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L-*O*-Caffeoylhomoserine from *Matteuccia struthiopteris*

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Abstract

A caffeic acid derivative was isolated from *Matteuccia struthiopteris* (ostrich fern) as a major radical scavenger. The compound consisted of caffeic acid and L-homoserine. NMR and MS analysis revealed the structure as L-*O*-caffeoylhomoserine.

Keywords: *Matteuccia struthiopteris*; Dryopteridaceae; Ostrich fern; L-*O*-Caffeoylhomoserine; Radical scavenger

1. Introduction

During the past decade, pathophysiological functions of antioxidative food constituents in humans have been receiving much attention, and consequently, much work on the screening of potentially natural antioxidants has been done. Today, a great number of the prospective antioxidants which could act as effective radical scavengers in vitro, have been discovered from plant species mainly distributed in relatively warm regions (e.g., flavonoids in tea leaves and citrus, lignans in sesame and curcuminoide in turmeric). Recently, to find other natural sources of antioxidants from relatively cool regions (e.g. the northern part of Japan), we investigated 200 edible Japanese plants. One of these plants is *Matteuccia struthiopteris* (ostrich fern), an edible fern widely distributed in the temperate regions of the northern hemisphere.

According to our previous study (Kimura et al., 2002), a DMSO extract of *M. struthiopteris* revealed strong radical scavenging activity, which was assessed by chemiluminescence and DPPH methods, and its efficacy was comparable to the extracts from well-known antioxidative plants (e.g., blueberry and grape). Since phenolics are the major plant constituents in fern species (reviewed by Soeder, 1985; Cooper-Driver, 1989), they could be responsible for the higher antioxidant capacity of *M. struthiopteris*. However, the structural analysis of phenolic constituents in *M. struthiopteris* has never been done, except for Hiraoka's taxonomic study (1978). Therefore, in this study, two phenolics (compounds **1** and **2**) were purified from *M. struthiopteris* by HPLC, and their chemical structures were clarified as chlorogenic acid (**1**) and L-*O*-caffeoylhomoserine (**2**), the latter of which has not been previously characterized. These compounds are the main phenolic constituents in *M. struthiopteris* and possess strong radical scavenging ability. As far as we know, this is the first report on the analysis of phenolics in *M. struthiopteris*. Here we focused on the new phenolic compound, L-*O*-caffeoylhomoserine (**2**), and describe its isolation and structural determination.

2. Results and discussion

The yield of compound **2** was 10 mg from fresh plant material (2 kg). The negative-ion High Resolution ESI (HRESI) MS spectrum gave a molecular ion peak at $[M-H]^-$ m/z 280.0835 (calc. 280.0821 for $C_{13}H_{14}NO_6$), consistent with the molecular formula $C_{13}H_{15}NO_6$. ^1H and ^{13}C NMR data are shown in Table 1. From these data, caffeic acid and homoserine moieties were found as parts of this compound. The ^1H NMR spectrum was

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Table 1
^{1}H NMR spectral data for the compound **2** (500 MHz, MeOH-d_4)

No. of atom	δ (ppm)	Multiplicity	J (Hz)
2	3.69	*dd*	5.1, 7.7
3a	2.15	*dddd*	7.7, 15.3, 5.4, 6.1
3b	2.35	*dddd*	5.1, 15.3, 6.9, 6.1
4a	4.33	*ddd*	6.1, 6.1, 11.5
4b	4.35	*ddd*	6.9, 5.4, 11.5
2′	7.05	*d*	2.0
5′	6.78	*d*	8.2
6′	6.95	*dd*	2.0, 8.2
7′	7.58	*d*	15.9
8′	6.30	*d*	15.9

TMS was used as the internal standard.

consistent with literature values of caffeic acid and homoserine (Calatayud et al., 2001; Pouchert and Behnke, 1993). By comparison with L-homoserine data, the signal for H-4 was clearly shifted downfield. The binding position of the caffeoyl group was considered to be 4-*O* of homoserine from HMBC data. Thus, compound **2** was characterized as a 4-*O*-caffeoyl ester of homoserine (Fig. 1). The fragment ions at HRESIMS obtained by in-source Collision Induced Dissociation (CID) supported the structure. The absolute stereochemistry of compound **2** was then determined by using chiral TLC. Compound **2** was hydrolyzed in an alkali condition, and homoserine was isolated, which coincided with L-homoserine (data not shown). Therefore, the structure of compound **2** was assigned as L-*O*-caffeoylhomoserine.

Esters of caffeic acid with alcohols, phenols, saccharides and flavonoids are common phytochemical constituents (Herrmann, 1978). Nevertheless, the caffeic acid esters with the OH group of the side chain of amino acids are rare. To date, there are only three reports: *O*-caffeoyltyrosine from *Aonidiella aurantii* (California red scale) and *O*-caffeoylserine from *Phenacoccus herreni* (Cassava mealybug), both produced by insects and identified as kairomones of their parasitoids (Miller, and Hare, 1993; Calatayud et al., 2001), and synthesized *O*-caffeoylserine (Lin et al., 1999). Thus, L-*O*-caffeoylhomoserine (**2**) is a new compound and this is the first report of this compound group originating from plants.

In plant cells, homoserine is phosphorylated into *O*-phosphohomoserine during threonine synthesis. It is

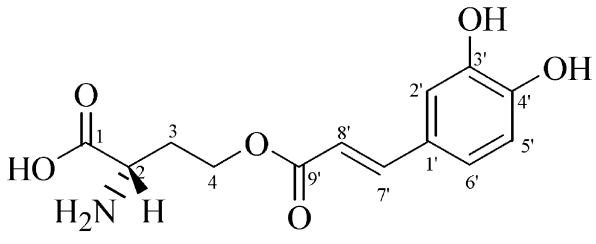

Fig. 1. Chemical structure of L-*O*-caffeoylhomoserine (**2**).

therefore plausible that *O*-phosphohomoserine reacts with caffeic acid to yield L-*O*-caffeoylhomoserine (**2**) in *M. struthiopteris*. If the reaction occurs enzymatically, L-*O*-caffeoylhomoserine could play some metabolic roles. The content of L-*O*-caffeoylhomoserine (**2**) (500 μg/g dry wt.) in the fern was comparable to chlorogenic acid (**1**) (360 μg/g dry wt), suggesting that like chlorogenic acid, L-*O*-caffeoylhomoserine might be produced enzymatically.

Like (−)-epigallocatechin gallate in tea leaves (Fujiki et al., 2002) or *trans*-resveratrol in red wine (Frankel et al., 1993; Pace-Asciak et al., 1995), dietary phenolic compounds have been considered with interest since they would remove oxidative stress and prevent life-style-related diseases such as cancer or heart diseases. Caffeic acid and its derivatives such as chlorogenic acid (**1**), which are the phenolic compounds commonly seen in vegetables or fruits, have also been researched for their efficacy as antioxidants in vitro (Rice-Evans et al., 1997) as well as in vivo (Kasai et al., 2000; Tanaka et al., 1993). In the present study, L-*O*-caffeoylhomoserine (**2**) exhibited almost the same radical scavenging activity as chlorogenic acid (**1**) and caffeic acid (Table 2). Hence, radical scavenging activity of L-*O*-caffeoylhomoserine (**2**) would be derived from the caffeoyl moiety in its molecule. In addition, L-*O*-caffeoylhomoserine (**2**) has a unique hydrophilic property caused by ampholite ions.

3. Experimental

3.1. General experimental procedure

MPs uncorr.; optical rotations: Perkin-Elmer model 241 polarimeter; HPLC was done on a Shimadzu LC-10 system. Preparative HPLC was performed on an Inertsil Prep-ODS (250×20 mm i.d.) at 7 ml/min using H_2O–MeOH gradient as the mobile phase. The composition of the mobile phase was changed linearly through 40 min from H_2O–MeOH (9:1) to (0:10). The peak was monitored by absorbance at 280 nm and an electrochemical detector (ECD) (esa Coulochem II, ESA Inc) set at 600 mV. ^{1}H, ^{13}C, HMBC NMR experiments were conducted on a Bruker AVANCE500 spectrometer. The resonant frequencies used were 500 MHz in ^{1}H and 125 MHz in ^{13}C. ESIMS spectra were obtained on a Mariner API-TOF Workstation (Applied Biosystems). TLC analysis was carried out on a chiral plate (HPTLC plated CHIR, Merck) using MeOH–H_2O–MeCN (10:10:40) as eluent. The amino group was detected with ninhydrin reagent (0.5% in EtOH–HOAc, 98:2).

3.2. Plant material

Fresh crosiers of *M. struthiopteris* were obtained in Fukushima, Japan, in April 1999. A voucher specimen,

Table 2
Radical scavenging activity of the compounds **1** and **2**

	Chemiluminescence method (IC50, mM)[a]	DPPH radical degradation method (IC50, mM)[a]
Chlorogenic acid (**1**)	0.31±0.01	0.13±0.01
L-*O*-Caffeoylhomoserine (**2**)	0.45±0.05	0.30±0.00
Rutin	0.11±0.01	0.15±0.00
Quercetin	0.53±0.01	0.26±0.02
Caffeic acid	0.66±0.07	0.39±0.01
Galic acid	0.74±0.06	0.36±0.02

[a] Concentration of the experimental substance to halve the radical species (IC50). Values are means ±S.D., $n=3$.

voucher no. kogomi 01, has been deposited in the National Agricultural Research Center for Tohoku Region, Fukushima, Japan.

3.3. Extraction and isolation

The fresh plant material (2 kg) was homogenized with MeOH (3 l) and filtered. The filtrate was concentrated under reduced pressure. The concentrate was centrifuged (17,000 *g*), and the resultant pellet was removed. The supernatant was added to H_2O–MeOH (9:1), the initial mobile phase of the following HPLC procedures. The separation and fractionation were done by HPLC-ECD. Two major reductive peaks were collected and purified, then recrystallised.

3.4. Radical scavenging activity

Radical scavenging activity of each purified compound was evaluated. Two methods which measures different radical species were employed (Kimura et al., 2002). In a chemiluminescence method, super oxide anions which were generated by xanthine and xanthine oxidase were measured by chemiluminescence. In the DPPH radical degradation method, the DPPH radical was measured by its absorbance at 550 nm. The results are expressed as the concentration of the experimental substance that reduces the radical species by 50% (IC50).

3.5. Alkaline hydrolysis and chiral TLC analysis

Compound **2** (0.1 mg) was dissolved in 2 M NaOH in MeOH–H_2O (3:1, 2 ml), and then heated at 70 °C for 3 h. Homoserine in the reaction mixture (4 ml) was analyzed by a chiral TLC and co-migrated with an authentic L-homoserine (1% w/v solutions).

3.6. L-*O*-Caffeoylhomoserine (**2**)

Colorless prism; mp 224.0–225.0 °C; specific rotation $[\alpha]_D^{20}-38.24°$ ($c=0.09$; 80%MeOH); for ^{1}H NMR spectral data, see Table 1; ^{13}C NMR (125 MHz, MeOH-d_4) δ:173.8 (C-1), 169.1 (C-9′), 149.7 (C-4′), 147.4 (C-7′), 146.9 (C-3′), 127.8 (C-1′), 123.1 (C-6′), 116.6 (C-5′), 115.4 (C-2′), 114.9 (C-8′), 62.2 (C-4), 54.0 (C-2), 31.6 (C-3); HMBC correlations: H-2/C-1, C-3, C-4; H-3/C-1, C-2, C-4; H-4/C-2, C-3, CO of caffeoyl; Hα/CO of caffeoyl, Cβ, C-1′; Hβ/CO of caffeoyl, CαC-1′, C-2′, C-6′; H-2′/Cβ, C-3′, C-4′, C-6′; H-5′/C-1′, C-3′, C-4′, C-6′;H-6′/Cβ,C-2′, C-4′, C-5′; HRESIMS *m*/*z*: $[M-H]^-$ 280.0835 (calc. 280.08156); the fragment peaks at HRESIMS (200 V, in-source CID) *m*/*z*: 280.0862 (calc. 280.08156, $C_{13}H_{14}NO_6$) (rel. int. 100), 179.0376 (calc. 179.03389, $C_9H_7O_4$) (rel. int.10), 135.0484 (calc. 135.04406, $C_8H_7O_2$) (rel. int.78).

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