Stability of Capsinoid in Various Solvents

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To investigate the stability of capsinoid in solvents, the quantitative change of vanillyl nonanoate, a synthetic model capsinoid, in various solvents was measured by HPLC. Vanillyl nonanoate was stable in nonpolar solvents, whereas it was labile in polar solvents. In particular, vanillyl nonanoate tended to decompose in protic solvents such as alcohol and water. Structures of the decomposition products from vanillyl nonanoate in methanol and ethanol were determined to be methyl and ethyl vanillyl ethers, respectively. To clarify the decomposition mechanism of capsinoid, six analogues of vanillyl nonanoate were tested. The stability of the analogues in organic solvents suggested that the hydroxyl group in the para-position of the benzene ring largely contributes to the decomposition of capsinoid.

Keywords: *Capsinoid; capsiate; vanillyl nonanoate; stability; solvolysis*

INTRODUCTION

Recently three homologues of nonpungent compounds, capsiate, dihydrocapsiate, and nordihydrocapsiate, have been isolated from the fruits of a sweet cultivar of pepper, CH-19 Sweet (*Capsicum annuum* L.) (*1*, *2*). They were fatty acid esters of vanillyl alcohol, and the compound group was named capsinoid (*2*). The major natural capsinoid was capsiate [4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate (**1**)] (Figure 1). Capsaicinoid is a pungent principal of *Capsicum* plants (*3*, *4*), and it has various physiological activities (*5*, *6*). Capsinoid bears a marked structural resemblance to capsaicinoid, except for the central linkage; that is, an amide moiety is found in capsaicinoid, and an ester moiety is found in capsinoid. Capsinoid has no pungency upon oral tasting. Therefore, it is expected that capsinoid has physiological functions similar to those of capsaicinoid and causes less harmful stimulation. Such characteristics are important for its wide usage as a food or medicine. It has been reported that in a feeding experiment of dry fruits of CH-19 Sweet capsinoid raises the body temperature in humans (*7*). However, as compared with capsaicinoid, capsinoid easily decomposes in methanol or during the purification process by silica gel column chromatography. The decomposition mechanism is not understood. To undertake further experimentation in which capsinoid will be used as a solution, it is necessary to know its stability in various solvents.

To investigate the stability of capsinoid in various solvents, the quantitative change of vanillyl nonanoate (**2**), a synthetic model capsinoid, in various solvents was measured by HPLC. The decomposition mechanism of capsinoid was determined from the structures of the decomposition products from vanillyl nonanoate and the stability of synthesized vanillyl nonanoate analogues having varied substituents in the aromatic portion.

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MATERIALS AND METHODS

Chemicals. Capsaicin, vanillyl alcohol, nonanoyl chloride, 3-methoxybenzyl alcohol, *o*-hydroxybenzyl alcohol, and *m*-hydroxybenzyl alcohol were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Acetic anhydride, 3,4-dimethoxybenzyl alcohol, and *p*-hydroxybenzyl alcohol were obtained from Wako Pure Chemical Industries (Osaka, Japan). Capsiate was isolated from the fruits of *C. annuum* L. cv. CH-19 Sweet (*1*). The rest of the chemicals used in this study were of analytical grade.

Instruments. Spectroscopic measurements were done with the following instruments: NMR, Alpha-400 (JEOL, Japan), ^{1}H 399.65 MHz, ^{13}C 100.40 MHz, $CDCl_3$, TMS as internal standard; HRMS, JMS-AX500 (JEOL); HPLC system composed of a pump, PU-980 (Jasco, Japan), a fluorescence detector, FP-920 (Jasco), and a UV detector, UV-970 (Jasco).

Stability of Capsinoid in Solvents. A methanol solution (1 mL) of 0.5 mM capsaicin was added as an internal standard into a 5-mL volume screw-top vial, and the methanol was evaporated under a nitrogen stream. Then, capsinoid (0.8 mg) was added to the vial and a 2 mM capsinoid solution was prepared with addition of the testing solvents. Physiological saline solution containing 2% ethanol and 10% Tween 80 was used as an aqueous solvent (aqueous surfactant vehicle). Immediately after the preparation, the samples were kept at 25 °C. An aliquot of the sample was taken at adequate time intervals and was subjected to HPLC analysis.

HPLC was carried out under the following conditions: column, J'sphere ODS-H80, 150 mm × 4.6 mm i.d. (YMC); solvent, 80% methanol; flow rate, 0.5 mL/min; detection, fluorescence, excitation 280 nm, emission 320 nm; UV, 280 nm.

Chemical Syntheses of Vanillyl Nonanoate Analogues. *Vanillyl Nonanoate (**2**).* Vanillyl alcohol (1543 mg) was dissolved in dehydrated pyridine (10 mL), and nonanoyl chloride (0.9 mL) was added dropwise to the solution under ice cooling. The mixture was stirred for 2 h under ice cooling, followed by the addition of H_2O (20 mL) to terminate the reaction and 2 N HCl (20 mL) to acidify the solution. The solution was extracted three times with 60 mL each of ethyl acetate, and the resulting organic portion was washed with water and dehydrated with sodium sulfate. After evaporation, the residue was rapidly fractionated by silica gel column chromatography under the following conditions: silica gel 60 (Merck), 30 g; column, 800 mm × 36 mm i.d.; eluent, hexane/ethyl acetate

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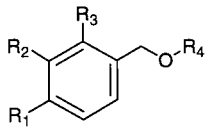

		R_1	R_2	R_3	R_4
1	Capsiate	OH	OCH_3	H	-C(=O)-$(CH_2)_4$CH=CHCH$(CH_3)_2$
2	Vanillyl nonanoate	OH	OCH_3	H	-C(=O)-$(CH_2)_7CH_3$
3	4-Acetoxy-3-methoxybenzyl nonanoate	OAc	OCH_3	H	-C(=O)-$(CH_2)_7CH_3$
4	3,4-Dimethoxybenzyl nonanoate	OCH_3	OCH_3	H	-C(=O)-$(CH_2)_7CH_3$
5	3-Methoxybenzyl nonanoate	H	OCH_3	H	-C(=O)-$(CH_2)_7CH_3$
6	*o*-Hydroxybenzyl nonanoate	H	H	OH	-C(=O)-$(CH_2)_7CH_3$
7	*m*-Hydroxybenzyl nonanoate	H	OH	H	-C(=O)-$(CH_2)_7CH_3$
8	*p*-Hydroxybenzyl nonanoate	OH	H	H	-C(=O)-$(CH_2)_7CH_3$
9	Methyl vanillyl ether	OH	OCH_3	H	CH_3
10	Ethyl vanillyl ether	OH	OCH_3	H	C_2H_5

Figure 1. Structures of capsiate (**1**), vanillyl nonanoate (**2**), 4-acetoxy-3-methoxybenzyl nonanoate (**3**), 3,4-dimethoxybenzyl nonanoate (**4**), 3-methoxybenzyl nonanoate (**5**), *o*-hydroxybenzyl nonanoate (**6**), *m*-hydroxybenzyl nonanoate (**7**), *p*-hydroxybenzyl nonanoate (**8**), and decomposition products methyl vanillyl ether (**9**) and ethyl vanillyl ether (**10**).

(8:2). The fraction containing the desired compound was purified by reversed-phase column chromatography under the following conditions: Wakosil 25C18 (Wako), 20 g; column, 120 mm × 21 mm i.d.; eluent, methanol/H_2O (4:1). The eluent gave a colorless oil (**2**, 191.6 mg, 13% yield). Spectral data of HRMS and ^{1}H NMR of **2** completely agreed with published data (*8*).

*4-Acetoxy-3-methoxybenzyl Nonanoate (**3**).* The mixture of vanillyl nonanoate (9.68 mg), acetic anhydride (10 mL), and dehydrated pyridine (10 mL) was heated for 20 min under reflux, and then the solvent was evaporated in vacuo. The residue was purified by HPLC under the following conditions: column, J'sphere ODS-H80, 150 mm × 20 mm i.d. (YMC); solvent, 80% methanol; flow rate, 8.0 mL/min; detection, UV 280 nm. The fraction gave a colorless oil (**3**, 9.63 mg, 87% yield). Spectral data of **3** were as follows: HRMS, *m/z* (M^+) calcd for $C_{19}H_{28}O_5$ 336.1937, found 336.1956; ^{1}H NMR δ 7.01 (1H, d, J = 7.6 Hz, 5′-H), 6.94 (1H, dd, J = 7.6, 1.6 Hz, 6′-H), 6.93 (1H, d, J = 1.6 Hz, 2′-H), 5.08 (2H, s, 1′-CH_2), 3.84 (3H, s, 3′-OCH_3), 2.35 (2H, t, J = 7.6 Hz, 2-H), 2.31 (3H, s, 4′-$OCOCH_3$), 1.64 (2H, quint, J = 7.6 Hz, 3-H), 1.28 (10H, m, 4−8-H), 0.88 (3H, t, J = 6.8 Hz, 9-H); ^{13}C NMR δ 173.6 (C-1), 169.0 (4′-$\underline{O}\underline{C}OCH_3$), 151.1 (C-3′), 139.6 (C-4′), 135.1 (C-1′), 122.8 (C-5′), 120.6 (C-6′), 112.4 (C-2′), 65.7 (1′-CH_2), 55.9 (3′-OCH_3), 34.3 (C-2), 31.8 (C-7), 29.2 (C-4), 29.1 (C-5,6), 25.0 (C-3), 22.6 (C-8), 20.7 (4′-$OCO\underline{C}H_3$), 14.1 (C-9).

*3,4-Dimethoxybenzyl nonanoate (**4**).* 3,4-Dimethoxybenzyl nonanoate was synthesized from 3,4-dimethoxybenzyl alcohol (0.8 mL) and nonanoyl chloride (0.895 mL) at room temperature in a manner similar to that used for **2**. The resulting residue was purified by silica gel column chromatography under the following conditions: silica gel 60 (Merck), 30 g; column, 800 mm × 36 mm i.d.; eluent, hexane/ethyl acetate (91:9). The eluent gave a colorless oil (**4**, 1342.6 mg, 92% yield). Spectral data of **4** were as follows: HRMS, *m/z* (M^+) calcd for $C_{18}H_{28}O_4$ 308.1988, found 308.1963; ^{1}H NMR δ 6.88 (3H, m, 2′,5′,6′-H), 5.05 (2H, s, 1′-CH_2), 3.88 (3H, s, 4′-OCH_3), 3.87 (3H, s, 3′-OCH_3), 2.33 (2H, t, J = 7.6 Hz, 2-H), 1.63 (2H, quint, J = 7.6 Hz, 3-H), 1.26 (10H, m, 4−8-H), 0.87 (3H, t, J = 7.0 Hz, 9-H); ^{13}C NMR δ 173.8 (C-1), 149.1 (C-4′), 149.0 (C-3′), 128.8 (C-1′), 121.2 (C-6′), 111.8 (C-5′), 111.1 (C-2′), 66.2 (1′-CH_2), 56.0 (4′-OCH_3), 55.9 (3′-OCH_3), 34.4 (C-2), 31.8 (C-7), 29.2 (C-4), 29.1 (C-5,6), 25.0 (C-3), 22.7 (C-8), 14.1 (C-9).

*3-Methoxybenzyl Nonanoate (**5**).* 3-Methoxybenzyl nonanoate (**5**, 1398.1 mg, 95% yield) was obtained from 3-methoxybenzyl alcohol (1.0 mL) and nonanoyl chloride (0.987 mL) in a manner similar to that used for **4**. Spectral data of **5** were as follows: HRMS, *m/z* (M^+) calcd for $C_{17}H_{26}O_3$ 278.1882, found 278.1870; ^{1}H NMR δ 7.27 (1H, t, J = 7.8 Hz, 5′-H), 6.90 (3H, m, 2′,4′,6′-H), 5.09 (2H, s, 1′-CH_2), 3.81 (3H, s, 3′-OCH_3), 2.36 (2H, t, J = 7.6 Hz, 2-H), 1.64 (2H, quint, J = 7.6 Hz, 3-H), 1.26 (10H, m, 4−8-H), 0.87 (3H, t, J = 6.8 Hz, 9-H); ^{13}C NMR δ 173.7 (C-1), 159.7 (C-3′), 137.7 (C-1′), 129.6 (C-5′), 120.3 (C-6′), 113.6 (C-2′,4′), 65.9 (1′-CH_2), 55.2 (3′-OCH_3), 34.4 (C-2), 31.8 (C-7), 29.2 (C-4), 29.1 (C-5,6), 25.0 (C-3), 22.6 (C-8), 14.1 (C-9).

*o-Hydroxybenzyl Nonanoate (**6**).* *o*-Hydroxybenzyl nonanoate (**6**, 985.3 mg, yield 35%) was obtained from *o*-hydroxybenzyl alcohol (2 g) and nonanoyl chloride (1.997 mL) under ice cooling in a manner similar to that used for **4**. Spectral data of **6** were as follows: HRMS, *m/z* (M^+) calcd for $C_{16}H_{24}O_3$ 264.1725, found 264.1720; ^{1}H NMR δ 7.88 (1H, s, OH), 7.27 (2H, m, 4′,6′-H), 6.92 (2H, m, 3′,5′-H), 5.12 (2H, s, 1′-CH_2), 2.35 (2H, t, J = 7.6 Hz, 2-H), 1.61 (2H, quint, J = 7.6 Hz, 3-H), 1.25 (10H, m, 4−8-H), 0.87 (3H, t, J = 7.0 Hz, 9-H); ^{13}C NMR δ 176.6 (C-1), 155.6 (C-2′), 132.2 (C-6′), 131.1 (C-4′), 121.8 (C-1′), 120.5 (C-5′), 117.9 (C-3′), 63.2 (1′-CH_2), 34.2 (C-2), 31.8 (C-7), 29.1 (C-4), 29.0 (C-5,6), 24.8 (C-3), 22.7 (C-8), 14.1 (C-9).

*m-Hydroxybenzyl Nonanoate (**7**).* *m*-Hydroxybenzyl nonanoate (**7**, 1252.5 mg, 44% yield) was obtained from *m*-hydroxybenzyl alcohol (2 g) and nonanoyl chloride (2.017 mL) under ice cooling in a manner similar to that used for **4**. Spectral data of **7** were as follows: HRMS, *m/z* (M^+) calcd for $C_{16}H_{24}O_3$ 264.1726, found 264.1707; ^{1}H NMR δ 7.22 (1H, t, J = 7.6 Hz, 5′-H), 6.90 (1H, d, J = 7.6 Hz, 6′-H), 6.81 (2H, m, 2′,4′-H), 5.07 (2H, s, 1′-CH_2), 2.36 (2H, t, J = 7.6 Hz, 2-H), 1.64 (2H, quint, J = 7.6 Hz, 3-H), 1.26 (10H, m, 4−8-H), 0.87 (3H, t, J = 7.0 Hz, 9-H); ^{13}C NMR δ 174.1 (C-1), 155.9 (C-3′), 137.8 (C-1′), 129.8 (C-5′), 120.3 (C-6′), 115.2 (C-2′), 115.0 (C-4′), 65.9 (1′-CH_2), 34.4 (C-2), 31.8 (C-7), 29.2 (C-4), 29.1 (C-5,6), 25.0 (C-3), 22.6 (C-8), 14.1 (C-9).

*p-Hydroxybenzyl Nonanoate (**8**).* *p*-Hydroxybenzyl nonanoate (**8**, 1792.4 mg, 65% yield) was obtained from *p*-hydroxybenzyl alcohol (2 g) and nonanoyl chloride (1.956 mL) under ice cooling in a manner similar to that used for **4**. Spectral data of **8** were as follows: HRMS, *m/z* (M^+) calcd for $C_{16}H_{24}O_3$ 264.1831, found 264.1805; ^{1}H NMR δ 7.23 (2H, d, J = 8.4 Hz, 3′,5′-H), 6.82 (2H, d, J = 8.4 Hz, 2′,6′-H), 5.64 (1H, s, OH), 5.04 (2H, s, 1′-CH_2), 2.33 (2H, t, J = 7.6 Hz, 2-H), 1.62 (2H, quint, J = 7.6 Hz, 3-H), 1.26 (10H, m, 4−8-H), 0.87 (3H, t, J = 6.8 Hz, 9-H); ^{13}C NMR δ 174.2 (C-1), 155.9 (C-4′), 130.2

(C-2′,6′), 128.2 (C-1′), 115.4 (C-3′,5′), 66.0 (1′-CH_2), 34.5 (C-2), 31.8 (C-7), 29.2 (C-4), 29.1 (C-5,6), 25.0 (C-3), 22.6 (C-8), 14.1 (C-9).

Decomposition of Vanillyl Nonanoate with Methanol. Vanillyl nonanoate (30.05 mg) was dissolved in methanol (30 mL), and the solution was kept at room temperature. After the complete decomposition of the vanillyl nonanoate had been confirmed by HPLC analysis, the solvent was evaporated in vacuo. Then chloroform (10 mL) was added to the residue. The resulting white insoluble precipitate was collected by centrifugation. The supernatant was purified by activated alumina column chromatography under the following conditions: activated alumina (Wako), 4 g; column, 500 mm × 20 mm i.d.; eluent, methanol (50 mL). The eluent gave a colorless oil (**9**, 9.01 mg).

*Methyl Vanillyl Ether (**9**).* Spectral data of **9** were as follows: HRMS, *m/z* (M^+) calcd for $C_9H_{12}O_3$ 168.0786, found 168.0742; ^{1}H NMR δ 6.85 (3H, m, 2′, 5′, 6′-H), 5.60 (1H, s, OH), 4.37 (2H, s, 1′-CH_2), 3.90 (3H, s, OCH_3), 3.36 (3H, s, 3′-OCH_3); ^{13}C NMR δ 146.6 (C-3′), 145.3 (C-4′), 130.1 (C-1′), 121.2 (C-6′), 114.1 (C-5′), 110.5 (C-2′), 74.8 (1′-CH_2), 57.8 (OCH_3), 55.9 (3′-OCH_3).

Decomposition of Vanillyl Nonanoate with Ethanol. Vanillyl nonanoate (28.01 mg) was dissolved in ethanol (30 mL), and the solution was kept at room temperature. The subsequent procedures were performed in much the same way as the decomposition of vanillyl nonanoate with methanol. The eluent gave a pale yellow oil (**10**, 17.75 mg).

*Ethyl Vanillyl Ether (**10**).* Spectral data of **10** were as follows: HRMS, *m/z* (M^+) calcd for $C_{10}H_{14}O_3$ 182.0943, found 182.0911; ^{1}H NMR δ 6.85 (3H, m, 2′,5′,6′-H), 4.42 (2H, s, 1′-CH_2), 3.90 (3H, s, 3′-OCH_3), 3.52 (2H, q, J = 7.2 Hz, OCH_2), 1.23 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR δ 146.6 (C-3′), 145.2 (C-4′), 130.5 (C-1′), 122.0 (C-6′), 121.1 (C-5′), 110.6 (C-2′), 72.8 (1′-CH_2), 65.5 (OCH_2), 55.9 (3′-OCH_3), 15.2 (CH_3).

RESULTS

Stability of Vanillyl Nonanoate in Various Solvents. Figure 2A shows the change in vanillyl nonanoate with time in various solvents at 25 °C. Vanillyl nonanoate was stable in ethyl acetate, chloroform, hexane, acetone, and nonsolvent conditions over 2 weeks. In contrast, vanillyl nonanoate was labile in methanol, ethanol, dimethyl sulfoxide (DMSO), and aqueous surfactant vehicle, and the half-life periods of vanillyl nonanoate in these solvents were about 90, 70, 400, and 115 h, respectively. From these data, vanillyl nonanoate was stable in nonpolar solvents, whereas it was labile in polar solvents.

Figure 2B shows the change in vanillyl nonanoate with time in several solvents containing water at 25 °C. The half-life periods of vanillyl nonanoate in methanol and ethanol containing 1% water were about 105 and 90 h, respectively. The decomposition rates of vanillyl nonanoate in the solvents were little different from those in only methanol and ethanol, respectively. However, the decomposition rate of vanillyl nonanoate in methanol containing 50% water was faster than that in methanol alone, and the half-life period was ∼45 h. In contrast, vanillyl nonanoate was stable even in ethyl acetate containing 1% or saturated (3.1%) water.

Decomposition Products of Vanillyl Nonanoate with Methanol and Ethanol. Dissolution of vanillyl nonanoate in methanol and ethanol gave decomposition products, compounds **9** and **10**, respectively. Compound **9** was determined to be $C_9H_{12}O_3$ by HRMS measurement. The ^{1}H NMR spectrum of **9** showed three aromatic protons (δ 6.85 m) of which the chemical shift values and signal patterns were very closely similar to those of vanillyl nonanoate. There were three substituent groups in the aromatic ring, a hydroxyl group (δ 5.60 s), an oxymethylene group (δ 4.37 s), and a methoxyl group (δ 3.90 s). An aliphatic methoxyl group was observed in the spectrum (δ 3.36 s). From these data, the structure of **9** was established to be 4-hydroxy-3-methoxybenzyl methyl ether, that is, methyl vanillyl ether (Figure 1). Compound **10** was determined as $C_{10}H_{14}O_3$ by HRMS measurement. This molecular formula indicated an addition of CH_2 to that of **9**. Moreover, the ^{1}H NMR spectrum of **10** was similar to that of **9** except for an ethyl ether group (δ 3.52 q, δ 1.23 t). From these data, compound **10** was determined to be 4-hydroxy-3-methoxybenzyl ethyl ether, that is, ethyl vanillyl ether (Figure 1). Both concomitant products obtained as white insoluble material during the decomposition of vanillyl nonanoate by methanol and ethanol were determined to be nonanoic acid by ^{1}H NMR analysis (data not shown).

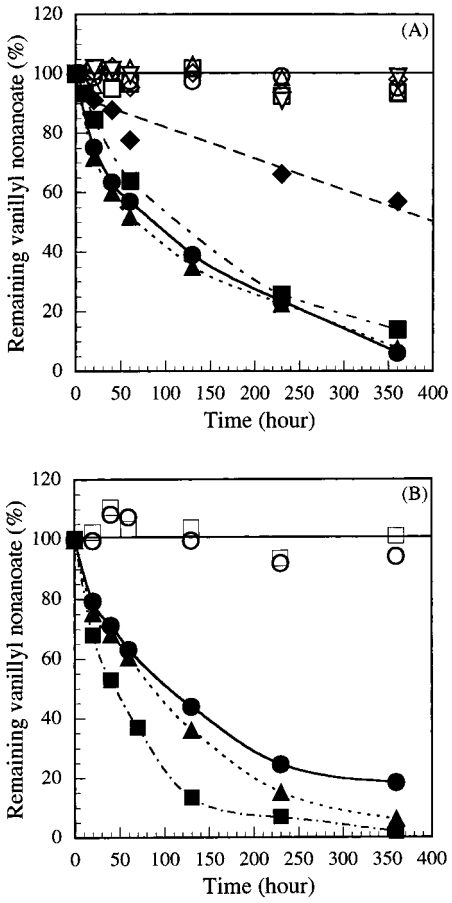

Figure 2. Change in vanillyl nonanoate with time in various solvents (A) and several solvents containing water (B) at 25 °C: (A) (□) nonsolvent, (○) ethyl acetate, (◇) chloroform, (△) hexane, (▽) acetone, (◆) dimethyl sulfoxide, (●) methanol, (▲) ethanol, and (■) aqueous surfactant vehicle (0.9% NaCl aqueous solution containing 2% ethanol and 10% Tween 80); (B) (□) ethyl acetate with 1% water, (○) water-saturated ethyl acetate, (●) methanol with 1% water, (▲) ethanol with 1% water, and (■) methanol with 50% water.

Stability of Capsiate in Organic Solvents. Figure 3 shows the change in capsiate (**1**), a major naturally occurring capsinoid, with time in ethyl acetate and methanol at 25 °C. Capsiate was stable in ethyl acetate, whereas it was labile in methanol, and the half-life period was ∼50 h. The decomposition product from capsiate was identified as methyl vanillyl ether by various spectral data. These results were similar to

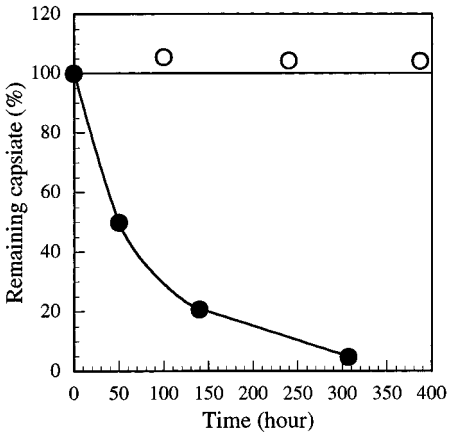

Figure 3. Change in capsiate with time in organic solvents at 25 °C: (○) ethyl acetate; (●) methanol.

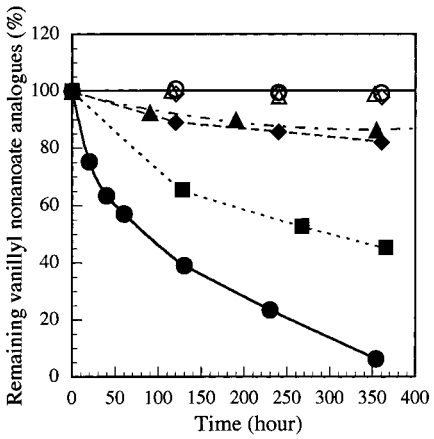

Figure 4. Change in vanillyl nonanoate analogues with time in methanol at 25 °C: (●) vanillyl nonanoate; (▲) 4-acetoxy-3-methoxybenzyl nonanoate; (△) 3,4-dimethoxybenzyl nonanoate; (◇) 3-methoxybenzyl nonanoate; (■) *p*-hydroxybenzyl nonanoate; (○) *m*-hydroxybenzyl nonanoate; (◆) *o*-hydroxybenzyl nonanoate.

those found for vanillyl nonanoate. Therefore, natural capsinoid is probably stable in nonpolar solvents and labile in polar solvents and probably tends to decompose in protic solvents such as alcohol and water.

Stability of Vanillyl Nonanoate Analogues in Organic Solvents. The stability of vanillyl nonanoate analogues in ethyl acetate and methanol was measured at 25 °C. Figure 4 shows the change in vanillyl nonanoate and its six analogues with time in methanol. Three analogues, 4-acetoxy-3-methoxybenzyl nonanoate, *o*-hydroxybenzyl nonanoate, and *p*-hydroxybenzyl nonanoate, were labile in methanol, whereas the others were stable in methanol. The decomposition rates of the three vanillyl nonanoate analogues were slower than that of vanillyl nonanoate. The half-life period of *p*-hydroxybenzyl nonanoate (310 h) was ~3-fold longer than that of vanillyl nonanoate. The half-life periods of 4-acetoxy-3-methoxybenzyl nonanoate and *o*-hydroxybenzyl nonanoate were not observed within 400 h. On the other hand, all vanillyl nonanoate analogues were stable in ethyl acetate (data not shown).

DISCUSSION

Vanillyl nonanoate was stable in ethyl acetate, chloroform, hexane, and acetone, whereas it was labile in methanol, ethanol, DMSO, and aqueous surfactant vehicle (Figure 2A). The decomposition rates of vanillyl nonanoate in methanol, ethanol, and aqueous surfactant vehicle were the same and were faster than that in DMSO. Therefore, vanillyl nonanoate probably decomposes more easily in a protic than in an aprotic solvent. High polarity of a solvent is probably responsible for the decomposition of vanillyl nonanoate because among the aprotic solvents used, the polarity, namely, the dielectric constant, of DMSO (45.0) is the highest (*9*). The decomposition rate of vanillyl nonanoate in methanol containing 50% water was faster than that in methanol alone (Figure 2B). This phenomenon can also be explained by the higher polarity of H_2O (dielectric constant = 78.5) than that of methanol (32.6). On the other hand, vanillyl nonanoate was stable even in water-saturated (3.1%) ethyl acetate. This suggested that vanillyl nonanoate was protected from an attack of water by excess ethyl acetate. Interestingly, the decomposition rate of vanillyl nonanoate in aqueous surfactant vehicle, an almost aqueous solvent, was little different from those in only methanol and ethanol. Because Tween 80, a surface active agent, has a very low critical micelle concentration (0.012 mM), it is certainly present as micelles in the aqueous surfactant vehicle. In addition, vanillyl nonanoate would be incorporated into the micelles. Therefore, the Tween 80 probably acts as a shield of vanillyl nonanoate against the attack of the aqueous solvent. In the case of a general solvolysis of simple esters, for example, alcoholysis, the resulting products are a corresponding alkyl carboxylate and an alcohol. However, the decomposition products of vanillyl nonanoate induced by methanol were methyl vanillyl ether and nonanoic acid, whereas ethyl vanillyl ether and nonanoic acid were induced by ethanol. In brief, the solvolysis of capsinoid by an alcohol gave a corresponding alkyl ether and a carboxylic acid. Therefore, the decomposition reaction of capsinoid is different from the usual solvolysis of simple esters.

The above considerations strongly support the theory that the decomposition process of capsinoid involves S_N1 solvolysis through nucleophilic displacement of the benzyl cation. The conjectural process of capsinoid decomposition is as follows: first, the polarization between the α-methylene of the benzyl moiety and the neighboring oxygen atom in capsinoid is increased by the presence of protic and/or polar solvents; then, the polarized portion is easily cleaved by means of heterolytic cleavage to give a benzyl cation and a carboxylate anion; finally, the solvent attacks the benzyl cation as a nucleophilic reagent, in the case of methanol, and methyl vanillyl ether is formed. In this process, the formation of the benzyl cation is the rate-determining step. Hence, the decomposition rate depends on the stability of the benzyl cation formed. It is natural that no difference in the decomposition rates between vanillyl nonanoate and capsiate was observed (Figure 3), because the structural difference in the two compounds is only in their acyl moieties.

In our preliminary experiment, no decomposition of benzyl nonanoate occurred in any solvents. Therefore, it is apparent that the substitution groups in the benzene ring of capsinoid would greatly contribute to the formation and stabilization of the benzyl cation. Our experiments to determine the stability of vanillyl nonanoate analogues (Figure 4) revealed the following: (i) a phenolic hydroxyl group is essential for the decomposition of capsinoid because no or only slight decom-

position was observed for nonphenolic analogues such as 3,4-dimethoxybenzyl nonanoate, 4-acetoxy-3-methoxybenzyl nonanoate, and 3-methoxybenzyl nonanoate; (ii) from the results for positional isomers, *o*-, *m*-, and *p*-hydroxybenzyl nonanoate, the effect of the phenolic hydroxyl group is maximally displayed by its para-position, followed by the ortho- and meta-positions; (iii) the methoxyl group of the meta-position in capsinoid prompts the decomposition because the decomposition rate of vanillyl nonanoate is faster than that of *p*-hydroxybenzyl nonanoate.

These considerations strongly support the hypothesis that the decomposition of capsinoid depends on the stability of the formed benzyl cation. In general, the substitution of an electron-donating group such as a hydroxyl group on the benzene ring disperses the positive charge at the α-methylene of the benzyl moiety, consequently stabilizing the benzyl cation. The hydroxyl group in a phenol is an ortho−para directing group, so that the benzyl cations of *o*- and *p*-benzyl phenols are highly stabilized.

In conclusion, capsinoid was stable in nonpolar solvents, whereas it was labile in polar solvents and especially tended to decompose in protic solvents such as alcohol and water. The decomposition proceeds by S_N1 solvolysis via the formed benzyl cation, and the *p*-hydroxyl group in the benzyl moiety of capsinoid contributes to the formation and stabilization of the benzyl cation. It is necessary for further use of capsinoid to prevent its decomposition, that is, to prevent the generation of the benzyl cation. Protection by a nonpolar or low-polar solvent and a detergent from the attack of active solvents could be a useful way to achieve stabilization of capsinoid in solvents.

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