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RAPID COMMUNICATIONS

Novel Capsaicinoid-like Substances, Capsiate and Dihydrocapsiate, from the Fruits of a Nonpungent Cultivar, CH-19 Sweet, of Pepper (*Capsicum annuum* L.)

Keywords: *Capsicum annuum L.; capsaicinoid-like substances; capsiate; dihydrocapsiate; vanillyl alcohol*

INTRODUCTION

Capsicum species, peppers, are important plants and have been used worldwide as foods, spices, and medicines. The major pungent components in fruits of *Capsicum* plants are capsaicin, (*E*)-*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide, and dihydrocapsaicin, the structure of which is a 6,7-dihydro derivative of capsaicin. More than 12 other capsaicinoids have been found as minor components (Suzuki and Iwai, 1984; Constant and Cordell, 1996). Capsaicin has many bioactivities (Buck and Burks, 1986; Suzuki and Iwai, 1984; Szolcsányi, 1982). However, its usage as a food additive or a drug is limited by its strong pungency and nociceptive activity. Several studies have reported on the pungency and bioactivity of various capsaicinoids. Watanabe et al. (1994) reported that several nonpungent capsaicinoids enhance adrenal catecholamine secretion as well as capsaicin. These nonpungent capsaicinoids are interesting from the viewpoint of wide application to foods and drugs.

Yazawa et al. (1989) reported that the fruits of a nonpungent cultivar of pepper, named CH-19 Sweet, contain only a small amount of capsaicinoids but considerable capsaicinoid-like substances (CLSs). CLSs develop an intense blue color by 2,6-dichloroquinone-4-chloroimide and ammonia on thin-layer chromatography, similarly to capsaicinoid. However, CLSs differ from capsaicinoid in their R_f values. There are two main R_f values in CLSs, and they are tentatively named CLS-A and CLS-B fractions, respectively. Yazawa et al. assumed that CLSs would be one of the precursors of capsaicinoid biosynthesis.

We expected that the CLSs would be a kind of nonpungent capsaicinoid occurring in natural resources. The purpose of the present paper is to characterize the structures of the CLSs in the fruits of cv. CH-19 Sweet. The two novel CLSs (**1** and **2**) have been obtained from the CLS-B fraction by repeated chromatography of the ethyl acetate extract from cv. CH-19 Sweet fruits, together with vanillyl alcohol (**3**) from the CLS-A fraction. The structure of **1** was determined as 4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate, named capsiate, from spectroscopic methods and comparison with the spectral data of an authentic capsaicin (Lin et al., 1993). The structure of **2** was determined as a 6,7-dihydro derivative of **1**, that is, 4-hydroxy-3-methoxybenzyl 8-methylnonanoate, named dihydrocapsiate, determined from identification with an authentic chemically synthesized sample.

EXPERIMENTAL PROCEDURES

Plant Material. Fruits of cv. CH-19 Sweet (*C. annuum* L.) were grown at the experimental farm of Kyoto University. CH-19 Sweet is a fixed, nonpungent cultivar that was selected and cultivated from a pungent cultivar, CH-19, of pepper obtained from Thailand (Yazawa et al., 1989).

Reagents. Capsaicin and 8-methylnonanoic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Vanillyl alcohol (4-hydroxy-3-methoxybenzyl alcohol) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Silica gel 60 was from Merck (Darmstadt, Germany), and reversed phase silica gel, Wakosil 25C18, was from Wako Pure Chem. Ind., Ltd. (Osaka, Japan).

Spectroscopic Analysis. ^{1}H- and ^{13}C-NMR spectra (TMS as the internal standard) were recorded on a JEOL α-400 instrument. IR spectra were recorded on a Hitachi 270-50 infrared spectrophotometer, and UV spectra were recorded on a JASCO Uvidec 660 spectrophotometer. HRMS spectra were recorded on a JEOL JMS-700 apparatus.

Isolation of Compounds 1−3. After fresh fruits (1.00 kg) of cv. CH-19 Sweet were freeze-dried, its seeds and calyces

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Table 1. ^{1}H-NMR Data for Compounds 1 and 2 and Capsaicin (399.65 MHz, $CDCl_3$)

position	capsaicin[a] δ (J, Hz)	**1** δ (J, Hz)	**2** δ (J, Hz)
2	2.19, t (7.6)	2.33, t (7.6)	2.33, t (7.6)
3	1.65, quint (7.6)	1.63, quint (7.6)	1.63, quint (7.6)
4	1.38, quint (7.6)	1.37, quint (7.6)	1.25, m
5	1.98, q (7.0)	1.97, q (6.8)	1.30, m
6	5.30, dt (15.6, 6.0)	5.30, dt (15.6, 6.4)	1.25, m
7	5.37, dd (15.6, 6.0)	5.37, dd (15.6, 6.0)	1.13, q (6.5)
8	2.20, oct (6.8)	2.21, oct (6.8)	1.50, m
9	0.95, d (6.8)	0.95, d (6.8)	0.85, d (6.8)
10	0.95, d (6.8)	0.95, d (6.8)	0.85, d (6.8)
2′	6.79, d (1.2)	6.87, d (1.5)	6.87, d (1.5)
5′	6.85, d (7.6)	6.90, d (8.3)	6.90, d (8.3)
6′	6.74, dd (7.6, 1.2)	6.86, dd (8.3, 1.5)	6.86, dd (8.3, 1.5)
7′	4.33, d (5.6)	5.03, s	5.03, s
OMe	3.85, s	3.90, s	3.90, s
OH	5.87, s	5.64, s	5.63, s

[a] Other signal: NH δ 5.84 (br t, J = 5.6 Hz).

were removed. The residue was then extracted three times with 1.8 L each of EtOAc for 5 min by use of a universal homogenizer (Nihon Seiki Seisakusho, Japan). The EtOAc extract was evaporated under reduced pressure to afford an oleoresin (7.3 g). The oleoresin was chromatographed on silica gel (36 × 200 mm) with stepwise elution of *n*-hexane and EtOAc. The fraction eluted with EtOAc was rechromatographed on reversed phase silica gel (20 × 50 mm) with 75% MeOH eluent to afford compound **3** (4.5 mg). The fraction eluted with *n*-hexane/EtOAc (80:20) was rechromatographed on reversed phase silica gel (20 × 90 mm) with 75% MeOH eluent, and a mixture containing compound **1** was obtained; the following fraction gave compound **2** as a colorless oil (59.7 mg).

The mixture containing compound **1** was rechromatographed on reversed phase silica gel (20 × 90 mm) with 75% MeOH containing 0.05 M $AgNO_3$. The collected eluent was partitioned with $CHCl_3$ three times, and the $CHCl_3$ fractions were collected and dried using anhydrous Na_2SO_4 and then filtered. The filtrate was evaporated under reduced pressure to afford compound **1** as a colorless oil (98.5 mg).

Chemical Synthesis of 4-Hydroxy-3-methoxybenzyl 8-Methylnonanoate (Dihydrocapsiate, 2). A mixture of 8-methylnonanoic acid (500 mg, 2.9 mmol) and thionyl chloride (3.5 g, 29 mmol) was stirred magnetically overnight at room temperature under drying with $CaCl_2$. After evaporation under reduced pressure, a brown oil was obtained. The oil was added dropwise into 5 mL of a pyridine solution of vanillyl alcohol (893 mg, 5.8 mmol). The mixture was stirred magnetically at 0 °C for 2 h. After water and 2 N HCl to acidify were added, the mixture was partitioned with 30 mL each of EtOAc three times, and the EtOAc fractions collected were washed with water, dried using anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure to afford a residue. The residue was chromatographed on silica gel (36 × 60 mm). The fraction eluted with *n*-hexane/EtOAc (90:10) gave a colorless oil (**2**, 329 mg, 36.8% yield).

RESULTS AND DISCUSSION

Structural Determination of Compound 1 (Capsiate). Spectral data of compound **1** were as follows: HRMS, m/z (M^+) calcd for $C_{18}H_{26}O_4$ 306.1831, found 306.1798; IR ν_{max} (film) 3450, 1740, 1615, 1610, 1520, 1470, 1435, 1275, 1160, 1120, 1035, 970, 850, 815, 795, 560 cm^{-1}; UV λ_{max} (MeOH) 280 (ϵ = 2400), 231 nm (ϵ = 6200). The ^{1}H- and ^{13}C-NMR spectral data are shown in Tables 1 and 2, respectively.

The molecular formula of **1** was determined as $C_{18}H_{26}O_4$ by HRMS measurement. The IR spectrum showed a hydroxyl absorption (3450 cm^{-1}) and an ester carbonyl absorption (1740 cm^{-1}). The ^{1}H-NMR spectrum of **1** showed three aromatic protons (δ 6.90 d, 6.86 dd, and 6.87 d), and these coupling constants and patterns indicated the typical 1-, 2-, and 4-substituted phenyl group. The phenyl group gave rise to the signals of δ 146.5, 145.8, 128.0, 122.0, 114.3, and 111.2 in ^{13}C-NMR data. In ^{1}H-NMR data of **1**, two olefinic methine protons (δ 5.37 dd and 5.30 dt), coupled to each other with 15.6 Hz, indicated the presence of an ethylenic moiety of trans configuration. A methoxyl group (δ 3.90 s) and an isopropyl group (δ 0.95 d, 0.95 d, and 2.21 oct) were observed in ^{1}H-NMR data of **1**.

Table 2. ^{13}C-NMR Data for Compounds 1 and 2 and Capsaicin (100.40 MHz, $CDCl_3$)

position	capsaicin δ	**1** δ	**2** δ
1	172.9	173.7	173.8
2	36.7	34.3	34.4
3	25.3	24.5	25.0
4	29.3	29.1	29.5
5	32.2	32.1	29.2
6	126.5	126.5	27.2
7	138.1	138.1	38.9
8	31.0	31.0	27.9
9	22.7	22.6	22.6
10	22.7	22.6	22.6
1′	130.3	128.0	128.0
2′	110.7	111.2	111.3
3′	146.8	146.5	146.5
4′	145.2	145.8	145.8
5′	114.4	114.3	114.4
6′	120.7	122.0	122.0
7′	43.5	66.3	66.3
OMe	55.9	55.9	55.9

As shown in Table 2, the ^{13}C-NMR spectrum of **1** was extremely similar to that of authentic capsaicin except for a methylene carbon at the C-7′ position (δ 43.5 of capsaicin and δ 66.3 of **1**). The ^{1}H-NMR spectrum of **1** was also similar to that of capsaicin. However, the chemical shift value of the methylene protons (δ 5.03 s) of **1** was different from that of the C-7′ position of capsaicin. These NMR signals of the methylene of **1** indicated the methylene group was caught between the phenyl group and the oxygen of the ester from their chemical shift values. These results suggested that the structure of **1** has an ester moiety instead of an amide moiety of capsaicin. Furthermore, it was suggested that compound **1** has the same acyl residue as capsaicin, that is, (*E*)-8-methyl-6-nonenoyl group, because ^{1}H- and ^{13}C-NMR data for the acyl group of **1** agreed excellently with those of capsaicin.

Therefore, we concluded the structure of **1** to be 4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate as shown in Figure 1, and this novel compound **1** was named capsiate.

Structural Determination of Compound 2 (Dihydrocapsiate). Spectral data of compound **2** were as follows: HRMS, m/z (M^+) calcd for $C_{18}H_{28}O_4$ 308.1987, found 308.2008; IR ν_{max} (film) 3450, 1740, 1615, 1610, 1520, 1470, 1435, 1275, 1160, 1120, 1035, 970, 850, 815, 795, 560 cm^{-1}; UV λ_{max} (MeOH) 279 (ϵ = 3700), 231 nm (ϵ = 8700). The ^{1}H- and ^{13}C-NMR spectral data are shown in Tables 1 and 2, respectively.

The molecular formula of **2** was determined as $C_{18}H_{28}O_4$ by HRMS measurement. The IR spectral data of **2** were similar to that of capsiate (**1**). It was therefore presumed that compound **2** has structural resemblance to **1**. ^{1}H- and ^{13}C-NMR data of **2** were also similar to those of **1**. In the ^{13}C-NMR spectrum of **2**, however, the presence of two alkanic methylene carbons (δ 27.2 and 38.9) instead of two olefinic methine carbons of **1** (C-6 and C-7) was observed. The ^{1}H-NMR data of **2** also

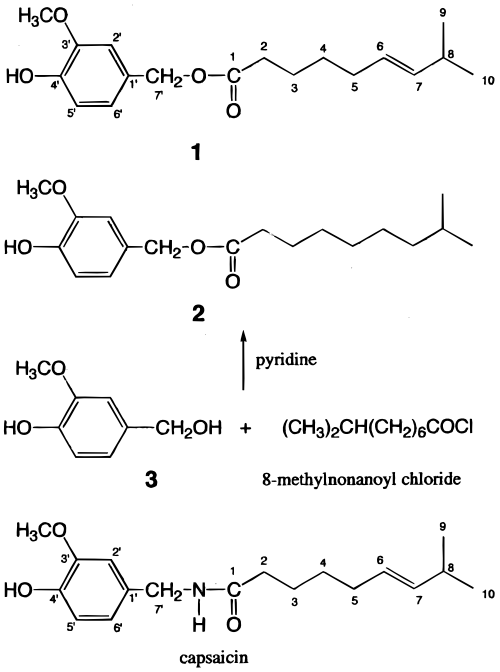

Figure 1. Structures of capsiate (**1**), dihydrocapsiate (**2**), vanillyl alcohol (**3**), and capsaicin and scheme of chemical synthesis of **2** by condensation of **3** and 8-methylnonanoyl chloride.

showed no signals of olefinic protons such as observed in **1**. These results suggested the structure of **2** to be a 6,7-dihydro derivative of **1**, that is, 4-hydroxy-3-methoxybenzyl 8-methylnonanoate.

To confirm the structure of **2**, the chemical synthesis of compound **2** was carried out by condensation of vanillyl alcohol with 8-methylnonanoyl chloride derived from 8-methylnonanoic acid and thiohyl chloride (Figure 1). The IR and ^{1}H- and ^{13}C-NMR data of the chemically synthesized product completely agreed with those of **2**. Therefore, the structure of **2** was confirmed as 4-hydroxy-3-methoxybenzyl 8-methylnonanoate (Figure 1), and the novel compound **2** was named dihydrocapsiate because **2** is a 6,7-dihydro derivative of capsiate (**1**).

Compound **3** was identified as vanillyl alcohol by comparison of its spectral data with those of authentic reagent.

This paper is the first report on the natural products of capsaicin analogues that have an ester moiety in their center linkage.

As expected, capsiate and dihydrocapsiate had no or very slight pungency upon our oral tasting.

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