Antioxidant Activity of a New Capsaicin Derivative from *Capsicum annuum*

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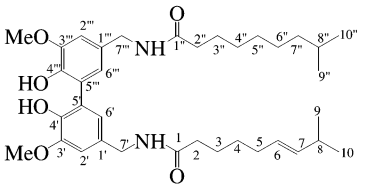
A new capsaicin derivative, 6″,7″-dihydro-5′,5‴-dicapsaicin (**1**), and a known capsaicin metabolite, *ω*-hydroxycapsaicin (**2**),[1] were isolated from the fruit of *Capsicum annuum*. Their structures were established on the basis of spectroscopic evidence. Compound **1** showed almost the same antioxidant activity as capsaicin, but did not have a pungent taste.

Capsicum species (Solanaceae), or hot peppers, are important plants and have been used worldwide as foods, spices, and medicines. The pungent principal component of red peppers is a group of acid amides of vanillylamine and C_8 to C_{13} fatty acids, which are known generally as capsaicin. More than 16 other capsaicinoids have been found as minor components.[2] Capsaicin has many useful properties. However, its use as a spice or drug is limited by its strong pungency and nociceptive activity.[3] Recently, nonpungent capsaicinoid-type compounds were isolated from *C. annuum* L. (CH-19 Sweet).[3] We have been trying to obtain a new capsaicinoid from *C. annuum* L. (Banshou) and isolated one new and a known nonpungent capsaicin derivative (**1** and **2**). In this paper, we describe their isolation, structural determination, and antioxidant activity.



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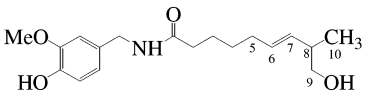

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The air-dried powdered fruits of *C. annuum* (Banshou) was extracted with MeOH. After the solvent was removed, the residue was partitioned between H_2O and EtOAc. The EtOAc-soluble fraction was fractionated using column chromatography and HPLC to give compounds **1** and **2**.

Compound **1**, obtained as a light yellow oil, showed hydroxy and amide bands at 3412 and 1645 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum (Table 1) showed signals due to two amides (δ_C 173.1, 173.0), 12 aromatic carbons (δ_C 147.5×2, 142.3×2, 130.5×2, 124.2×2, 122.9×2, 110.4×2), two methoxyl carbons (δ_C 56.4×2), four methyl carbons (δ_C 22.8×4), 12 methylenes, and four methines. The ^1H NMR spectrum also showed the presence of two doublet methyls [δ_H 0.85 (6H, d, J = 6.6 Hz), δ_H 0.95 (6H, d, J = 6.6 Hz)] and two aromatic signals [δ_H 6.88 (2H, brs), δ_H 6.90 (2H, brs)]. The positive-ion HRFABMS of compound **1** gave a quasi-molecular ion peak at *m/z* 611.4091 [M + H]$^+$, suggesting a molecular formula of $C_{36}H_{54}N_2O_6$. In the HMBC spectrum, correlations were observed for δ_H 6.90 (2′, 2‴-H) with δ_C 43.7 (C-7′, 7‴), 130.5 (C-1′, 1‴), 147.5 (C-3′, 3‴), 142.3 (C-4′, 4‴), and 122.9 (C-6′, 6‴), δ_H 6.88 (6′, 6‴-H) with δ_C 142.3 (C-4′, 4‴), δ_H 0.95 (9, 10-H) with δ_C 31.1 (C-8) and 138.3 (C-7), δ_H 0.85 (9″, 10″-H) with δ_C 28.1 (C-8″) and 39.1 (C-7″), δ_H 1.99 (5-H) with δ_C 126.7 (C-6) and 138.3 (C-7), and δ_H 3.91 (OMe×2) with δ_C 147.5 (C-3′, 3‴). These data indicated that **1** was a dimer of capsaicinoids. The ^1H NMR and ^{13}C NMR spectra of **1** are very similar to those of capsaicin[2] and dihydrocapsaicin,[3] and the difference between them is the peak of aromatic protons and the chemical shifts of C-5′ and -5‴. On the basis of the molecular formula and the ^1H and ^{13}C NMR

Table 1. NMR ($CDCl_3$) Data for Compound **1**

position	δ_C, mult[a]	δ_H (mult, J)[a]	HMBC	COSY
1	173.1, s[b]		2, 3, 7′	
2	37.0, t	2.20 (m)	3	
3	25.4, t	1.65 (m)	2, 5	2, 4
4	29.5, t	1.39 (m)	2	3, 5
5	32.4, t	1.99 (m)	6, 7	4, 6
6	126.7, d	5.34 (dd, J = 15.5, 6.0)	5, 8	5, 7
7	138.3, d	5.40 (dt, J = 15.5, 5.7)	5, 9, 10	6, 8
8	31.1, d	2.24 (m)	9, 10	7, 9, 10
9	22.8, q	0.95 (d, J = 6.6)		
10	22.8, q	0.95 (d, J = 6.6)		
1′	130.5, s		2′, 7′	
2′	110.4, d	6.90 (brs)	6′, 7′	
3′	147.5, s		2′, OMe	
4′	142.3, s		2′, 6′	
5′	124.2, s		6′	
6′	122.9, d	6.88 (brs)	2′, 7′	
7′	43.7, t	4.39 (d, J = 5.6)	2′, 6′	
OMe	56.4, s	3.91 (s)		
1″	170.3, s[b]		2″, 3″, 7‴	
2″	36.9, t	2.20 (m)	3″	
3″	25.4, t	1.65 (m)	2″	2″, 4″
4″	29.5, t	1.30 (m)		3″, 5″
5″	26.0, t	1.28 (m)		4″, 6″
6″	27.4, t	1.27 (m)		5″, 7″
7″	39.1, t	4.39 (d, J = 5.6)	9″, 10″	6″, 8″
8″	28.1, d	1.50 (m)	9″, 10″	7″, 9″, 10″
9″	22.8, q	0.85 (d, J = 6.6)		
10″	22.8, q	0.85 (d, J = 6.6)		
1‴	130.5, s		2‴, 7‴	
2‴	110.4, d	6.90 (brs)	6‴, 7‴	
3‴	147.5, s		2‴, OMe	
4‴	142.3, s		2‴, 6‴	
5‴	124.2, s		6‴	
6‴	122.9, d	6.88 (brs)	2‴, 7‴	
7‴	43.7, t		2‴, 6‴	
OMe	56.4, s	3.91 (s)		

[a] Assignments made by the HSQC and HMBC experiments. [b] Value may be interchanged.

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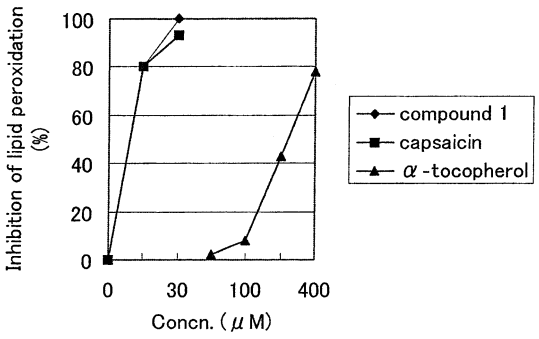

Figure 1. Inhibitory effects of compound **1**, capsaicin, and α-tocopherol on the lipid peroxidation of EyPC liposomes induced by ADP/Fe^{2+}.

spectra, the structure of **1** was assigned to be as shown. 5′,5‴-Dicapsaicin, the oxidative coupling product of capsaicin upon treatment with pepper peroxidase, has been reported.[5] This is the first isolation of a capsaicinoid dimer from a natural source.

Compound **2**, obtained as a light yellow oil, showed hydroxy and amide bands at 3339 and 1647 cm^{-1} in its IR spectrum. The ^{13}C NMR spectrum showed signals due to amide (δ_C 172.9), six aromatic carbons (δ_C 146.9, 145.3, 130.6, 121.0, 114.6, 110.9), a methoxyl carbon (δ_C 56.1), a methyl carbon (δ_C 16.8), six methylenes, and three methines. The positive-ion FABMS of compound **2** gave a quasi-molecular ion peak at *m/z* 322.2 $[M + H]^+$. The ^{13}C NMR spectrum of **2** is very similar to that of capsaicin, and the clearest difference between them is the chemical shifts at C-9 [capsaicin: δ_C 22.7 (CH_3); **2**: δ_C 67.5 (CH_2)]. In the HMBC spectrum of **2**, correlations were observed for the proton signal at δ_H 0.97 (H-10) with the carbon signals at δ_C 133.2 (C-7), 39.9 (C-8), and 67.5 (C-9), and for the proton signal at δ_H 2.05 (H-5) with the carbon signals at δ_C 131.5 (C-6) and 133.2 (C-7). These findings show that C-9 of compound **2** is hydroxymethylene. Thus, the structure of **2** was determined to be as shown. Compound **2** has been previously reported as a metabolite of capsaicin in rabbit[6] and a microorganism,[7] but this is the first report from a natural source. In contrast to capsaicin, compounds **1** and **2** are not pungent.

We have interest in the antioxidant activity of capsaicin. Thus we measured the antioxidant activity of **1**. Compound **1** showed antioxidant activity comparable to that of capsaicin. We compared the effect of **1** on ADP/Fe^{2+}-induced liposomal lipid peroxidation with that of capsaicin and the well-known antioxidant α-tocopherol (Figure 1). All three compounds inhibited lipid peroxidation in a concentration-dependent manner. However, **1** was about 25 times more potent than α-tocopherol; that is, the concentration of **1** needed for 50% inhibition (IC_{50}) was about 10 μM, while that of α-tocopherol was 250 μM. In addition, the IC_{50} of **1** was almost the same as that of capsaicin.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/as internal standard) were measured on a Bruker AM 400 spectrometer and MS spectra on a JEOL JMS D-300 instrument (IR-420). NMR: 400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR, both use TMS. Column chromatography: silica gel 60 (Merck), Toyopearl HW 40 (TOSHO), and Sephadex-LH 20 (Pharmacia). HPLC: silica gel (YMC-pack SIL-06 SH-043-5-06, 250 × 20 mm, Hibar RT 250-25 Si 60).

Plant Material. The dried fruit of *Capsicum annuum* (Banshou) (4.2 kg) was purchased from Koshiro Seiyaku Co., Japan. A voucher specimen (M0026) was deposited in the Herbarium of Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

Table 2. NMR ($CDCl_3$) Data for Compound **2**

position	δ_C, mult[a]	δ_H (mult, *J*)[a]	HMBC
1	172.9, s[b]		2, 3, 7′
2	36.7, t	2.20 (t, *J* = 4.5)	3
3	25.2, t	1.68 (m)	2, 5
4	29.0, t	1.42 (m)	2
5	32.2, t	2.05 (m)	6, 7
6	131.5, d	5.49 (m)	5
7	133.2, d	5.27 (dd, *J* = 15.5, 7.8)	5, 9, 10
8	39.9, d	2.29 (m)	10
9	67.5, t	3.42 (m)	
10	16.8, q	0.97 (d, *J* = 6.5)	
1′	130.6, s		2′, 5′,7′
2′	110.9, d	6.82 (s)	6′, 7′
3′	146.9, s		2′, 5′, OMe
4′	145.3, s		2′, 6′
5′	114.6, d	6.87 (d, *J* = 8.0)	
6′	121.0, d	6.77 (d, *J* = 8.0)	2′, 7′
7′	43.7, t	4.36 (d, *J* = 5.5)	2′, 6′
OMe	56.1, s	3.89 (s)	

[a] Assignments made by the HSQC and HMBC experiments. [b] Value may be interchanged.

Extraction and Isolation. The dried fruits of *C. annuum* (Banshou) (4.2 kg) were extracted three times with MeOH at 60 °C. The MeOH extract was concentrated to give a residue (721 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (153 g), which was subjected to column chromatography on silica gel with different solvents of increasing polarity (*n*-hexane−EtOAc; EtOAc−MeOH) to give 18 fractions (1−18). Fraction 15 was applied to a Toyopearl HW-40 column with $CHCl_3$−MeOH (2:1) as an eluent to give six fractions (15.1−15.6). Fraction 15.2 (427 mg) was applied to a Sephadex-LH 20 column with MeOH as an eluent to give three fractions (15.2.1−15.2.3). Fraction 15.2.2 (284 mg) was loaded on a silica gel column and eluted with different solvents of increasing polarity ($CHCl_3$−MeOH) to give four fractions (15.2.2.1−15.2.2.4). Fraction 15.2.2.1 was subjected to HPLC (silica gel, $CHCl_3$−MeOH, 95:5) to give **1** (10 mg). Fraction 15.3 was applied to a Toyopearl HW-40 column with $CHCl_3$−MeOH (2:1) as an eluent to give three fractions (15.3.1−15.3.3). Fraction 15.3.2 (457 mg) was loaded on a silica gel column and eluted with different solvents of increasing polarity ($CHCl_3$−MeOH) to give five fractions (15.3.2.1−15.3.2.5). Fraction 15.3.2.2 (140 mg) was loaded on a silica gel column and eluted with different solvents of increasing polarity ($CHCl_3$−MeOH) to give eight fractions (15.3.2.2.1−15.3.2.2.8). Fraction 15.3.2.2.7 (23.7 mg) was subjected to HPLC (silica gel, $CHCl_3$−MeOH, 95:5) to give **2** (3 mg).

6″,7″-Dihydro-5′,5‴-dicapsaicin (1): light yellow oil; IR (KBr) ν_{max} 3412, 2928, 1645, 1544, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (6H, d, *J* = 6.6 Hz, H-9″,10″), 0.95 (6H, d, *J* = 6.6 Hz, H-9, 10), 3.91 (6H, s, OMe × 2), 4.39 (4H, d, *J* = 5.6 Hz, H-7′,7‴), 5.34 (1H, dt, *J* = 15.5, 6.0 Hz, H-6), 5.40 (1H, dd, *J* = 15.5, 5.7 Hz, H-7), 6.88 (2H, brs, H-6′, 6‴), 6.90 (2H, brs, H-2′, 2‴); ^{13}C NMR data, see Table 1; HR FABMS *m/z* 611.4091 $[M + H]^+$ (calcd for $C_{36}H_{54}N_2O_6$, 611.4060).

***ω*-Hydroxycapsaicin (2):** light yellow oil; $[\alpha]_D$ +3.5° (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 3339, 2929, 1647, 1517, 1462 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 0.97 (3H, d, *J* = 6.5 Hz, H-10), 3.89 (3H, s, OMe), 4.36 (2H, d, *J* = 5.5 Hz, H-7′), 5.49 (1H, m, H-6), 5.27 (1H, dd, *J* = 15.5, 7.8, H-7), 6.77 (1H, d, *J* = 8.0 Hz, H-6′), 6.82 (1H, s, H-2′), 6.87 (1H, d, *J* = 8.0 Hz, H-5′); ^{13}C NMR data, see Table 2; FABMS *m/z* 322.2.

Preparation of Liposomes. Liposomes were prepared by drying a chloroform solution of egg yolk phosphatidylcholine (EyPC) under a stream of N_2 gas. The thin lipid film was then hydrated with 10 mM Tris-HCl buffer (pH 7.4, 25 °C) and sonicated in a bath-type sonicator.

Lipid Oxidation Assay. EyPC liposomes (final concentration, 0.2 μmol lipid/mL) were suspended in 10 mM Tris-HCl

buffer (pH 7.4, 25 °C). Test compounds dissolved in DMSO were added to the liposomal suspension and incubated for 5 min. Peroxidation was initiated by adding 1 mM ADP and 0.1 mM $FeSO_4$. After incubation for 15 min, an ethanol solution of 4.5 mM butylated hydroxytoluene was added to terminate lipid peroxidation. The amount of lipid peroxides was determined in terms of thiobarbituric acid-reactive substances (TBARS) from the absorbance at 532 nm, with tetraethoxypropane as an external standard.

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