JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3627–3631 3627
DOI:10.1021/jf904280z

Long-Chain *N*-Vanillyl-acylamides from *Capsicum* Oleoresin

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N-Vanillyl-acylamides (NVAs) naturally occur as capsaicinoids in *Capsicum* plants. NVAs with a longer chain acyl moiety (LCNVAs) have been developed as attractive tools for medicinal usage because of their capsaicin-like bioactive and physiological properties, without harmful irritancy. In this study, we isolated four LCNVAs from *Capsicum* oleoresin. Their structures were determined to be *N*-vanillyl-hexadecanamide (palvanil, **2**), *N*-vanillyl-octadecanamide (stevanil, **3**), *N*-vanillyl-9*E*-octadecenamide (olvanil, **4**), and *N*-vanillyl-9*E*,12*E*-octadecadienamide (livanil, **5**) by spectroscopic analysis and gas chromatography−mass spectrometry analysis of their methanolysis products. Furthermore, the existence of two LCNVAs in oleoresin, *N*-vanillyl-tetradecanamide (myrvanil, **1**) and *N*-vanillyl-9*E*,12*E*,15*E*-octadecatrienamide (linvanil, **6**), was suggested. The contents of these LCNVAs and the major capsaicinoids—capsaicin and dihydrocapsaicin—in three *Capsicum* oleoresins and the fresh fruits of two hot peppers were measured by a liquid chromatography−tandem mass spectrometry system. The content ratios of the total LCNVAs, except for myrvanil, versus the capsaicin in the oleoresins (0.1−41%) was significantly larger than that in fresh fruits (<0.01%). The composition of these LCNVAs in each oleoresin was similar to that of fatty acids in the oil fraction of each oleoresin. We observed no relationship between the composition of these LCNVAs in the fresh fruits.

KEYWORDS: Capsaicinoids; long-chain *N*-vanillyl-acylamides (LCNVAs); olvanil; *Capsicum* oleoresin; LC-MS/MS

INTRODUCTION

When consuming *Capsicum* fruits, the burning sensation (pungency in the mouth or irritation of the skin and mucosa) is caused by the presence of capsaicinoids. Capsaicinoids is a general term for a group of *N*-vanillyl-acylamides (NVAs) (*1*). The acyl chain length of naturally occurring NVAs ranges from 8 to 10 carbons (*2*). The most abundant NVAs in nature are capsaicin (CAP) and its dihydro analogue, dihydrocapsaicin (DC). Studies on the relationship between the acyl chain length and the pungency of NVAs revealed that a chain length of around nine carbons, such as CAP and DC, causes the strongest sensation of pungency in humans (*3*, *4*). NVAs with a longer or shorter acyl chain than CAP have less pungency, and NVAs with a chain length of more than 18 carbons chain length do not generate any stimulus. The burning sensation caused by CAP is induced by the direct activation of a nonselective cation channel—transient receptor potential vanilloid 1 (TRPV1)—which is located at the end of sensory nerves (*5*). It has been revealed that several physiological activities caused by CAP are also related to the activation of TRPV1 (*6*).

Long acyl chain NVAs (LCNVAs) have been developed as synthetic CAP analogues with CAP-like physiological activities and with no, or less, harmful stimuli (*7*). Since the late 1980s, olvanil, *N*-vanillyl-9*E*-octadecenamide, has mostly been studied as an attractive LCNVA because of its high CAP-like activities: It is anti-inflammatory (*8*) and antinociceptive (*9*), and it enhances adrenaline secretion (*10*), despite its lack of irritancy or pungency. Furthermore, several studies have shown that the potency of olvanil to activate TRPV1 is comparable to that of CAP (*5*, *11*, *12*). The paradoxical relationship between the high potency of olvanil to activate TRPV1 and its lack of pungency might be due to its lower accessibility to TRPV1 in the tongue because of its higher lipophilicity than CAP (*12*). LCNVAs with ubiquitously occurring natural fatty acid moieties, such as stearic (C18:0), linoleic (C18:2), and linolenic (C18:3) acids, have been developed as stevanil, livanil, and linvanil, respectively (*13*−*15*). LCNVAs with arachidonic (C20:4) and docosahexanoic (C22:6) acids have also been investigated (*16*, *17*).

In the course of our survey on various capsaicinoids from natural sources, we found several LCNVAs in a foodstuff

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Published on Web 03/01/2010

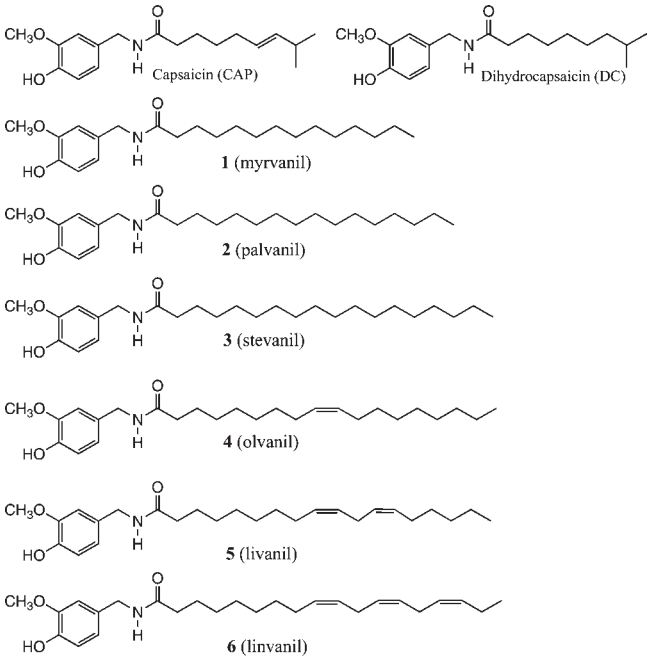

Figure 1. Chemical structures of capsaicinoids (CAP and DC) and LCNVAs.

commonly used as a seasoning, *Capsicum* oleoresin. The six LCNVAs were identified to be myrvanil, palvanil, stevanil, olvanil, livanil, and linvanil (**Figure 1**) by spectroscopic analysis together with their chemical derivatization and/or by comparison of the data with authentic compounds. The contents of these LCNVAs in three oleoresins and the fruits of two hot peppers were determined by a liquid chromatography−tandem mass spectrometry (LC-MS/MS) analysis. On the basis of the relationship between the contents of the LCNVAs and the fatty acid composition of the oleoresins and the fruits, we discussed the origin of the LCNVAs in the oleoresins.

MATERIALS AND METHODS

Materials. Three types of *Capsicum* oleoresin (A−C) were obtained from a Chinese market. The fresh fruits of *Capsicum annuum* cv. Takanotsume and *Capsicum chinense* cv. Habanero were harvested from the experimental farm at the University of Shizuoka (Japan). Authentic CAP and DC were purchased from Sigma (St. Louis, MO). Authentic LCNVAs were prepared according to a previous report (*18*). The other reagents were of guaranteed grade.

Apparatus. ^{1}H and ^{13}C NMR spectra (tetramethylsilane was used as the internal standard) were recorded on a JEOL α-400 instrument (JEOL, Tokyo, Japan) at 399.65 and 100.40 MHz, respectively. LC-atmospheric pressure chemical ionization (APCI)-MS/MS analysis was performed with the API2000 LC-MS/MS system (Applied Biosystems, Carlsbad, CA) equipped with a semimicro high-performance liquid chromatography (HPLC) system (Nanospace SI-1, Shiseido, Tokyo, Japan). Gas chromatography−mass spectrometry (GC-MS) analysis was performed with the Agilent 6890 GC and 5975 MSD system (Agilent Technologies, Santa Clara, CA).

Isolation of LCNVAs from *Capsicum* Oleoresin. *Capsicum* oleoresin (sample A, 87.8 g) was extracted with MeOH (200 mL × 4) to obtain an LCNVA-containing extract (8.7 g). The extract was chromatographed on a silica gel column (70 mm i.d. × 200 mm) with the stepwise elution of *n*-hexane and EtOAc [*n*-hexane/EtOAc = 90:10 (1 L, fractions 1 and 2) → 80:20 (1 L, fractions 3 and 4) → 70:30 (1 L, fractions 5 and 6) → 60:40 (1 L, fractions 7 and 8) → 50:50 (3.5 L, fractions 9−15)]. Two fractions (fractions 12 and 13) were the LCNVAs-containing fractions. Fraction 12 was chromatographed with an MPLC system (Yamazen Co., Osaka, Japan) using a reversed-phase silica gel column (UltraPack ODS-50B, 26 mm i.d. × 300 mm, Yamazen) with a stepwise elution of MeOH and water [70% MeOH (100 mL) → 80% MeOH (900 mL) → 85% MeOH (500 mL) → 90% MeOH (500 mL)]. The 80% MeOH elution was purified by an HPLC system (Shimadzu, Kyoto, Japan) using a reversed-phase silica gel column (J'sphare ODS-H80, 20 mm i.d. × 150 mm, YMC, Kyoto, Japan) with 95% MeOH to attain compound **5** (53.0 mg). Further purification of the 90% MeOH elution by the same HPLC conditions yielded compound **3** (5.7 mg). The same HPLC system equipped with a recycle valve (HPV-Rc, GL Sciences Inc., Tokyo, Japan) enabled the isolation of compound **2** (23.8 mg) and compound **4** (12.4 mg) from the 85% MeOH elution.

Fraction 13 was chromatographed with the same MPLC conditions as described above. The fraction eluted with 85% MeOH was subjected to the same HPLC conditions to yield a combination of compounds **1** and **6** (0.6 mg).

*Compound **2*** (*N-Vanillyl-hexadecanamide, Palvanil*). Colorless amorphous. Positive-ion APCI-MS: *m*/*z* 392 $[M + H]^+$, 268, 256, 137. ^{1}H NMR: δ 6.86 (1H, d), 6.80 (1H, d), 6.75 (1, dd), 5.71 (1H, br, NH), 4.35 (2H, d), 3.87 (3H, s, OMe), 2.19 (2H, t), 1.63 (2H, quint), 1.25 (24H, m), 0.88 (3H, t). ^{13}C NMR: δ 173.0, 146.7, 145.1, 130.4, 120.8, 114.4, 110.7, 55.9, 43.5, 36.9, 31.9, 29.7 (multiplet), 29.6, 29.5, 29.4, 29.4, 29.3, 25.8, 22.7, 14.1.

*Compound **3*** (*N-Vanillyl-octadecanamide, Stevanil*). Colorless amorphous. Positive-ion APCI-MS: *m*/*z* 420 $[M + H]^+$, 296, 284, 137. ^{1}H NMR: δ 6.86 (1H, d), 6.80 (1H, d), 6.75 (1, dd), 5.63 (1H, br, NH), 4.35 (2H, d), 3.88 (3H, s, OMe), 2.19 (2H, t), 2.01 (4H, m), 1.65 (2H, m), 1.28 (20H, m), 0.88 (3H, t). ^{13}C NMR: δ 172.9, 146.7, 145.1, 130.4, 120.8, 114.3, 110.7, 55.9, 43.5, 36.9, 31.9, 29.8, 29.7, 29.5, 29.3 (multiplet), 29.2, 27.2, 27.2, 25.8, 22.7, 14.1.

*Compound **4*** (*N-Vanillyl-9E-octadecenamide, Olvanil*). Colorless oil. Positive-ion APCI-MS: *m*/*z* 418 $[M + H]^+$, 294, 282, 137. ^{1}H NMR: δ 6.86 (1H, d), 6.80 (1H, d), 6.75 (1, dd), 5.66 (1H, br, NH), 5.34 (2H, m), 4.35 (2H, d), 3.87 (3H, s, OMe), 2.19 (2H, t), 1.63 (2H, quint), 1.25 (24H, m), 0.88 (3H, t). ^{13}C NMR: δ 172.9, 146.7, 145.1, 130.4, 130.0, 129.7, 120.8, 114.4, 110.7, 55.9, 43.5, 36.9, 31.9, 29.8, 29.7, 29.5, 29.3, 29.3, 29.3, 29.3, 29.2, 27.2, 27.2, 25.8, 22.7, 14.1.

*Compound **5*** (*N-Vanillyl-9E,12E-octadecadienamide, Livanil*). Colorless oil. Positive-ion APCI-MS: *m*/*z* 416 $[M + H]^+$, 292, 280, 137. ^{1}H NMR: δ 6.86 (1H, d), 6.80 (1H, d), 6.75 (1, dd), 5.77 (1H, br, NH), 5.35 (4H, m), 4.34 (2H, d), 3.87 (3H, s, OMe), 2.77 (2H, t), 2.19 (2H, t), 2.04 (4H, m), 1.63 (2H, quint), 1.35 (14H, m), 0.89 (3H, t). ^{13}C NMR: δ 173.0, 146.7, 145.1, 130.3, 130.2, 130.0, 128.1, 127.9, 120.8, 114.4, 110.7, 55.9, 43.5, 36.8, 31.5, 29.6, 29.4, 29.3, 29.3, 29.2, 29.1, 27.2, 25.8, 25.6, 22.6, 14.1.

Methanolysis of LCNVAs for GC-MS Analysis. A small amount (ca. 0.5 mg) of each of the compounds (**2**−**5**) and the mixture of compounds **1** and **6** were dissolved in ca. 1 mL of MeOH/concentrated HCl (7:3); they were then heated at 100 °C for 20 h. After extraction with *n*-hexane, an aliquot of the *n*-hexane fraction was subjected to GC-MS analysis. The GC-MS conditions were as follows: column, HP-5MS, 0.25 mm i.d. × 30 m (Agilent Technology); injector temperature, 260 °C; oven temperature, initial temperature, 160 °C increased at 3 °C/min to 240 °C; mobile phase, He, 2 mL/min; injection, splitless; and injection volume, 1 μL. The operation of the apparatus was performed with the ChemStation software (Agilent), and the database analysis was by the NIST05.

LC-MS/MS Quantification of LCNVAs in Samples. Each of the *Capsicum* oleoresins (A, 1.0 g; B, 1.4 g; and C, 2.9 g) was extracted with MeOH (10 mL × 3). The MeOH fractions were dried by evaporation; the residues were again dissolved and diluted with MeOH containing 0.1% AcOH for LC-MS/MS analyses.

The fresh fruits of Habanero (20.6 g) and Takanotsume (10.2 g) were freeze-dried, and their seeds and calyces were removed. The residues (4.14 g of Habanero and 6.24 g of Takanotsume) were ground and then soaked with EtOAc (41.4 mL for Habanero and 62.4 mL for Takanotsume) for 1 month. After centrifugation, an aliquot of the supernatants was subjected to LC-MS/MS analysis, as described below, to quantify the LCNVAs. Another aliquot of each of the supernatants was dried to weigh the oleoresin of the pepper fruits. The weights of the Habanero and Takanotsume oleoresins were estimated to be 0.28 and 1.09 g from 20.6 and 10.2 g of the fresh fruits, respectively.

The LC-MS/MS conditions were as follows: LC column, a reversed-phase silica gel column, Unison UKC-8, 2 mm i.d. × 150 mm (Imtakt Co.,

Table 1. Contents of Capsaicinoids (CAP and DC) and LCNVAs (**1**−**6**) in *Capsicum* Oleoresins and Fruit Extracts[a]

	oleoresins (μg/g)			fruit extracts (μg/g DW)	
	A	B	C	Habanero	Takanotsume
CAP	5790 (100)	3.33 (100)	6490 (100)	90500 (100)	9020 (100)
DC	4170 (72)	2.96 (89)	4750 (73)	33400 (37)	6670 (74)
1	19.5 (0.34)	0.0060 (0.18)	4.21 (0.07)	36.1 (0.04)	4.41 (0.05)
2	392 (6.80)	0.0025 (0.08)	1.05 (0.02)	2.80 (<0.01)	0.578 (<0.01)
3	45.9 (0.79)	0.0016 (0.05)	0.13 (<0.01)	0.0155 (<0.01)	0.0025 (<0.01)
4	544 (9.40)	0.0007 (0.02)	1.02 (0.02)	0.0346 (<0.01)	0.0030 (<0.01)
5	1370 (24.0)	0.0037 (0.10)	3.15 (0.05)	ND	0.0017 (<0.01)
6	17.4 (0.30)	0.0013 (0.04)	0.649 (0.01)	0.0530 (<0.01)	0.0009 (<0.01)

[a] ND, not detected. Parentheses show the percentage content of each compound against CAP.

Kyoto, Japan); solvent, 80−100% MeOH containing 0.1% AcOH (0−15 min), 100% MeOH containing 0.1% AcOH (15−25 min); flow rate, 0.2 mL/min; injection volume, 5 μL; MS/MS, ion source, APCI; polarity, positive; detection mode, multiple reaction monitoring (MRM); detected ions, precursor/product, 306/137 for CAP, 308/137 for DC, 364/137 for **1**, 392/137 for **2**, 420/137 for **3**, 418/137 for **4**, 416/137 for **5**, and 414/137 for **6**. These ions were observed in the mass chromatogram at 9.6, 12.9, 15.7, 13.6, 11.8, and 10.1 min, respectively. The optimum parameters for the detection of each compound were tuned automatically using authentic samples by the Analyst software (Applied Biosystems). The samples were analyzed in duplicate, and each compound was quantified by the use of the calibration curves from the authentic samples.

GC-MS Analysis of Fatty Acid Compositions in the Oil Fractions of Samples. Approximately 10 mg of each sample (the oleoresins and the pepper fruit extracts) was dissolved in 25 μL of $CHCl_3$ solution containing 2% (w/v) pentadecanoic acid as an internal standard. The mixture was dried under a nitrogen stream. After the residue was heated at 100 °C for 1 min with 250 μL of 0.5 M NaOH in MeOH, the mixture was further heated at 100 °C for 2 min with 300 μL of 14% BF_3 in MeOH. After petroleum ether and water were added to the cooled mixture, the organic layer was collected and dried under a nitrogen stream. The residue was diluted with 100 mL of *n*-hexane for GC-MS analysis. The conditions of GC-MS have been described above.

RESULTS AND DISCUSSION

Isolation of LCNVAs from *Capsicum* Oleoresin and the Structural Elucidation of the LCNVAs. It is difficult to isolate capsaicinoids from *Capsicum* oleoresin by chromatographic methods because oleoresin mainly consists of oils (triacylglycerols). In a preliminary experiment, the liquid−liquid partition of the oleoresin with methanol was determined to be suitable for the extraction of natural capsaicinoids (CAP and DC) and spiked olvanil quantitatively into methanol fractions. In the present study, therefore, we used methanol to extract the capsaicinoids and LCNVAs from a *Capsicum* oleoresin sample (sample A). Silica gel TLC analysis of the extract showed a typical color development with Gibbs reagent caused by phenolic compounds; the extract had a higher R_f value than CAP and DC, suggesting the existence of capsaicinoids that were more hydrophobic than CAP and DC.

We isolated four compounds (**2**−**5**) from the extract by several chromatographic methods (see the Materials and Methods). Their ^{1}H NMR spectra showed the typical signals of the vanillyl moiety of capsaicinoids, that is, a 1,2,4-substituted benzene (δ 6.86, 6.80, and 6.75), a methylene (δ 4.35), and a methoxy group (δ 3.88) attached to the benzene ring. Although the higher magnetic fields of their spectra indicated the existence of long-chain acyl moieties, it was difficult to estimate their exact structure from the data. However, it appeared that only one olefin group (δ_H 5.34, 2H; δ_C 130.0 and 129.7) was in the acyl moiety of **4**, and two olefin groups (δ_H 5.35, 4H; δ_C 130.2, 130.0, 128.1, and 127.9) were in the acyl moiety of **5**. To confirm the structures of the acyl moieties, GC-MS analyses of the methanolysis products of each of the compounds were performed. The NIST database determined the methanolysis products of **2**−**5** to be methyl esters of hexadecanoic, octadecanoic, 9*E*-octadecenoic, and 9*E*,12*E*-octadecadienoic acids, respectively. From these data, the structures of **2**−**5** were elucidated to be *N*-vanillyl-hexadecanamide (palvanil), *N*-vanillyl-octadecanamide (stevanil), *N*-vanillyl-9*E*-octadecenamide (olvanil), and *N*-vanillyl-9*E*,12*E*-octadecadienamide (livanil), respectively (**Figure 1**). The APCI-MS spectra on the positive mode for these compounds showed mass peaks at *m*/*z* 392 for **2**, 420 for **3**, 418 for **4**, and 416 for **5**. These protonated molecular ion peaks of **2**−**5** strongly supported their structures. Furthermore, the common fragment ion of **2**−**5** at *m*/*z* 137 indicated the typical vanillylamine moiety caused by the cleavage of capsaicinoids (*19*, *20*). All of the data for **2**−**5** were in complete agreement with the chemically synthesized authentic compounds (*18*).

We were also able to obtain a very small quantity of the mixture of compounds **1** and **6** from the oleoresin. Although further purification of the compounds from the mixture could not be achieved, the ^{1}H NMR spectrum of the mixture conclusively indicated the existence of capsaicinoids (data not shown). GC-MS analysis of the methanolysis products of the mixture revealed the existence of methyl esters of two fatty acids, tetradecanoic and 9*E*,12*E*,15*E*-octadecatrienoic acids. We, therefore, estimated the structures of **1** and **6** to be *N*-vanillyl-tetradecanamide (myrvanil) and *N*-vanillyl-9*E*,12*E*,15*E*-octadecatrienamide (linvanil), respectively (**Figure 1**). HPLC analysis of the mixture showed two peaks whose retention times were in complete agreement with the chemically synthesized authentic compounds (*18*).

Contents of LCNVAs in *Capsicum* Oleoresins and Fruits. Various methods for capsaicinoids analysis have been developed in the last century (*2*). Recently, the LC-MS technique has been applied to capsaicinoids analysis (*19*−*21*). Although electronic spray ionization (ESI) has been mainly used as the ionization method for capsaicinoids, we selected the APCI method for the LCNVAs analysis because APCI is effective for the ionization of higher hydrophobic compounds like LCNVAs. The positive-ion APCI-MS spectra of each LCNVA showed a corresponding protonated molecular mass ($[M + H]^+$) as the major peak (see the Materials and Methods). The successive fragmentation of the peak for each LCNVA by neutral gas collision (MS/MS analysis) conclusively showed a common peak at *m*/*z* 137, which presents the vanillyl moiety derived from the cleavage of NVAs at their amide bond (*19*, *20*). Therefore, we chose these two characteristic ions (MRM) on an LC-APCI-MS/MS to identify and quantify each LCNVA (see the Materials and Methods). In the MRM chromatogram of the mixture of authentic CAP, DC, and LCNAVs (**1**−**6**), the baseline resolution was achieved at a relatively higher quantity of the compounds (50 pmol each). The detection limit was approximately 0.01 pmol, and the

dynamic range was 0.05−500 pmol under the conditions employed.

Table 1 shows the contents of the LCNVAs (**1**−**6**), CAP, and DC from *Capsicum* oleoresin samples (A−C) and extracts from the pepper fruits (Habanero and Takanotsume), measured by LC-APCI-MS/MS. In all of the samples, CAP and DC were the dominant components of NVAs. The total amounts of CAP and DC in the dry fruits of Habanero and Takanotsume were calculated as 8380 and 2740 μg/g dw, respectively, which were within the ordinary amounts for these varieties (*22*). The total amounts of CAP and DC in the oleoresins A and C were similar to those of the fruit extract from Takanotsume. The ratio of DC to CAP in these oleoresins was also similar to that observed in Takanotsume. Therefore, the oleoresins A and C might be extracts from a Takanotsume-like variety.

Table 2. Fatty Acid Composition (mg/g) of the Oil Fraction in *Capsicum* Oleoresins and Fruit Extracts[a]

	oleoresins			fruit extracts	
	A	B	C	Habanero	Takanotsume
C12:0	ND	3	ND	ND	ND
C14:0	5	8	6	4	ND
C16:0	200	82	78	96	110
C18:0	20	15	19	20	9
C18:1	150	150	140	96	57
C18:2	750	520	470	500	770
C18:3	ND	ND	ND	ND	ND

[a] ND, not detected; C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; and C18:3, linolenic acid.

The contents of LCNVAs in the samples were very small, except for oleoresin A. Only a negligible amount of the LCNVAs **2**−**6** was detected in the fruit extracts, and the amount ratios of each LCNVA to CAP were extremely small (< 0.01% each). In contrast, oleoresin A contained a large amount of total LCNVAs (**2**−**6**), 2370 μg/g, and its amount ratio to CAP was over 41%. Although the amounts of **2**−**6** in the other oleoresins (samples B and C) were also very small, their total amount ratios to CAP were obviously remarkable when compared to those of the fruit extracts (0.3% for B and 0.1% for C). On the other hand, *N*-vanillyl-tetradecanamide (myrvanil, **1**) and *N*-vanillyl-hexadecanamide (palvanil, **2**) were significantly abundant in the fresh pepper fruit extracts. Therefore, it is possible that intact fruits of *Capsicum* plants naturally possess these LCNVAs (**1** and **2**). The other LCNVAs are probably generated and/or increased in *Capsicum* oleoresin by an undetermined mechanism.

Relationship between the Composition of LCNVAs and Fatty Acids in *Capsicum* Oleoresins and Fruits. The oil fraction of plants or their products primarily consists of glyceric esters of fatty acids (triacylglycerol). **Table 2** shows the fatty acid composition of the

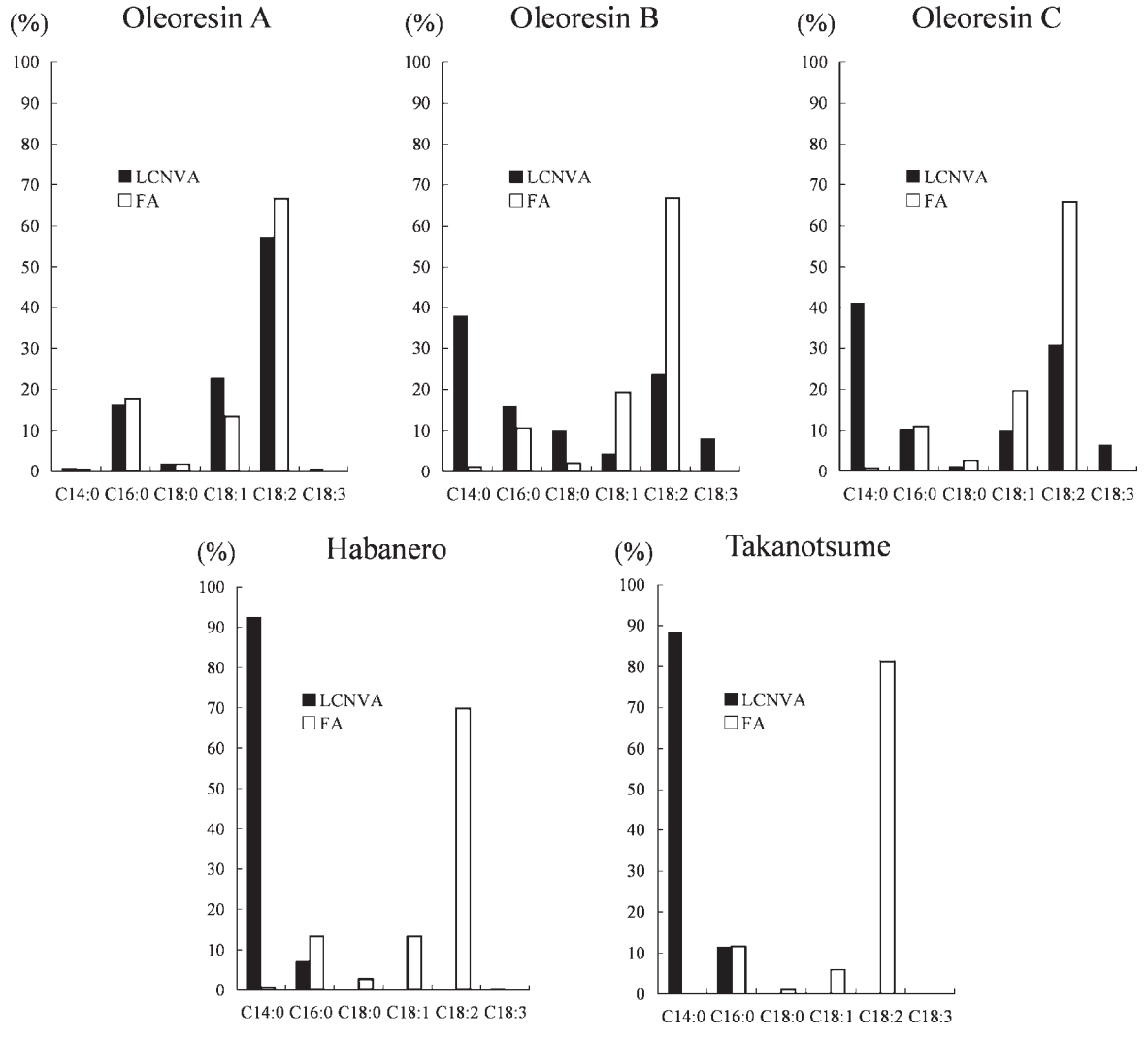

Figure 2. Comparison of the relative contents of LCNVAs (**1**−**6**) and the fatty acid (FA) composition of the oil fraction in *Capsicum* oleoresins (A−C) and fruits (Habanero and Takanotsume).

oil fractions in the oleoresins and pepper fruits, measured by GC-MS analysis after methanolysis of the oil fractions. The richest fatty acid in all of the samples was linoleic acid (C18:2), followed by oleic (C18:1) or palmitic (C16:0) acids. In terms of composition, the samples were similar to each other and also to the compositions of common peppers (*22*). Therefore, the oleoresins we used must be the products processed by simple extraction from some peppers.

Figure 2 shows the comparison of the percent ratios of the fatty acid composition and LCNVAs content for the samples. In oleoresin A, the pattern of the ratio of fatty acids closely resembled those of LCNVAs. The patterns for oleoresins B and C were also alike, especially when myristic acid (C14:0) and myrvanil (**1**) were excluded. On the other hand, no resemblance was observed with the fruit samples even when C14:0 and **1** were excluded. These results suggest that myrvanil (**1**) and palvanil (**2**) naturally occur in intact peppers, while the others (**3**−**6**) would be generated and accumulate in the oil fraction extracted from the peppers and that the generation of LCNVAs would be affected by the fatty acid composition of the oil fraction. This suggestion was consistent with the close resemblance between the patterns of LCNVAs and fatty acids that was observed in oleoresin A, the sample with the highest accumulated amount of LCNVAs. There might be a positive correlation between the amount of LCNVAs and the storage and/or maturation period of oleoresin.

Transacylation of triacylglycerols with natural capsaicinoids like CAP and DC to generate LCNVAs probably occurred spontaneously during the storage of the *Capsicum* oleoresins. A nucleophilic amine could react with a carboxylic group, such as glyceride, to generate an amide in ambient conditions. Therefore, the vanillylamine in the pepper fruits could also be a possible source of the vanillyl moiety of LCNVAs. This possibility could be supported by our previous report on the existence of olvanil in olive oil flavored with *Capsicum* pepper (*23*). A trace amount of linvanil (**6**) was detected despite the absence of linolenic acid (C18:3) in the oleoresins. The acyl moiety of this LCNVA might be donated from an extremely small amount of linolenic acid that would be undetectable by GC-MS analysis. Further investigation into the mechanism responsible for the generation of LCNVAs in *Capsicum* oleoresin is now in progress.

We found several LCNVAs from natural sources. These LCNVAs might be spontaneously generated from the major capsaicinoids (CAP and DC) and plant oils during the storage and/or maturation of these sources.

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Received for review December 3, 2009. Revised manuscript received February 9, 2010. Accepted February 13, 2010. This work was supported by the Grant-in-Aid for Scientific Research (C) (21580152) from the JPSP (Japan) and the Global Center of Excellence (COE) program from the MEXT (Japan).