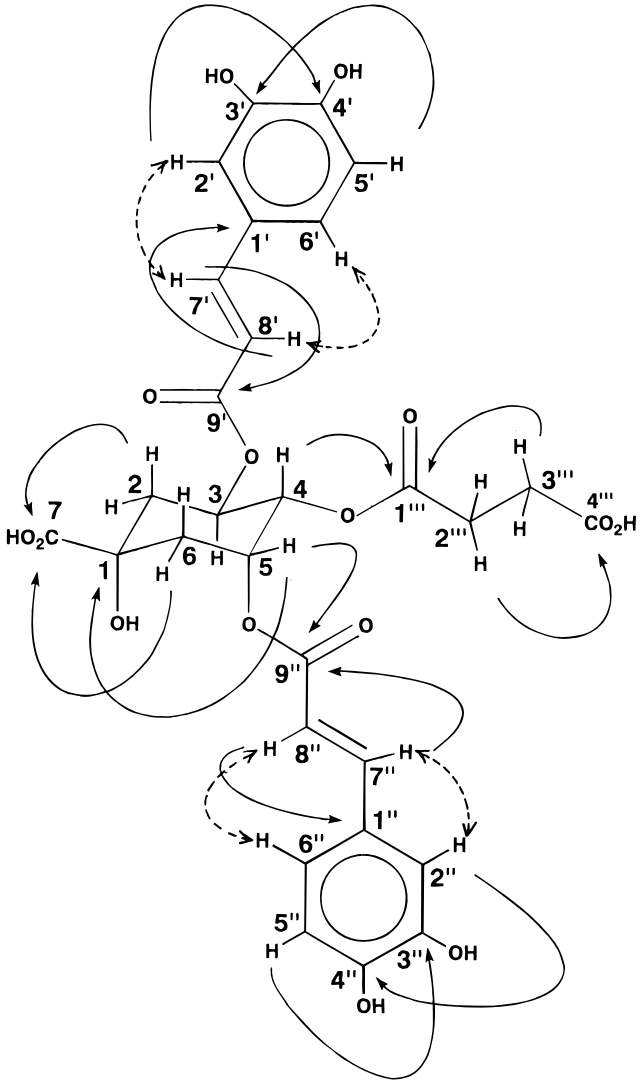

Figure 1. NOESY and HMBC of 4-succinyl-3,5-dicaffeoylquinic acid. (← – –→) NOESY; (→) HMBC.

ppm) and the other signal without axial coupling was assigned to H-5 (5.464 ppm). Two pairs of geminal protons, H-2 (ax, eq) and H-6 (ax, eq), also occupied symmetrical positions of the molecule and were observed between 2.2 and 2.35 ppm as one pair of double doublet signals and a broad triplet signal. Relative to the caffeoyl group on C-5, the conformation of H-6_{ax} was in the anti form, and that of H-6_{eq} was gauche. Both the conformations of H-2_{ax} and H-2_{eq} were in the same gauche forms relative to the caffeoyl group on C-3. Hence, H-2_{ax} and H-2_{eq} were assigned to the broad triplet signal, and H-6_{ax} and H-6_{eq} were assigned to the pair of double doublet signals. One of the double doublet signals of H-6 was broader than the other. Such broadened peaks indicate the existence of W-type long-range coupling between H-6_{eq} and H-2_{eq}.

Two caffeoyl groups were observed as a pair of nonequivalent signals with similar coupling patterns. The *trans* olefinic structure was assigned by the coupling constants (16.0 and 16.9 Hz), and the coupling patterns of the aromatic region showed that the benzene rings were 1,3,4-trisubstituted. These ^{1}H NMR assignments were also supported by the following NMR experiments: ^{13}C NMR; distortionless enhancement by polarization transfer (DEPT); ^{1}H−^{1}H nuclear Overhauser enhancement and exchange spectroscopy (NOESY, Figure 1); and heteronuclear multiple bond correlation (HMBC, Figure 1). All ^{1}H and ^{13}C NMR signals

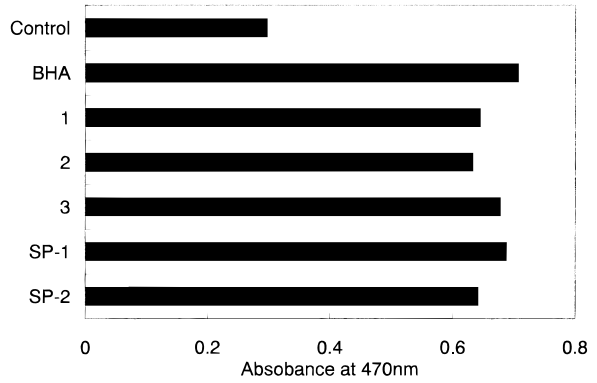

Figure 2. Antioxidant activity of the 1,3-dicaffeoylquinic acid (**1**), 4,5-dicaffeoylquinic acid (**2**), 3,4-dicaffeoylquinic acid (**3**), SP-1, SP-2, and BHA denoted in the absorbance (470 nm) at 30 min, and control (free from antioxidant) compounds **1**, **2**, and **3**, SP-1, and SP-2 were at 1 ppm, and BHA was at 0.1 ppm.

were assigned (shown in Tables 1 and 2), and SP-1 was identified as 3,5-dicaffeoylquinic acid.

The ^{1}H and ^{13}C NMR spectra of SP-2 were similar to those of SP-1 except that the chemical shift of H-4 (5.266 ppm) was observed at a higher frequency and an A_2B_2 signal appeared around 2.6 ppm. The ^{13}C NMR spectrum showed two carbonyl (ester/carboxyl region) and two methylene carbon signals in addition to similar signals for SP-1. With respect to C-4, the chemical shift of SP-2 (71.8 ppm) was observed at a higher frequency region than that of SP-1 (70.2 ppm) in contrast to no shift for C-1. Taking into account the results of MS and NMR spectral data, the structure of SP-2 was established to be 4-succinyl-3,5-dicaffeoylquinic acid.

The CD spectra of SP-1 and SP-2 did not exhibit the typical exciton split CD Cotton effects because of unsymmetrically substituted chromophores. However, the UV spectra allowed assignment of the region of Cotton curves that arised from the long-axis polarized transition of the chromophores. The negative first and positive second signs of SP-1 led to the 3*R*, 5*R* absolute configuration (Harada and Nakanishi, 1983). A similar spectrum, including sign, position, and intensity, was observed in SP-2, indicating the equivalent stereochemistry of two caffeoyl moieties. The data implied that both SP-1 and SP-2 had identical conformations in solution and confirmed the assignment of NMR signals of SP-2 by reference to those of SP-1.

Evaluation of Antioxidant Activity. The decay curves of β-carotene (0−60 min) in the presence of SP-1 or SP-2 were approximately equivalent to other three dicaffeoylquinic acids (i.e., 1,3-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid). The absorbance at 30 min is represented in Figure 2. Judging from the results, we concluded that the antioxidative activity of all examined dicaffeoylquinic acids (1 ppm) was nearly equal to that of 0.1 ppm BHA (3-*tert*-butyl-4-hydroxyanisole). Maruta et al. (1995) also reported antioxidative activity of four dicaffeoylquinic acids and a tricaffeoylquinic acid using a different method. Their results approximately agreed with ours.

In conclusion we elucidated that SP-2 was a novel dicaffeoylquinic acid derivative with antioxidative ability equivalent to that of dicaffeoylquinic acid. It has been previously reported that both di- and tricaffeoyquinic acids derivatives showed antioxidant activity in burdock (*Arctium lappa* L.; Nakabayashi, 1968; Maruta et al., 1995). Both garland and burdock belong to the same family of Asteraceae. These facts suggest that various dicaffoylquinic acid derivatives contributing to the plant protection systems are contained in the family Asteraceae.

Caffeoylquinic acid is recognized as a common antioxidant in plants such as sweet potato (Hayase and Kato, 1984), apple (Miller et al., 1995), and cacao bean (Naito et al., 1982). Dicaffeoylquinic acids are known as coffee components (Correia et al., 1992; De Menezes, 1992). These acids and their derivatives have been identified in sunflower (Christopher et al., 1991) and pear (Wieslaw et al., 1994). Recently, Shimozono et al. (1996) reported that steamed sweet potato contained chlorogenic acid and three kinds of dicaffoylquinic acids, which suppressed melanogenesis in mouse melanoma cells. Thus, the effect of dicaffeoylquinic acids on human health is important in view of their widespread occurrence in food materials.

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