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Nordihydrocapsiate, a New Capsinoid from the Fruits of a Nonpungent Pepper, *Capsicum annuum*

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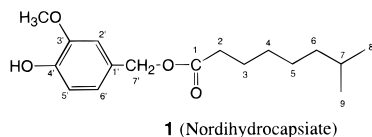
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A new capsiate-like substance, named nordihydrocapsiate (**1**), has been isolated from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (*Capsicum annuum*). The structure of **1** was determined to be 4-hydroxy-3-methoxybenzyl 7-methyloctanoate by spectroscopic methods.

The pungent principal of red peppers (*Capsicum* spp., Solanaceae) is a group of acid amides of vanillylamine and C₈ to C₁₃ fatty acids, which are known generally as capsaicinoids. The major capsaicinoids in red peppers are capsaicin,¹ (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide, and dihydrocapsaicin,² and the structure of the latter is a 6,7-dihydro analogue of capsaicin. Nordihydrocapsaicin is recognized as the third major capsaicinoid, and the structure is a mono-nor homologue of the acyl residue of dihydrocapsaicin, that is, *N*-(4-hydroxy-3-methoxybenzyl)-7-methyloctanamide.³ More than 10 capsaicinoids occur in red peppers as minor components.^{4–6}

Yazawa et al. reported that the fruits of a nonpungent cultivar of *Capsicum annuum* L., named CH-19 Sweet, contains only a small amount of capsaicinoids but has considerable capsaicinoid-like substances (CLSs).⁷ In our previous work, the structures of two major CLSs were determined to be 4-hydroxy-3-methoxybenzyl (*E*)-8-methyl-6-nonenoate and 4-hydroxy-3-methoxybenzyl 8-methyl-nonanoate, and the two novel compounds were named capsiate and dihydrocapsiate, respectively.⁸ The acyl residues of capsiate and dihydrocapsiate were the same as those of capsaicin and dihydrocapsaicin, respectively, although their aromatic portions were not vanillylamine such as capsaicinoids but vanillyl alcohol. They had no pungency upon our oral tasting. The present paper describes the isolation of a new substance, nordihydrocapsiate (**1**), from the fruits of *C. annuum* L. cv. CH-19 Sweet as the third major component, and the structure of **1** was determined to be 4-hydroxy-3-methoxybenzyl 7-methyloctanoate by spectroscopic methods.



An oleoresin from the fruits of cv. CH-19 Sweet was chromatographed successively on Si gel and reversed-phase Si gel, followed by reversed-phase HPLC purification with silver nitrate eluent to afford compound **1**. The molecular

formula of **1** was found to be C₁₇H₂₆O₄ by HRMS. The IR spectrum showed hydroxyl (3450 cm⁻¹) and ester carbonyl absorptions (1740 cm⁻¹). The ¹H NMR spectrum of **1** indicated the typical 1-, 2-, and 4-substituted phenyl group by the coupling constants and patterns of three aromatic protons (δ 6.90 d, 6.86 dd, and 6.87 d) and showed a phenolic hydroxyl group (δ 5.62 br s). A methoxyl group (δ 3.90 s and 56.0) and a methylene group (δ 5.03 s and 66.3) between the phenyl group and the oxygen were also observed in the ¹H and ¹³C NMR spectra of **1**. These results indicated the presence of a vanillyl-oxy moiety in **1**.

In the ¹H NMR data, characteristic signals for an isopropyl group (δ 0.85 d, 0.85 d, and 1.50 m) were indicated. The isopropyl group gave rise to the signals of δ 22.6, 22.6, and 27.9 in the ¹³C NMR data. Furthermore, the ¹³C NMR data showed a carbonyl carbon (δ 173.8) and five aliphatic methylene carbons (δ 25.0, 27.0, 29.4, 34.4, and 38.8). From these results, it is obvious that compound **1** has a 7-methyloctanoyl moiety as an acyl residue.

Although the IR and UV spectra of **1** were similar to those of capsiate and dihydrocapsiate, the molecular formula of **1** indicated the lack of one carbon and two protons in comparison with that of dihydrocapsiate.⁸ Furthermore, the ¹H and ¹³C NMR spectra of **1** were extremely similar to those of dihydrocapsiate except for the lack of a signal of a methylene group.⁸

Therefore, we concluded the structure of **1** to be a mono-nor homologue of the acyl residue of dihydrocapsiate, that is, 4-hydroxy-3-methoxybenzyl 7-methyloctanoate. The new compound **1** was named nordihydrocapsiate. Like capsiate and dihydrocapsiate, nordihydrocapsiate had no pungency upon our oral tasting.

Nordihydrocapsiate (**1**) was isolated as the third major component after capsiate and dihydrocapsiate from the fruits of *C. annuum* L. cv. CH-19 Sweet, and the structure of **1** was determined to be an ester of vanillyl alcohol and 7-methyloctanoic acid. The acyl residue of **1** was the same as that of nordihydrocapsaicin. Considering our previous results,⁸ the content ratio of capsiate, dihydrocapsiate, and **1** in cv. CH-19 Sweet was approximately 5:3:1. This value was similar to that of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin in several varieties of *Capsicum* species.⁹ The acyl residues of capsaicinoids are biosynthesized from L-valine, L-leucine, and L-isoleucine,^{10,11} and the vanillylamine moiety is biosynthesized from L-phenylalanine via cinnamic, caffeic, and ferulic acids.^{4,6,10} We speculate that the biosynthetic pathway of the three compounds

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is closely related to that of capsaicinoids except for a part of the pathway for the aromatic portion.

Our preliminary work indicated the existence of several capsiate-like substances as minor components in cv. CH-19 Sweet (data not shown). They may be analogues and homologues of the acyl residues of these compounds, and they may also be several analogues and homologues involving the acyl residues. Yazawa et al. reported that CLSs occur in several cultivars of *C. annuum*.⁷ We propose that the compound group of capsiate-like substances that are esters of vanillyl alcohol and fatty acids be named capsinoid(s).

Experimental Section

General Experimental Procedures. ¹H and ¹³C NMR spectra (TMS as the internal standard) were recorded on a JEOL α-400 instrument at 399.65 and 100.40 MHz, respectively. IR spectra were recorded on a Hitachi 270–50 IR spectrophotometer, and UV spectra were recorded on a JASCO Uvidec 660 spectrophotometer. HRMS and EIMS measurements were carried out on a JEOL JMS-AX500 apparatus. Si gel 60 was purchased from Merck (Darmstadt, Germany), and reversed-phase Si gel, Wakosil 25C18, was from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). The packed column for HPLC, J'sphere ODS–H80 (20 × 150 mm), was purchased from YMC Co., Ltd. (Kyoto, Japan).

Plant Material. Fruits of cv. CH-19 Sweet (*C. annuum* L.) were grown at the experimental farm of Kyoto University. The CH-19 Sweet is a nonpungent cultivar that was selected and cultivated from a pungent cultivar, CH-19, of pepper obtained from Thailand.⁷

Isolation of 1. An oleoresin (7.3 g) from fresh fruits (1.00 kg) of cv. CH-19 Sweet was obtained by our previously described method.⁸ The oleoresin was chromatographed on Si gel (36 × 200 mm) with stepwise elution of *n*-hexane and EtOAc. The fraction eluted with *n*-hexane–EtOAc (80:20) was rechromatographed on reversed-phase Si gel (20 × 90 mm) with 75% MeOH eluent, and a mixture containing **1** was obtained. The mixture was purified by a preparative HPLC system. HPLC conditions were as follows; column: J'sphere ODS–H80 (20 × 150 mm); eluent: 80% MeOH containing 0.05 M AgNO₃; flow rate: 8.0 mL/min; detection: fluorescence, Excitation 280 nm, Emission 320 nm. The fraction represented

by a characteristic peak was collected. The eluent was partitioned with CHCl₃ three times; the CHCl₃ fractions were dried using anhydrous Na₂SO₄ and then filtered. The filtrate was evaporated under reduced pressure to afford compound **1** (25.5 mg).

Compound 1 (nordihydrocapsiate): colorless oil; HREIMS *m/z* 294.1872 (calcd for C₁₇H₂₆O₄, 294.1831); EIMS (70 eV) *m/z* 294 [M⁺] (10), 252 (5), 236 (3), 154 (27), 137 (46), 107 (14), 73 (47), 44 (100), 40 (90); IR (film) ν_{\max} 3450, 1740, 1615, 1610, 1520, 1470, 1435, 1275, 1160, 1120, 1035, 970, 850, 815, 795, 560 cm⁻¹; UV(MeOH) λ_{\max} (ε) 281 (2400), 231 (6800) nm; ¹H NMR (CDCl₃, 399.65 MHz) δ 6.90 (1H, d, *J* = 8.3 Hz, H-5'), 6.87 (1H, d, *J* = 1.5 Hz, H-2'), 6.86 (1H, dd, *J* = 8.3, 1.5 Hz, H-6'), 5.62 (1H, bs, OH), 5.03 (2H, s, H-7'), 3.90 (3H, s, OCH₃), 2.33 (2H, t, *J* = 7.6 Hz, H-2), 1.63 (2H, quint, *J* = 7.6 Hz, H-3), 1.50 (1H, m, *J* = 6.8 Hz, H-7), 1.25 (2H, m, H-4), 1.25 (2H, m, H-5), 1.14 (2H, q, *J* = 6.8 Hz, H-6), 0.85 (3H, d, *J* = 6.8 Hz, H-8), 0.85 (3H, d, *J* = 6.8 Hz, H-9); ¹³C NMR (CDCl₃, 100.40 MHz) δ 173.8 (C-1), 146.5 (C-3'), 145.8 (C-4'), 128.1 (C-1'), 122.0 (C-6'), 114.4 (C-5'), 111.3 (C-2'), 66.3 (C-7'), 56.0 (OCH₃), 38.8 (C-6), 34.4 (C-2), 29.4 (C-4), 27.9 (C-7), 27.0 (C-5), 25.0 (C-3), 22.6 (C-8), 22.6 (C-9).

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