JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Purification and Structure Determination of Glucosides of Capsaicin and Dihydrocapsaicin from Various *Capsicum* Fruits

FUMIHARU HIGASHIGUCHI,* HIROYASU NAKAMURA, HIDEO HAYASHI,‡ AND TAKASHI KOMETANI

Central Research Laboratory, Ezaki Glico Company, Ltd., 4-6-5 Utajima, Nishiyodogawa-Ku, Osaka, Japan 555-8502

Two new glucosides, capsaicin-β-D-glucopyranoside (**1**) and dihydrocapsaicin-β-D-glucopyranoside (**2**), were discovered in the fruit of the *Capsicum annuum* cultivar 'High Heat'. They were sequentially purified by acetone extraction, *n*-hexane extraction, and acetonitrile extraction, followed by medium-pressure liquid chromatography and high-performance liquid chromatography performed on an octadecylsilane column. Their chemical structures were elucidated by proton nuclear magnetic resonance, carbon nuclear magnetic resonance, and hydrolysis with α- and β-glucosidases. The glucosides were also detected in various pungent cultivars of *C. annuum*, *Capsicum frutescens,* and *Capsicum chinense* by liquid chromatography−mass spectrometry. However, they were not detected in nonpungent cultivars of *C. annuum*. Furthermore, a positive correlation was observed between the quantity of the capsaicinoids, capsaicin, and dihydrocapsaicin and their glucosides.

KEYWORDS: ***Capsicum*; pepper; capsaicin; dihydrocapsaicin; glucoside**

INTRODUCTION

Plants of the *Capsicum* species belong to the Solanaceae family and are important sources of food and medicine all over the world. The major pungent components in the fruits of *Capsicum* plants are capsaicin and dihydrocapsaicin. There are many reports of these components, for example, biosynthesis (*1*−*5*) and metabolism (*6*−*10*) in peppers. With regard to its nutritional benefits, capsaicin has been reported to reduce the perirenal adipose tissue weight and serum triglyceride concentration in rats by enhancing energy metabolism through an in vivo β-adrenergic action (*11*). Furthermore, it was demonstrated that the enhancement of energy metabolism occurred through catecholamine secretion from the adrenal medulla as a result of the activation of the central nervous system (*12*). In addition, there are many reports relative to body heat generation (*13*), lipid and energy metabolism (*14*, *15*), increase in swimming endurance capacity (*16*, *17*), antioxidant activity (*18*−*20*), antibacterial activity (*21*), anodyne effect (*22*−*26*), and perspiration effect (*27*).

However, the use of *Capsicum* as a food, food additive, or medicine is limited by its strong pungency and nociceptive activity. Therefore, glucosylation of capsaicin has been attempted to reduce its pungency and enhance its water solubility. For example, glucosylation of vanillylnonanamide by chemosynthetic methods (*28*) and glucosylation of capsaicin by cell suspension cultures of *Coffea arabica* (*29*) have been attempted. It has been reported that the pungency of capsaicin-β-D-glucopyranoside was $^1/_{100}$ that of capsaicin (*29*) and that the pungency of vanillylnonanamide-β-D-glucoside was $^1/_{500}$ (*30*). These facts will be proved on the grounds that glucosides of capsaicinoids are glucosylated at the phenolic OH but stringent structural requirements for capsaicin-like activity would be the aromatic portion of capsaicin molecule (*23*).

With regard to the physiological functions of capsaicinoid glucosides, Tani et al. (*30*) reported that vanillylnonanamide-β-D-glucoside, which was synthesized chemically, had a remarkable in vivo effect on lipid metabolism in rats. The levels of liver lipid and serum lipid were lowered when vanillylnonanamide-β-D-glucoside was orally administered. Furthermore, the levels of total cholesterol and the arteriosclerosis index also were also lowered after supplementation with this glucoside. Because the pungency of the glucosides of capsaicinoids is significantly lower than that of capsaicin, they will be more suitable for eating and may improve lipid metabolism in humans.

However, the glucosides synthesized chemically have not been used in the food, pharmaceutical, and cosmetic industries, so we have tried to research the glucosides of capsaicinoids in natural peppers. Although many types of glycosides have been reported in natural plants, no studies have reported the presence of capsaicin glucosides in nature. This is the first report of the purification and structure determination of capsaicinoid glucosides.

MATERIALS AND METHODS

Plant Material. Pungent cultivars of *Capsicum annum*, *Capsicum frutescens*, and *Capsicum chinense* (**Table 1**) were received as gifts

* Author to whom correspondence should be addressed (telephone 81-6-6477-8424; fax 81-6-6477-8271; e-mail higashiguchi-fumiharu@glico.co.jp).
‡ Present address: Graduate School of Life and Environmental Sciences, Osaka Prefecture University.

10.1021/jf0607720 CCC: $33.50
Published on Web 07/12/2006

Table 1. Pepper Samples and Quantity of Capsaicinoids and Glucosides in Samples

taste	trade name	species	place of production	glucoside (ppb)		capsaicinoid (ppm)	
				CG	DCG	C	DC
pungent	High Heat	*Capsicum annuum*	India	2647	557	3254	1378
	Sannam	*Capsicum annuum*	India	2386	834	1712	1121
	Habaneros	*Capsicum chinense*	Mexico	1557	312	4818	2311
	Medium Heat	*Capsicum annuum*	India	1082	385	808	707
	Bird's Eye	*Capsicum frutescens*	India	820	200	2238	1100
	Unnan	*Capsicum annuum*	China	481	98	1149	490
	Jikutsuki	*Capsicum annuum*	China	322	71	2404	1110
	Bowto	*Capsicum annuum*	China	178	64	344	176
	Kankoku	*Capsicum annuum*	Korea	160	75	128	77
	Hontaka	*Capsicum annuum*	China	110	35	832	623
	Ekito	*Capsicum annuum*	China	105	42	261	194
nonpungent	Shishito	*Capsicum annuum*	Japan	nd	nd	nd	nd
	Shishito	*Capsicum annuum*	Korea	nd	nd	nd	nd
	Manganji	*Capsicum annuum*	Japan	nd	nd	nd	nd
	Fushimiamanaga	*Capsicum annuum*	Japan	nd	nd	nd	nd

[a] CG, capsaicin-β-D-glucopyranoside; DCG, dihydrocapsaicin-β-D-glucopyranoside; C, capsaicin; DC, dihydrocapsaicin. nd, not determined.

from Hachi Foods Co., Ltd.. Nonpungent cultivars of these species were purchased at the market (Jusco, Kyoto, Japan).

Reagents. Capsaicin was purchased from Sigma-Aldrich, Inc., and dihydrocapsaicin was from Wako Pure Chemical Industries, Ltd. Acetonitrile, ethanol, and *n*-hexane were purchased from Wako Pure Chemical Industries, Ltd. Acetonitrile was of chromatographic grade, and other reagents were of analytical grade.

Liquid Chromatographic (LC) Conditions. Medium-pressure liquid chromatography (MPLC) (Yamazen Corp.) was performed on a glass column (200 × 10 mm) packed with Wakogel 50 C18 (38−63 μm) (Wako Pure Chemical Industries, Ltd.) eluted with acetonitrile/H_2O (1:1) at a flow rate of 4.0 mL/min. The high-performance liquid chromatography (HPLC) system consisted of a model 600E, a 996 photodiode array detector, and a 717 autosampler instrument (Waters Corp.) and was performed on an ODS column (300 × 7.5 mm) (Showa Denko K.K.) that was eluted with acetonitrile/H_2O (30:70) at a flow rate of 2.0 and on an ODS column (250 × 4.6 mm) (Showa Denko K.K.) that was eluted with acetonitrile/H_2O (40:60) at a flow rate of 1.0 mL/min; detection was carried out at 280 nm. Ion chromatography (IC) on a model DX500 instrument (Dionex Corp.) was performed on a PA1 column (250 × 4.6 mm) (Dionex Corp.) that was eluted with 200 mM NaOH at a flow rate of 1.0 mL/min; detection was carried out with an electrochemical detector.

Liquid chromatography−mass spectrometry (LC-MS) was performed using the QP8000α (Shimadzu Corp.) equipped with atmospheric pressure chemical ionization (APCI). HPLC was performed on an ODS column (250 × 4.6 mm) (Showa Denko K.K.) eluted with 10 mM ammonium acetate/acetonitrile (50:50, v/v) at a flow rate of 1.0 mL/min at 40 °C. The APCI operating parameters were as follows: nebulizer gas (air) flow, 2.5 L/min; APCI probe temperature, 400 °C; curved desolvation line (CDL) temperature, 250 °C; detector voltage, 1.8 kV; probe voltage, −3.0 V; CDL voltage, 30.0 V; and deflector voltage, −35.0 V.

Nuclear Magnetic Resonance (NMR) Conditions. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JNM-A400 (JEOL) spectrometer at 400 MHz. Chemical shifts were referenced to the residual solvent signal (methanol-d_4 δ_H 3.30, δ_C 49.0). ^{1}H NMR and ^{13}C NMR were performed with methanol-d_4 as the solvent and tetramethylsilane as the internal reference.

Sample Extraction. *Purification of Glucosides of the Capsaicinoids for Structure Determination*. Dry fruit of the *C. annum* 'High Heat' cultivar was used as the sample. It was pulverized in a grinder, and 500 g of the pulverized sample was extracted by dipping into 3 L of acetone in a glass container for about a week. The acetone extract was suction filtered and evaporated to an oily extract under reduced pressure. To this oily extract was added 200 mL of *n*-hexane. The mixture was stirred and then transferred into a separatory funnel. The *n*-hexane layer was collected and evaporated under reduced pressure. To the *n*-hexane extract was added 200 mL of acetonitrile, and the same procedure of separation was performed. After the acetonitrile layer was collected and evaporated, the acetonitrile extract was dissolved in 30 mL of acetonitrile/H_2O (1:1, v/v).

The above filtered final extract solution was chromatographed by MPLC. Five milliliters of the extract solution was applied onto a glass column packed with Wakogel. The fraction containing the glucosides of capsaicinoids was collected and rechromatographed by HPLC on an ODS column (300 × 7.5 mm). The fraction containing the glucosides of capsaicinoids was collected and rechromatographed by HPLC on an ODS column (250 × 4.6 mm). Finally, the fraction containing the glucosides of capsaicinoids was collected and subsequently freeze-dried.

Extraction for Confirming the Presence of the Capsaicinoid Glucosides in Various Capsicum Fruits. All of the samples listed in **Table 1** were pulverized in a grinder and kept in a refrigerator. Five grams of each pulverized sample was placed in 50-mL glass centrifuge tubes, and 30 mL of ethanol was added to the tubes. After sufficient mixing, the tubes were centrifuged (3000 rpm × 10 min), and the supernatant was collected. The same procedure was performed twice for the residue. The collected supernatant was evaporated, dissolved in 5 mL of ethanol, and then used as a sample for detecting glucosides.

Enzymatic Hydrolysis. To determine the structure of the purified glucosides, the compounds purified from the 'High Heat' cultivar were hydrolyzed by treatment with 10 units/mL of α-glucosidase (Wako Pure Chemical Industries, Ltd.) at 37 °C for 16 h with 0.1 M phosphate buffer (pH 7.0) and 1 unit/mL of β-glucosidase (Sigma-Aldrich Corp.) at 37 °C for 16 h with 0.1 M acetate buffer (pH 5.0). Subsequently, the released capsaicin and dihydrocapsaicin were measured on an ODS column by HPLC. The released glucose was measured by ion chromatography.

Statistical Analysis. A correlation between capsaicinoids and the glucosides were calculated with Pearson's method.

RESULTS

LC-MS Conditions of the Glucosides of Capsaicin and Dihydrocapsaicin. In the APCI mass spectra of nordihydrocapsaicin-β-D-glucopyranoside, which was a gift from Dr. T. Furuya (Prof. Emeritus, Kitasato University), the acetate-added ion appeared at *m*/*z* 514 $[M + CH_3COO]^-$ as the base peak (data not shown). Therefore, mass spectra of the peaks of the capsaicin and dihydrocapsaicin glucosides were assumed to be at *m*/*z* 526 $[M + CH_3COO]^-$ and 528 $[M + CH_3COO]^-$, respectively. In this study, peaks at *m*/*z* 526 (4.5 min) and *m*/*z* 528 (5.4 min) appeared free from any interfering contaminants in the selected ion monitoring (SIM) mode chromatograms. Furthermore, the ratio of the peak area at *m*/*z* 526 to that at *m*/*z* 528 was similar to the ratio of the peaks of capsaicin to dihydrocapsaicin. On the basis of these results, we deduced that

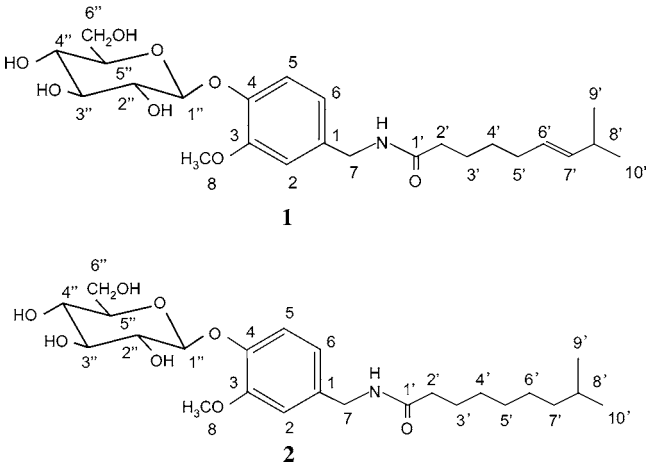

Figure 1. Structures of capsaicin-β-D-glucopyranoside (**1**) and dihydrocapsaicin-β-D-glucopyranoside (**2**).

the peaks at *m*/*z* 526 and 528 were the glucoside of the capsaicin and that of dihydrocapsaicin, respectively.

Purification of the Glucosides of Capsaicin and Dihydrocapsaicin with MPLC and HPLC. To verify that the compounds purified from the 'High Heat' cultivar were the glucosides of capsaicin and dihydrocapsaicin, ≈5 kg of 'High Heat' cultivar was extracted with acetone, *n*-hexane, and acetonitrile. The fractions containing the compounds with peaks at *m*/*z* 526 and 528 were selected. Purification of the extracts by MPLC and HPLC yielded approximately 7.2 mg of the compound with a peak at *m*/*z* 526 and 0.5 mg of the compound with a peak at *m*/*z* 528 (**Figure 2**).

NMR Analysis and Hydrolysis with α-and β-Glucosidase of Purified Glucosides of Capsaicin and Dihydrocapsaicin. The values obtained from the ^{1}H NMR and ^{13}C NMR spectra of the compound with a peak at *m*/*z* 526 were as in **Table 2**. Furthermore, on hydrolysis with β-glucosidase, the compound with a peak at *m*/*z* 526 yielded 0.175 mM capsaicin (**Figure 3**) and the corresponding amount of glucose. The molar ratio of capsaicin to glucose was 0.97. However, α-glucosidase did not hydrolyze the glucosides. Therefore, the compound was identified as capsaicin-β-D-glucopyranoside (**1**) (**Figure 1**). The values obtained from the ^{1}H NMR and ^{13}C NMR spectra of the compound with a peak at *m*/*z* 528 were as in **Table 2**. On hydrolysis with β-glucosidase, the compound with a peak at *m*/*z* 528 also yielded 0.24 mM dihydrocapsaicin (**Figure 3**) and 0.32 mM glucose, but α-glucosidase did not hydrolyze the glucosides. The molar ratio of dihydrocapsaicin to glucose was 0.73. Therefore, the compound was identified as dihydrocapsaicin-β-D-glucopyranoside (**2**) (**Figure 1**).

Relationship between the Content of Capsaicinoids and That of Its Glucosides in Various Species of *Capsicum*. Considering that glucosides of capsaicinoid had now been discovered in nature, we attempted to analyze other cultivars of *C. annuum*, *C. frutescens*, and *C. chinense* (**Table 1**) by LC-MS. The plots of concentration versus peak area ratio for capsaicin-β-D-glucopyranoside (**1**) and dihydrocapsaicin-β-D-glucopyranoside (**2**) were linear over the range of 0.5−100 μg/mL with a correlation coefficient of 0.9999. Capsaicin and dihydrocapsaicin in these cultivars were identified by HPLC with ultraviolet detection (HPLC-UV). Subsequently, it was shown that the peaks at *m*/*z* 526 and 528 deduced as capsaicin-β-D-glucopyranoside (**1**) and dihydrocapsaicin-β-D-glucopyranoside (**2**) were detected in the pungent cultivars, but not in the nonpungent cultivars (**Table 1**). When the solution extracted from the pungent cultivars was hydrolyzed with α-glucosidase, the peaks

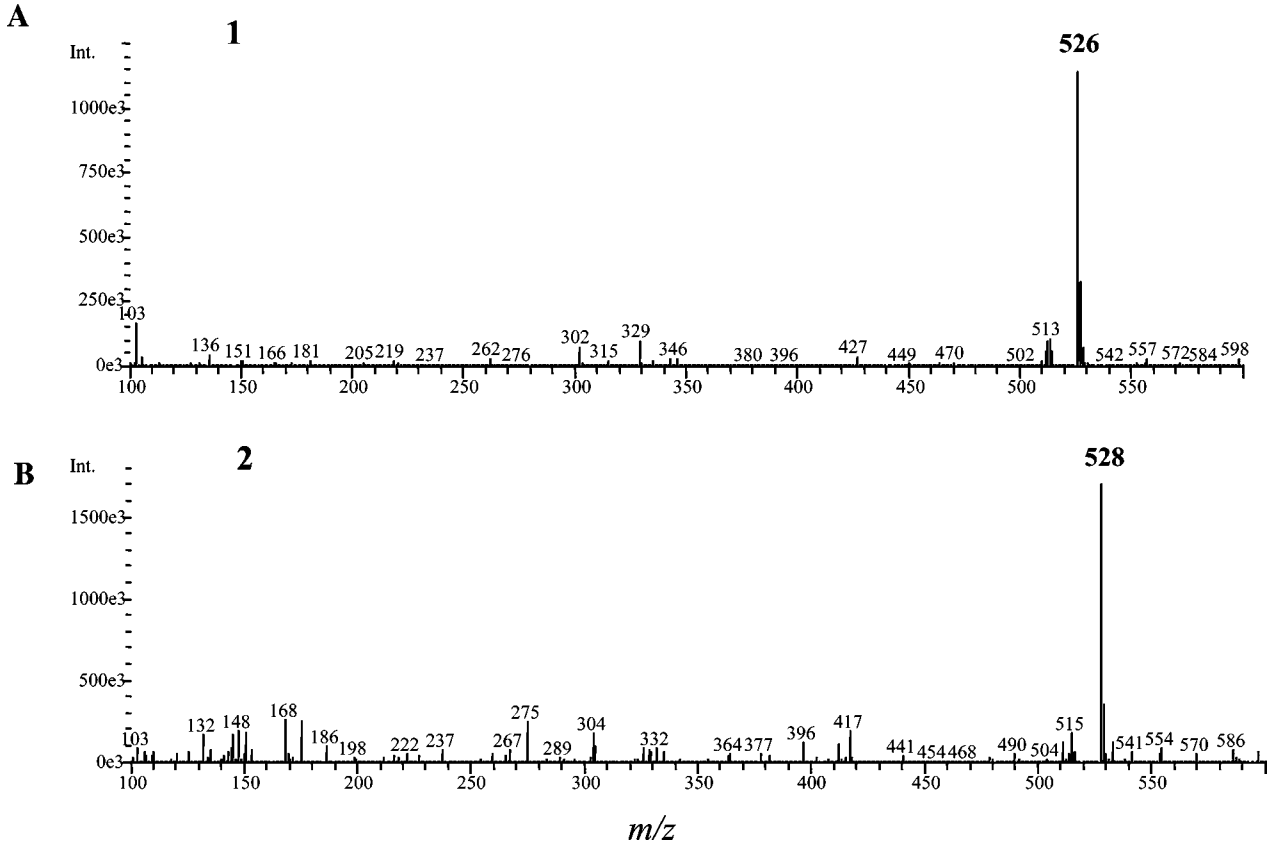

Figure 2. HPLC chromatogram of (**A-1**) purified capsaicin-β-D-glucopyranoside, (**A-2**) released capsaicin by hydrolysis with β-glucosidase, (**B-1**) purified dihydrocapsaicin-β-D-glucopyranoside, and (**B-2**) released dihydrocapsaicin by hydrolysis with β-glucosidase.

Table 2. ^{1}H NMR and ^{13}C NMR Data for Compounds *m/z* 526 and 528

	m/z 526 (*J*, Hz)		*m/z* 528	
position	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1		135.32		135.12
2	6.81	121.34	6.71	122.69
3		118.49		121.25
4		147.27		150.90
5	7.10	151.09	7.00	157.54
6	6.93	113.52	6.84	113.20
7	4.29	43.80	4.20	43.76
8		56.86		56.68
1′		176.05		176.14
2′	2.22	33.20	2.12	30.72
3′	1.62	26.52	1.49	27.11
4′	1.38	32.34	1.39	28.41
5′	1.99	32.22	1.06–1.21	28.28
6′	5.38	127.88		30.36
7′	5.38	139.18		29.14
8′	2.17	37.01	1.39	37.13
9′	0.95	23.08	0.78	23.08
10′	0.95	23.08	0.78	23.08
1″	4.84 (7.56)	103.17		102.93
2″	3.29–3.50	75.01	3.20–3.40	74.92
3″		77.94		77.86
4″		71.47		71.34
5″		78.23		78.20
6″	3.67, 3.69	62.63	3.56, 3.61	62.50
OMe	3.84	56.86	3.74	56.68
NH	4.65		4.77	

at *m/z* 526 and 528 did not disappear, whereas on hydrolysis with β-glucosidase, the peaks disappeared. On the basis of these results, we inferred that these compounds were capsaicin-β-D-glucopyranoside and dihydrocapsaicin-β-D-glucopyranoside. Additionally, a positive correlation was observed between the glucosides and the capsaicinoids (**Figure 4**). To confirm the presence of the glucosides, each solution extracted from other cultivars of the *Capsicum* species was evaporated and hydrolyzed by treatment with α-glucosidase or β-glucosidase, as described above. The disappearance of the peak at *m/z* 526 for the glucoside of capsaicin and at *m/z* 528 for that of dihydrocapsaicin, as seen in the LC-MS analysis, was investigated.

DISCUSSION

Two substances found in this study were not hydrolyzed with α-glucosidase, but hydrolyzed with β-glucosidase. Because glucosidase reaction is unique, the sugars of these glucosides will be estimated as the β-anomer. It was shown that the substances released by β-glucosidase were glucose and capsaicinoids (**Figure 2**), and the ratios of glucose/capsaicinoids were about 0.7−1 with HPLC and IC. The APCI mass spectra of synthesized nordihydrocapsaicin-β-D-glucopyranoside (molecular weight = 455) appeared at the acetate-added ion *m/z* 514 $[M + CH3COO]^-$ as the base peak, and the mass spectra of the peaks of the two glucosides were shown to be at *m/z* 526 $[M + CH3COO]^-$ and 528 $[M + CH3COO]^-$ (**Figure 3**), respectively. Therefore, the molecular weights of the glucosides were estimated as 467 and 469. Therefore, these glucosides would be estimated to be capsaicin-β-D-glucopyranoside and dihydrocapsaicin-β-D-glucopyranoside from the mass spectra data and the ^{1}H NMR and ^{13}C NMR spectra data.

Iwai et al. (*31*) reported that the total capsaicinoids content of strong pungent peppers, Karayatsubusa, Takanotsume, Red chili, and so on, was 0.90−10.80 mg/g of sample, and the composition of capsaicin and dihydrocapsaicin was about 1/1 to 2/1 (capsaicin/dihydrocapsaicin). In our study, the total amount of capsaicin and dihydrocapsaicin was 0.2−7.1 mg/g, and the composition was about 1/1 to 2/1 (**Table 1**). Therefore, our data almost coincided with Iwai's data.

Capsaicin is a pungent principle of the *Capsicum* species. The biosynthesis and metabolism of capsaicinoids in peppers have been reported by many researchers (*1*−*10*), but natural glucosides of capsaicinoids have not been reported. Sukrasno and Yeoman (*6*) have reported that the glycosides of phenylpropanoids such as cinnamoylglycoside and the glycoside of vanillic acid were formed as intermediates in the pathway leading to the conversion of phenylalanine to vanillylamine. They have reported that the glycosides of phenylpropanoid decreased as the biosynthesis of capsaicin increased; consequently, these glycosides were thought to be the precursors of capsaicinoids. Furthermore, Fujiwake et al. (*4*) have reported in detail that capsaicinoids are synthesized by the dehydration condensation reaction between vanillylamine and the acyl-CoA

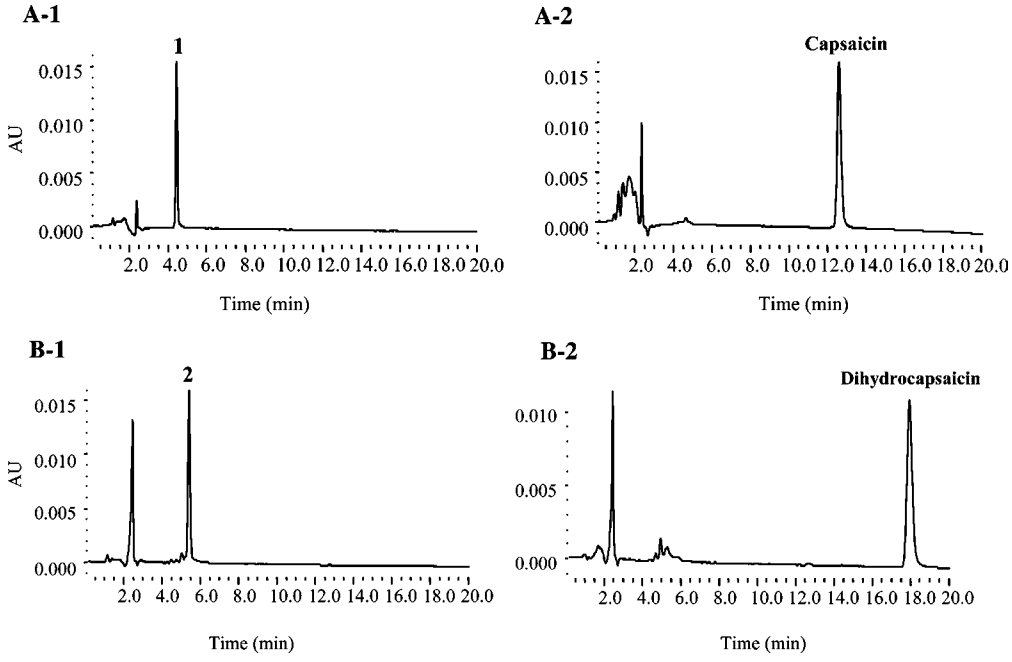

Figure 3. Full-scan mass spectra of (**A**) capsaicin-β-D-glucopyranoside and (**B**) dihydrocapsaicin-β-D-glucopyranoside.

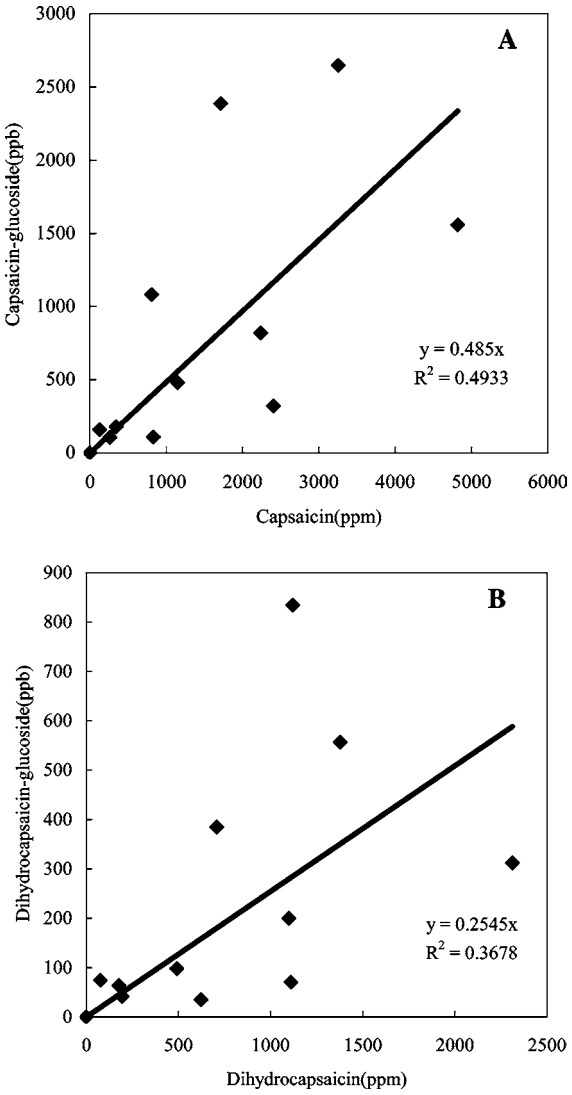

Figure 4. Scatter diagrams of quantity of (**A**) capsaicin-capsaicin-β-D-glucopyranoside, and (**B**) dihydrocapsaicin-dihydrocapsaicin-β-D-glucopyranoside.

that is derived from an iso-type fatty acid found on the outer surface of the tonoplast in *Capsicum* cells. Although studies have been conducted on capsaicinoids and their glycosides, which are formed as intermediates, their glucosides have not been reported. This study elucidated for the first time that glucosides of capsaicinoids exist in various pungent peppers and that a possibility of positive correlation exists between the content of the capsaicinoids, capsaicin and dihydrocapsaicin, and their glucosides in peppers.

The glucosides of capsaicinoids found in this study constitute only a very small quantity of the total pool of capsaicinoids in pepper fruits. These results could be caused by the harvest season and storage condition. The collected peppers were harvested after carotenoids increased sufficiently. Suzuki et al. (*3*) have reported in detail that the content of capsaicinoids would be highest at ≈40 days after flowering, following a decrease. Compared with that, the content of carotenoid in peppers would increase at ×bb≈40 days after flowering. Because it was thought that the contents of capsaicinoids and the glucosides possibly correlated, the content of glucosides already would have decreased when the peppers were collected. Bernal et al. (*7*) have reported capsaicin and the precursors coumaric acid, caffeic acid, ferulic acid, and vanillin could be oxidized by peroxidase in peppers. In their report, it was shown that these substances would be oxidized and become "lignin-like" substances. Our collected peppers were kept at normal temperature in local offices. Therefore, it is presumed that glucosides of capsaisinoids also would be metabolized by oxidation.

We are now studying how the contents of the glucosides of capsaicinoids change at different maturity stages in a variety of fruits of cultivars of *Capsicum annuum*, *Capsicum frutescens*, and *Capsicum chinense*.

It would be expected that the contents of the glucosides of capsaicinoids and correlation between the glucosides and capsaicinoids would be elucidated in detail.

ACKNOWLEDGMENT

We thank Dr. T. Furuya (Prof. Emeritus, Kitasato University) for a gift of the nordihydrocapsaicin-β-D-glucopyranoside.

LITERATURE CITED

(1) Kopp, B.; Jurenitsch, J. Biosynthesis of capsaicinoids in *Capsicum-annuum*-var-*annuum* 2. Formation of the fatty-acid moiety of the capsaicinoids from L valine, l leucine and L isoleucine. *Planta Med*. **1981**, *43*, 272−279.

(2) Kopp, B.; Jurenitsch, J. Biosynthesis of capsaicinoids in *Capsicum-annuum*-var-*annuum*. *Planta Med*. **1980**, *39*, 289.

(3) Suzuki, T.; Fujiwake, H.; Iwai, K. Intracellular localization of capsaicin and its analogues, capsaicinoid, in *Capsicum* fruit 1. Microscopic investigation of the structure of the placenta of *Capsicum annuum* var. *annuum* cv. Karayatsubusa. *Plant Cell. Physiol*. **1980**, *21*, 839−853.

(4) Fujiwake, H.; Suzuki, T.; Iwai, K. Capsaicinoid formation in the protoplast from the placenta of *Capsicum-annuum*-var-*annuum* cultivar Karayatsubusa fruits. *Agric. Biol. Chem*. **1982**, *46*, 2591−2592.

(5) Fujiwake, H.; Suzuki, T.; Iwai, K. Intra-cellular distributions of enzymes and intermediates involved in biosynthesis of capsaicin and its analogs in *Capsicum-annuum*-var-*annuum* cultivar. *Agric. Biol. Chem*. **1982**, *46*, 2685−2690.

(6) Sukrasno, N.; Yeoman, M, M. Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochemistry* **1993**, *32*, 839−844.

(7) Bernal, M.; Calderon, A.; Pedreno, M.; Munoz, R.; Ros Barcelo, A.; Merino de Caceres, F. Capsaicin oxidation by peroxidase from *Capsicum annuum* (variety *Annuum*) fruits. *J. Agric. Food Chem.* **1993**, *41*, 1041−1044.

(8) Bernal, M.; Calderon, A.; Ferrer, M.; Merino de Caceres, F.; Ros Barcelo, A. Oxidation of capsaicin and capsaicin phenolic precursors by the basic peroxidase isoenzyme B6 from hot pepper. *J. Agric. Food Chem.* **1995**, *43*, 352−355.

(9) Bernal, M.; Barcelo, R. 5,5′-dicapsaicin, 4′-*O*-5-dicapsaicin ether, and dehydrogenation polymers with high molecular weights are the main products of the oxidation of capsaicin by peroxidase from hot pepper. *J. Agric. Food Chem.* **1996**, *44*, 3085−3089.

(10) Contreras-Padilla, M.; Yahia, M. Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. *J. Agric. Food Chem.* **1998**, *46*, 2075−2079.

(11) Kawada, T.; Hagihara, K.; Iwai, K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J. Nutr*. **1985**, *116*, 1272−1278.

(12) Watanabe, T.; Kawada, T.; Yamamoto, M.; Iwai, K. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochem. Biophys. Res. Commun*. **1987**, *142*, 259−264.

(13) Kobayashi, A.; Osaka, T.; Namba, Y.; Inoue, S.; Lee, T. H; Kimura, S. Capsaicin activates heat loss and heat production simultaneously and independently in rats. *Am. J. Physiol*. **1998**, *275*, R92−R98.

(14) Kawada, T.; Watanabe, T.; Takaishi, T.; Tanaka, T.; Iwai, K. Capsaicin-induced β-adrenergic action on energy metabolism in rats. Influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc. Soc. Exp. Biol. Med.* **1986**, *183*, 250−256.

(15) Watanabe, T.; Kawada, T.; Iwai, K. Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. *Agric. Biol. Chem*. **1987**, *51*, 75−79.

(16) Kim, K.; Kawada, T.; Ishihara, K.; Inoue, K.; Fushiki, T. Increase in swimming endurance capacity of mice by capsaicin-induced adrenal catecholamine secretion. *Biosci., Biotechnol., Biochem*. **1997**, *61*, 1718−1723.

(17) Kim, K.; Kawada, T.; Ishihara, K.; Inoue, K.; Fushiki, T. Swimming capacity of mice is increased by oral administration of a nonpungent capsaicin analog, stearoyl vanillylamide. *J. Nutr*. **1998**, *128*, 1978−1983.

(18) Henderson, D. E.; Slickman, A. M.; Henderson, S. K. Quantitative HPLC determination of the antioxidant activity of capsaicin on the formation of lipid hydroperoxides of linoleic acid: a comparative study against BHT and melatonin. *J. Agric. Food Chem*. **1999**, *47*, 2563−2570.

(19) Perucka, I.; Materska, M. Antioxidant activity and content of capsaicinoids isolated from paprika fruits. *Pol. J. Food Nutr. Sci*. **2003**, *12*, 15−18.

(20) Materska, M.; Perucka, I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agric. Food Chem*. **2005**, *53*, 1750−1756.

(21) Gal, I. E. Antibacterial activity of the spice, paprika. Testing of capsicidin and capsaicin activity. *Z. Lebensm.-Unters. Forsch.* **1968**, *138*, 86−92.

(22) Janusz, J. M.; Buckwalter, B. L.; Young, P. A.; LaHann, T. R.; Farmer, R. W.; Kasting, G. B.; Loomans, M. E.; Kerckaert, G. A.; Maddin, C. S.; Berman, E. F.; Bohne, R. L.; Cupps, T. L.; Milstein, J. R. Vanilloids. 1. Analogs of capsaicin with antinociceptive and anti-inflammatory activity. *J. Med. Chem*. **1993**, *36*, 2595−2604.

(23) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Perkins, M. N.; Reid, D. J.; Winter, J. Analogues of capsaicin with agonist activity as novel analgesic agents; structure−activity studies. 1. The aromatic "A-region". *J. Med. Chem.* **1993**, *36*, 2362−2372.

(24) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. Analogues of capsaicin with agonist activity as novel analgesic agents; structure−activity studies. 2. The amide bond "B-region". *J. Med. Chem.* **1993**, *36*, 2373−2380.

(25) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. Analogues of capsaicin with agonist activity as novel analgesic agents; structure−activity studies. 3. The hydrophobic side-chain "C-region". *J. Med. Chem.* **1993**, *36*, 2381−2389.

(26) Wrigglesworth, R.; Walpole, C. S. J.; Bevan, S.; Campbell, E. A.; Dray, A.; Hughes, G. A.; James, I.; Masdin, K. J.; Winter, J. Analogues of capsaicin with agonist activity as novel analgesic agents; structure−activity studies. 4. Potent, orally active analgesics. *J. Med. Chem.* **1996**, *39*, 4942−4951.

(27) Lee, T. S. Physiological gustatory sweating in a warm climate. *J. Physiol*. **1954**, *124*, 528−542.

(28) Mihara, S.; Hiraoka, K.; Kameda, W. Synthesis of *N*-[4-(α-D-glucopyranosyloxy)-3-methoxybenzyl] nonanamide and its β-anomer. *J. Agric. Food Chem*. **1992**, *40*, 2057−2059.

(29) Kometani, T.; Tanimoto, H.; Nishimura, T.; Kanbara, I.; Okada, S. Glucosylation of capsaicin by cell suspension cultures of *Coffea arabica*. *Biosci., Biotechnol., Biochem*. **1993**, *57*, 2192−2193.

(30) Tani, Y.; Fujioka, T.; Hamada, H.; Kunimatsu, M.; Furuichi, Y. Favorable effects of vanillylnonanamide-[β]-D-glucoside on serum lipids in hyperlipidemic rats. *J. Jpn. Soc. Nutr. Food Sci*. **2003**, *56*, 181−187.

(31) Iwai, K.; Suzuki, T.; Fujiwake, H.; Oka, S. Simultaneous microdetermination of capsaicin and its four analogues by using high-performance liquid chromatography and gas chromatography−mass spectrometry. *J. Chromatogr*. **1979**, *172*, 303−311.

Received for review March 19, 2006. Revised manuscript received June 2, 2006. Accepted June 2, 2006.

JF0607720