Studies on the Pungent Principle of *Capsicum*

Part XIV Chemical Constitution of the Pungent Principle[†]

By Sadayoshi KOSUGE and Masaji FURUTA

Department of Agricultural Chemistry, Faculty of Agriculture, Gifu University

Received July 7, 1969

The chemical constitutions of the pungent principle of *Capsicum* were investigated. These principles are assumed to consist of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and two or more analogues of these materials. Thin-layer chromatography and open tubular gas chromatography showed that the natural pungent mixture contains no *cis*-isomer of capsaicin. The chemical structure of nordihydrocapsaicin was determined as N-(4-hydroxy-3-methoxybenzyl)-7-methyloctanamide by gas chromatography, infrared spectroscopy, mass spectrometry and nuclear magnetic resonance spectroscopy. In addition, homodihydrocapsaicin was identified as N-(4-hydroxy-3-methoxybenzyl)-9-methyl-decanamide. These identities were also proven by comparison with synthetic samples.

Capsaicin, the pungent principle of *Capsicum*, was denoted as N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-*trans*-6-enamide by Nelson¹⁾ and Crombie *et al.*²⁾ Kosuge *et al.*³⁾ demonstrated the existence of saturated capsaicin (dihydrocapsaicin). Lately, Bennett *et al.*⁴⁾ have suggested that the pungent principle is a mixture of at least five closely related vanillyl amides, which they named Capsaicin (mol. wt., 307–2) dihydrocapsaicin (307), nordihydrocapsaicin (307–14), homodihydrocapsaicin (307+14), homocapsaicin (307+14–2). In this paper, further investigation is made on the existence of the *cis*-isomer of capsaicin and the chemical structure of the homologues.

Crystalline pungent principles, isolated as described previously⁵⁾ from the Japanese *Capsicum*, were separated by silver nitrate thin-layer chromatography into capsaicin (*R_f*, 0.50), dihydrocapsaicin (*R_f*, 0.32) and two

spots (*R_f*, 0.42, 0.21). In thin-layer chromatography, no *cis*-capsaicin was detected in the natural pungent mixture. Methyl esters of fatty acids obtained from the natural mixture were analyzed by gas chromatography. In the gas chromatogram of the diethylene glycol succinate polyester (DEGS), Fig. 1, peak-3 and peak-4 were each identified as 8-methyl-nonanoate and 8-methylnon-*trans*-6-enoate by comparison with authentic samples. With the aid of published data,⁶⁾ the relationship between logarithms of the retention volume relative to *n*-decanoate and the number of carbon atoms in the molecule from methyl esters of fatty acids led us to assume that the unknown peaks having ECL, equivalent carbon length,⁷⁾ of 8.5 and the 10.5 on DEGS column are methyl esters of iso-C₉ acid and iso-C₁₁ acid, respectively. On the Apiezon M, peak-4 and peak-3

[†] Part of this work was presented at the meeting of the Chubu Division of the Agricultural Chemical Society of Japan held on November 31, 1968 at Nagoya, and at the Annual Meeting of the Society held in Tokyo, April, 2, 1969.

1) E. K. Nelson, *J. Am. Chem. Soc.*, **45**, 2179 (1923).

2) L. Crombie, S. H. Dandegaonker and K. S. Simpson, *J. Chem. Soc.*, **1955**, 1025.

3) S. Kosuge, Y. Inagaki and H. Okumura, *Nippon Nogeikagaku Kaishi*, **35**, 923 (1961).

4) D. J. Bennett and G. W. Kirby, *J. Chem. Soc.*, **1968**, 442.

5) S. Kosuge, Y. Inagaki and T. Uehara, *Nippon Nogeikagaku Kaishi*, **32**, 578 (1957).

6) R. G. Ackman, *J. Chromatog.*, **28**, 225 (1967).

7) T. K. Miwa, *J. Am. Oil Chemists' Soc.*, **40**, 309 (1963).

FIG. 1. Gas Chromatograms of Methyl Esters of the Fatty Acids from *Capsicum*.

Conditions (A): Column 3.5 mm $\times$ 200 cm, packing diethylene glycol succinate polyester 15%; temp. 110°C, He flow rate, 30 ml/min.

Conditions (B): Column 3.5 mm $\times$ 200 cm, packing Apiezon grease M 25%, temp. 160°C, He flow rate, 35 ml/min.

FIG. 2. Gas Chromatograms of Methyl Esters of the Fatty Acids from *Capsicum*.

Conditions: Golay column Q, (Apiezon grease L coated) 75 ft $\times$ 0.01 inch *i. d.* operated at 100°C and 15 psig, He. Perkin-Elmer Model 226, No. 2 injection splitter, attenuation 1/100X. *cis*: Synthetic methyl 8-methylnon-*cis*-6-enoate; *trans*: Synthetic methyl 8-methylnon-*trans*-6-enoate.

were identified as 8-methylnonanoate and 8-methylnon-*trans*-6-enoate by the peak shift with hydrogenation and by comparison with authentic samples. Peak-1 and peak-7 on the Apiezon M corresponded to peak-1 and peak-5 on the DEGS. The component of peak-1 had the same retention time as the synthesized 7-methyloctanoate. Likewise, peak-7 was identified as 9-methyldecanoate.

To investigate the existence of *cis*-isomer in the natural pungent mixture, gas chromatography of the fatty acid methyl ester obtained from a crystalline pungent mixture, was carried out using an open tubular column, Golay Q (Fig. 2). The number of carbon atoms in each fatty acid was determined by comparison of their relative retention time in the gas chromatogram using a grid established under the same conditions as with known

acids, Fig. 3. No isomerization between *cis*- and *trans*-form occurred during the gas chromatography based on tests of authentic acid samples. The unknown peaks, 7, 9, 10 and 13, had ECL of 9.8, 10.1, 10.3 and 10.8, respectively. These results suggest that the *cis*-isomer of capsaicin was not present in the natural material. In order to confirm the chemical structure of the iso acid, each component of the peaks was separated by preparative gas chromatography using a DEGS column. Infrared spectra of the isolated components were identical with those of the synthesized components. They had a doublet peak at 1380 cm^{-1} , which suggests the presence of an isopropyl group.⁸⁾ High resolution mass spectrometric studies offered further corroborating evidence for this postulation. The methyl ester of iso acid, containing a terminal isopropyl group, gives a mass spectrum that is similar to that of the normal chain isomer. The spectrum, however, contains a small peak at m/e : $M^+-(\text{CH}_3\text{OH}+\text{H}_2\text{O}+\text{CH}_3)$, which is absent in the normal chain isomer.⁹⁾ The mass spectra of synthetic iso- C_9 acid methylester and iso- C_{11} acid methyl ester had the same ion fragmentation patterns for the components of peak-1 and peak-5 (Figs. 4 and 5). In these mass spectra, the ketene ions m/e : M-47 were derived from the methoxycarbonyl type ions m/e : M-15 through loss of methanol. There were characteristic peaks of iso acid methyl ester at m/e : M⁺-65 arising through loss of water from the ketene ions. The peak at m/e : M⁺-29 which arises through loss of the ethyl group in normal fatty acid was, therefore, absent from these spectra. The result of NMR spectroscopy finally demonstrated the identity of the methyl esters isolated with the authentic methyl esters. The characteristic peaks were the same as those of authentic samples of 7-methyloctanoate and

FIG. 3. Relationship between $\log_{10}$ of the V_R Relative to Methyl Decanoate and the Number of Carbon Atoms in the Molecules of the Esters.

●—●, straight-chain acids; ●---●, iso acids; ●---●, mono-unsaturated iso acids.

8) D. L. Guertin, S. E. Wiberley and W. H. Bauer, *Anal. Chem.*, **28**, 1194 (1956).

9) R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **13**, 523 (1959).

FIG. 4. Mass Spectra of Natural and Synthetic Methyl 7-Methyloctanoate.

FIG. 5. Mass Spectra of Natural and Synthetic Methyl 9-Methyldecanoate.

9-methyldecanoate. The relative integral intensity of each signal and the assignments¹⁰⁾ for peak-1 and peak-5 are shown in Table I. Thus, the identity of the isolated methyl esters with the authentic methyl esters was

TABLE I. CHEMICAL SHIFT DATA FOR ISOLATED METHYLESTERS OF FATTY ACIDS

Chemical shift	Relative intensity		Group
	peak-1	peak-5	
0.85 (doublet)	6	6	CH ₃
1.20 (multiplet)	9	13	CH, CH ₂
2.30 (triplet)	2	2	CH ₂ ^{a)}
3.67 (singlet)	3	3	OCH ₃

^{a)} Methylene group attached to the carbon atom carrying a carbomethoxy group.

¹⁰⁾ T. Hashimoto, H. Shiina and H. Mamuro, *Kogyokagaku Zasshi*, **68**, 1434 (1965).

confirmed by gas chromatography, and infrared, mass and nuclear magnetic resonance spectroscopy. The main conclusion is as follows.

Besides the reported 8-methylnonanoic acid and 8-methylnon-*trans*-6-enoic acid, 7-methyloctanoic and 9-methyldecanoic acid exist as the side chain fatty acids of the vanillyl amide, pungent principle of *Capsicum*. In order to clearly demonstrate this, vanillyl 7-methyloctanamide (IIa) and vanillyl 9-methyldecanamide (IIb) were prepared from vanillyl amine and the authentic fatty acid chlorides respectively.

A thin-layer chromatogram on silica-gel impregnated with silver nitrate is shown in Fig. 6. *R_f*, 0.55 and 0.27 components behaved as capsaicin and dihydrocapsaicin. *R_f*, 0.42 and 0.14 components, respectively, were de-

FIG. 7. Infrared Spectra of Natural and Synthetic Nordihydrocapsaicin.

a=Synthetic nordihydrocapsaicin, b=Pungent principle isolated from a crystalline pungent mixture of *Capsicum*. (KBr).

FIG. 8. 100-MHz NMR Spectrum of Natural Nordihydrocapsaicin in the Range of δ 0.6~7.1ppm. Solvent: CDCl_3 , Standard: Me_4Si .

to a singlet after treatment with D_2O .

Isolation of homodihydrocapsaicin from the natural pungent mixture is difficult because of its scarceness and because of the formation of the critical pair with dihydrocapsaicin. Attempts to separate homodihydrocapsaicin from the pungent mixture were unsuccessful. Accurate mass measurements of the natural crystalline pungent mixture and isolated fatty acids are presented in Table II. The mass spectrum of the authentic pungent principle showed no $\text{M}^+ \pm \text{CH}_2$ peaks. Therefore, the peaks at m/e : 184 ($170+14$), 198 ($170+14 \times 2$) and 200 ($186+14$) suggest the

presence of homologues of the principal components. It was necessary to consider the possibility of the formation of these homologues in the course of isolating the pungent principles from natural *Capsicum*. To clarify this, thin-layer chromatography was carried out on silica-gel impregnated with a silver nitrate of acetone extract of seed pods of *Capsicum*. The chromatogram gave four distinct pungent spots. It is also possible that the formation of the homologues of fatty acids occur during decomposition of the crystalline pungent mixture. But gas chromatograms of fatty acid methyl esters, which were obtained

TABLE II. ACCURATE MASS MEASUREMENTS

Compound	Found	Calculated	Molecular formula
Pungent mixture of <i>Capsicum</i>	293.198	293.199	C ₁₇ H ₂₇ NO ₃
	305.204	305.199	C ₁₈ H ₂₇ NO ₃
	307.220	307.215	C ₁₈ H ₂₉ NO ₃
	319.212	319.215	C ₁₉ H ₂₉ NO ₃
	321.223	321.230	C ₁₉ H ₃₁ NO ₃
Fatty acids from pungent mixture	158.128	158.131	C ₉ H ₁₈ O ₂
	170.130	170.131	C ₁₀ H ₁₈ O ₂
	172.144	172.146	C ₁₀ H ₂₀ O ₂
	184.144	184.146	C ₁₁ H ₂₀ O ₂
	186.158	186.162	C ₁₁ H ₂₂ O ₂
	198.160	198.162	C ₁₂ H ₂₂ O ₂
	200.176	200.178	C ₁₂ H ₂₄ O ₂

from the authentic pungent principle by decomposition, showed no homologues peaks. This indicates that the formation of homologues does not occur in the course of decomposition. These results prove the existence of homologues in the natural pungent mixture. Infrared spectroscopy, mass spectroscopy and thin-layer chromatography of vanillyl residue isolated from natural crystalline pungent mixture confirmed that there are no homologues with varying vanillyl residue. The relative amounts of pungent principle were calculated from the densitometry of thin-layer chromatograms with silver nitrate-silica-gel, the intensities of the molecular ion peaks in the mass spectrum of the crystalline pungent mixture, and the intensities of peaks in the gas chromatograms of fatty acids isolated from the pungent mixture. The resulting approximate composition is capsaicin (48.6%), dihydrocapsaicin (36.0%), nordihydrocapsaicin (7.4%), homodihydrocapsaicin (2.0%), homocapsaicin (2.0%), nonoyl vanillylamide (1.0%) and decoyl vanillylamide (1.5%).

EXPERIMENTAL

All melting points were determined on a Yanagimoto micromelting point apparatus and were cor-

rected. Infrared spectral data were obtained with a JASCO (Japan Spectroscopic Co., Ltd.) DS-301 spectrophotometer. Ultraviolet spectra were obtained with a Shimadzu AUV-50 spectrophotometer. NMR were recorded with a Varian HA-100 spectrometer at 100-Mc and a Varian A-60D spectrometer at 60Mc in deuteriochloroform using tetramethylsilane as an internal reference. MS spectra were measured with a CEC-110B double focusing spectrometer using an ionization potential of 70 eV. In mass spectrometry, methyl esters of the fatty acids were introduced through a heated inlet system at 140 to 150°C. The pungent principles were introduced by direct insertion on a probe at 90°C; ion source temperature 115°C. Scanning graphs of thin-layer chromatograms were obtained with an OZUMOR-82 densitometer. Gas chromatography was carried out with a Yanagimoto GCG-5DH equipped with a thermal conductivity detector and an automatic preparative attachment. Open tubular gas chromatography was carried out with a Perkin Elmer model 226 equipped with a flame ionization detector.

Isolation of fatty acids. Seven grams of the pungent mixture (mp 64.5°C) was decomposed as described previously.³⁾ Carbon dioxide gas was bubbled through the resulting aqueous solution. The phenolic compounds were removed by ether extraction. The water-layer was acidified with dilute sulfuric acid and extracted with ether. Then the organic layer was washed with water and dried. The residue remaining after removal of ether was distilled. Bp 137~142°C/18 mm, Yield from pungent mixture, 3.03 g (77%). n_D^{20} 1.4380. $IR_{\nu_{\max}}^{NaCl}$ cm⁻¹: 3000~2500, 1707, 1462, 1410, 1385, 1365, 1290, 1235, 1115, 968, 937. The MS data in Table II and the methylated fatty acids were analyzed by gas chromatography, as shown in Figs. 1 and 2.

Gas chromatography. Methyl esters of the fatty acids were prepared by the method reported by Schlenk *et al.*¹¹⁾ The columns were 3.5 mm × 200 cm U-shape stainless steels packed with 15w/w%-DEGS coated on 80~100-mesh Celite 545 or with 25w/w%-Apiezon grease M coated on 40~60 mesh Fire-brick C-22. The open tubular column (Golay Q), Apiezon grease L coated, 75 ft in length and 0.01 inch *i. d.*, was purchased from Perkin Elmer. In preparative gas

11) H. Schlenk and J. L. Gellerman, *Anal. Chem.*, **32**, 1412 (1960).

chromatography, stainless steel columns, 400 cm in length and 1.4 cm *i. d.*, were packed with 15w/w%-DEGS.

Syntheses of fatty acids and pungent principles.

A) *7-Methyl octanoic acid (Ia)*. Grignard solution prepared in the usual way¹²⁾ from 50 g of isoamyl bromide and 8.1 g of magnesium was cooled in ice and 33 g of dried cadmium chloride was added. A solution of 40 g of β -carbomethoxypropionyl chloride in ether was added to the ether solution of organo-cadmium compound in about 5 min. The solution was stirred for 45 min at room temperature and for an additional hour with refluxing. After decomposition with ice and sulfuric acid, the ether layer was washed with 5% sodium carbonate solution and with water, then dried and distilled. The ketoester was collected at bp 100~105°C/10 mm. Yield, 15.5 g (32%).

Fifteen grams of the ketoester was reduced using the Clemmenson procedure¹³⁾ with 50 g of amalgamated zinc, 40 ml of water, and 100 ml of concentrated hydrochloric acid. Refluxing was continued for 46 hr, during which time 170 ml of concentrated hydrochloric acid and a 25 g charge of amalgamated zinc were added. The colorless product was extracted with hexane, and after this extract had been washed and dried, the acid was distilled. Bp 121~123°C/15 mm. Yield from the ketoester, 8.7 g (68%). n_D^{20} 1.4332. d_4^{20} 0.9245. *Anal.* Found: C, 68.51; H, 11.91. Calcd. for $C_9H_{18}O_2$: C, 68.40; H, 11.47. IR_{ν}^{NaCl} cm^{-1} : 3000~2500, 1702, 1465, 1412, 1385, 1365, 1280, 1232, 1165, 1110, 930, 720. Methyl ester's $NMR_{\delta}^{CDCl_3}$: 0.85 (6H, doublet, *gem*-CH₃), 1.20~1.85 (9H, multiplet, -(CH₂)₄CHMe₂), 2.30 (2H, triplet, -CH₂CO-), 3.67 (3H, singlet, -OCH₃). Amide, mp 106°C (lit.,¹⁴⁾ 106.5°C). MS spectrum of the methylester is shown in Fig. 4.

B) *N-(4-Hydroxy-3-methoxybenzyl)-7-methyloctanamide (IIa)*. Three milliliters of thionyl chloride was added to a solution of 0.7 g of 7-methyloctanoic acid in 5 ml absolute ether, and the solution was set aside for 18 hr at room temperature. It was then heated at 100°C for 1 hr and excess reagent was removed under reduced pressure. The residual acid chloride was

added to 1.4 g of freshly prepared vanillyl amine suspended in 50 ml of ether. This mixture was shaken for 3 days, then poured into water and extracted. Extracts were dried and evaporated producing 0.67 g of crude amide, which crystallized from light petroleum (bp 40~60°C). Mp 65.6°C, Yield, 0.41 g (16%). *Anal.* Found: C, 69.15; H, 9.45; N, 4.74. Calcd. for $C_{17}H_{27}NO_3$: C, 69.59; H, 9.28; N, 4.77. UV_{λ}^{EtOH} $m\mu$: 281. MS *m/e*: 293 (M⁺), 195 ($C_{10}H_{13}NO_3^+$), 137 ($C_8H_9O_2^+$, base peak). $NMR_{\delta}^{CDCl_3}$: 0.90 (6H, doublet, *gem*-CH₃), 1.20~1.85 (9H, multiplet, -(CH₂)₄CHMe₂), 2.30 (2H, triplet, -CH₂CO-), 3.80 (3H, singlet, -OCH₃), 4.20 (2H, doublet, benzyl), 6.0 (1H, singlet, -OH), 6.34 (1H, multiplet, -NH-), 6.70 (3H, multiplet, aryl). The IR spectrum is shown in Fig. 7.

C) *9-Methyldecanoic acid (Ib)*. 9-Methyldecanoic acid was prepared, by the same method as for Ia, from 38 g of methyl 6-keto-9-methyldecanoate which was obtained from di-isoamyl cadmium and δ -carbomethoxyvaleryl chloride in benzene. Bp 127~129°C/2.5 mm. Yield, 16.6 g (50.3%). *Anal.* Found: C, 71.24; H, 12.18. Calcd. for $C_{11}H_{22}O_2$: C, 70.92; H, 11.90. n_D^{20} 1.4403; d_4^{20} 0.8903. IR_{ν}^{NaCl} cm^{-1} : 3000~2500, 1705, 1463, 1408, 1383, 1365, 1285, 1165, 1110, 935, 720. Methyl ester's $NMR_{\delta}^{CDCl_3}$: 0.85 (6H, doublet, *gem*-CH₃), 1.20~1.85 (13H, multiplet, -(CH₂)₆CHMe₂), 2.30 (2H, triplet, -CH₂CO-), 3.67 (3H, singlet, -OCH₃). *p*-Bromophenacyl ester, mp 65.0°C (lit.,¹⁵⁾ 65.5°C). The MS spectrum of the methylester is shown in Fig. 5.

D) *N-(4-Hydroxy-3-methoxybenzyl)-9-methyldecanamide (IIb)*. The amide (IIb) was obtained by the method described for IIa from 1.2 g of 9-methyldecanoic acid chloride and 1.3 g of vanillyl amine. Mp 66.5°C. Yield, 0.47 g (21%). *Anal.* Found: C, 70.84; H, 9.80; N, 4.40. Calcd. for $C_{19}H_{31}NO_3$: C, 70.99; H, 9.72; N, 4.36. UV_{λ}^{EtOH} $m\mu$: 280.5. IR_{ν}^{KBr} cm^{-1} : 3440, 2930, 1628, 1512, 1415, 1348, 1284, 1205, 1120, 1030, 804. MS *m/e*: 321 (M⁺), 195 ($C_{10}H_{13}NO_3^+$), 137 ($C_8H_9O_2^+$). $NMR_{\delta}^{CDCl_3}$: 0.85 (6H, doublet, *gem*-CH₃), 1.20~1.85 (13H, -(CH₂)₆CHMe₂), 2.30 (2H, triplet, -CH₂CO-), 3.80 (3H, singlet, -OCH₃), 4.20 (2H, doublet, benzyl), 5.75 (1H, singlet, -OH), 6.20 (1H, multiplet, -NH-), 6.70 (3H, multiplet, aryl).

Isolation of IIa from the pungent mixture of Capsicum.

12) A. H. Blatt, "Organic Syntheses," Coll. Vol. I, 2nd ed., John Wiley and Sons, Inc., 1956, p. 75.

13) E. L. Martin, *J. Am. Chem. Soc.*, **58** 138 (1936).

14) P. A. Levene and C. H. Allen, *J. Biol. Chem.*, **27**, 433 (1916).

15) C. V. Wilson, *J. Am. Chem. Soc.*, **67**, 2161 (1945).

Catalytic hydrogenation was done on the pungent mixture isolated from *Capsicum* using Raney Ni W-6¹⁶⁾ as the catalyst. The hydrogenated pungent mixture was developed with 0.1 N sodium hydroxide as solvent for the paper chromatography. Spots of the pungent principles were observed on the paper chromatogram without using a color-producing reagent. The component of the spot *R_f*, 0.42 was extracted with 0.1 N sodium hydroxide. The collected alkaline aqueous solution was treated with sodium hydrocarbonate, then shaken with ether. The organic layer which separated, was washed with water and

dried. After removal of the solvent, the residue was recrystallized with petroleum ether (bp 40~60°C). Forty-four mg of the crystalline pungent principle was obtained from 2 g of the pungent mixture. Mp 64.5°C. MS *m/e*: 293.194 (M^+ , calcd. for $C_{17}H_{27}NO_3$, 293.199), 195 ($C_{10}H_{13}NO_3^+$, base peak). Synthetic IIa gave the same ion fragment pattern. IR and NMR data are in the text.

Acknowledgements. The authors wish to express their thanks to Dr. A. Hasegawa and Mr. S. Sawada, of Kyoto University, for NMR spectra measurement and elemental analyses. We are also indebted to Dr. N. Wasada, Government Chemical Industrial Research Institute, Tokyo, for the high resolution mass spectra measurements.

16) A. H. Blatt, "Organic Syntheses," Coll. Vol. III, 2nd ed., John Wiley and Sons, Inc., 1956, p. 181.