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Simultaneous Determination of Capsaicin, Dihydrocapsaicin and Nordihydrocapsaicin in Capsicum Fruit by Gas Chromatography[1]

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A new simple gas chromatographic method for the simultaneous determination of capsaicin, dihydrocapsaicin and nordihydrocapsaicin in capsicum fruit was developed. The three components extracted from capsicum fruit could be completely separated on a 3% Apiezon grease L column. Quantitative analysis could be achieved by the peak area ratio method using methyl arachidate as an internal standard. The results obtained by this method were in good accord with those obtained by mass chromatography.

Keywords——capsicum fruit; capsaicin; dihydrocapsaicin; nordihydrocapsaicin; gas chromatography; mass chromatography

Capsicum fruit has been used as a counter-irritant for the treatment of rheumatism, lumbago or neuralgia. The pungent components in capsicum fruit, such as capsaicin (CP), dihydrocapsaicin (DCP), nordihydrocapsaicin (NDCP), homocapsaicin and homodihydrocapsaicin are usually called capsaicinoids.

Although various reports[3] on the determination of capsaicinoids in plants have appeared, most of them have been restricted to the analysis of total capsaicinoids. In order to determine the amounts of individual capsaicinoids, analytical methods using mass chromatography[4] (MC), mass fragmentography[5] (MF), high-performance liquid chromatography[6] (HPLC) and gas chromatography[7] (GC) have been reported in recent years. However, the MC and MF methods may not be suitable for routine assay of the crude drug since they require the use of an expensive instrument. In the case of the HPLC method, not only does the separation between CP and NDCP seem to be insufficient, but also the reproducibility has not been established. Similarly, the trimethylsilyl derivatives of capsaicinoids have not been separated satisfactorily by GC. Accordingly, these methods also appear to be unsuitable for routine use at present. Therefore, we hoped to find a method to separate CP, DCP and NDCP simultaneously, and we established a rapid and simple GC method using 3% Apiezon grease L coated on Gas Chrom Q. The results obtained corresponded well to those obtained by MC. In this experiment, CP, DCP and NDCP isolated by preparative HPLC were used as reference standards.

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1) This work was reported at the 26th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, November, 1979, "Abstracts of Papers," p. 40.

2) Location: *1-403, Yoshino-cho, Omiya-shi, Saitama, 330, Japan.*

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Experimental

Materials and Reagents——Capsaicin (commercial "Capsaicin") purchased from Sigma Chemical Co. was used for the isolation of CP, DCP and NDCP. Air-dried red fruit of *Capsicum annuum* native to China (Unnan: 雲南) were purchased from Nippon Funmatsu Yakuhin Co. (Osaka). Methyl arachidate was purchased from Sigma Chemical Co.

Gas Chromatography——A Shimadzu GC-4BM gas chromatograph equipped with a flame ionization detector was used. A glass column (1 m × 3 mm I.D.) was packed with 3% Apiezon grease L coated on Gas Chrom Q (100—120 mesh). The temperatures of the column, the injection port and the detector were 235°, 280° and 280°, respectively. The nitrogen flow rate was 36 ml/min.

Mass Chromatography——A Shimadzu LKB 9000 combined gas chromatograph-mass spectrometer equipped with a MS-PAC 300DGB computer data system was used. A coiled glass column (1 m × 3 mm I.D.) was packed with 3% Apiezon grease L coated on Gas Chrom Q (100—120 mesh). The temperatures of the column, the separator and ion source were kept at 235°, 280° and 290°, respectively. The helium flow rate was 36 ml/min. The ionization energy, acceleration voltage, trap current and slits were set at 20 eV, 3.552 kV, 60 μA and 0.1 mm, respectively. Mass spectra were recorded automatically at intervals of 5 sec with a scanning speed of 8 from *m/e* 10 to 450. The off-set and stop time were set at 6 and 17 min, respectively.

Assay Procedure——About 0.4 g of dry powder of capsicum fruit was weighed accurately, mixed with 30 ml of acetone, and refluxed on a water bath at 60° for 60 min. The acetone extract was concentrated *in vacuo* and 20 ml of hexane was added to the residue. The hexane solution was extracted twice with 15 ml of 30% water–methanol solution. The water–methanol solution was concentrated *in vacuo* on a water bath, then 1 ml of the internal standard solution (0.2 mg of methyl arachidate per ml of chloroform) was added to the residue, and the solution (2 μl) was injected into the gas chromatograph. The determination of individual capsaicinoids was achieved by the peak area ratio method.

Results and Discussion

Isolation of CP, DCP and NDCP from Commercial "Capsaicin" by Preparative HPLC

Lee *et al.*[5] reported that the contents of CP, DCP and NDCP in capsaicin purchased from Sigma Chemical Co. were 71%, 26% and 3%, respectively, by MF. On the other hand, Sticher *et al.*[6] reported the determination of CP, DCP, NDCP and homodihydrocapsaicin in capsicum friut by HPLC employing μ-Bondapak C_{18} (30 cm × 4 mm I.D.) with a 47% water–methanol system. Based on these reports, we tried to isolate CP, DCP and NDCP from commercial "Capsaicin" by preparative HPLC. The three compounds were recrystallized from ether–petroleum ether (1: 4) for use as reference standards in GC and MC.

Determination of Individual Capsaicinoids in Capsicum Fruit by GC

Aliquots of the capsaicinoids extracted from capsicum fruit were injected into Gas Chrom Q columns packed with various liquid phases such as Apiezon grease L, SE-30, OV-1, OV-101, OV-17 and Carbowax 20M. Apiezon grease L was found to be the best stationary phase as regards separability of individual capsaicinoids. A chromatogram of capsaicinoids in capsicum fruit obtained by this method is shown in Fig. 1. The retention times of methyl arachidate (internal standard), NDCP, CP and DCP were 10, 12.6, 16 and 18 min, respectively. The small peak at 24 min was assumed to be due to homodihydrocapsaicin, judging from the molecular ion peak (*m/e*: 321) obtained by GC–MS measurement. Calibration plots for each capsaicinoid were obtained from 0.4 to 2.0 μg for CP, 0.2 to 1.0 μg for DCP and 0.1 to 0.8 μg for NDCP. The regression equations were as follows: $y=1.5973\,x-0.1205$ (r: 0.999) for CP, $y=1.4583\,x-0.0908$ (r: 0.999) for DCP and $y=0.9137\,x-0.0159$ (r: 0.999) for NDCP, where y is the ratio of the peak area of each capsacinoid to that of methyl arachidate (0.4 μg) and x is the concentration of each capsacinoid. The detection limits were 25 ng for NDCP, 25 ng for CP and 50 ng for DCP at a signal-to-noise ratio of 2: 1. To determine the recovery of the extraction procedure, known amounts of CP, DCP and NDCP were added to capsicum fruit, in which the content of each capsaicinoid had already been determined by the GC method. After extraction as described above, the sample was assayed by the GC method as well. The

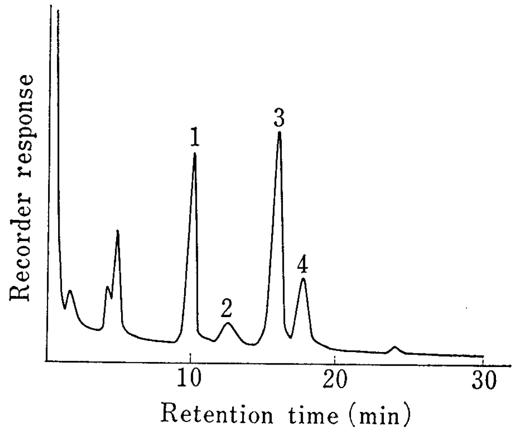

Fig. 1. Gas Chromatogram of Capsaicinoids in Capsicum Fruit

1. methyl arachidate (internal standard),
2. nordihydrocapsaicin,
3. capsaicin,
4. dihydrocapsaicin.

TABLE I. Recovery of Capsaicinoids after Addition of Standard Amounts

Capsaicinoid	Added mg	Recovery ($n=5$)		
		mg	%	CV (%)
Capsaicin	0.498	0.502	100.8	2.86
	1.195	1.211	101.3	3.23
	1.494	1.485	99.4	1.80
Dihydrocapsaicin	0.200	0.203	101.5	2.91
	0.400	0.402	100.5	1.74
	0.600	0.603	100.5	1.04
Nordihydrocapsaicin	0.105	0.104	99.0	3.52
	0.150	0.149	99.3	2.98
	0.210	0.211	100.5	2.74

TABLE II. Determination of Capsaicinoids in Capsicum Fruit by GC and MC

Capsaicinoid	GC		MC	
	mg/g[a]	%[b]	mg/g[a]	%[b]
Capsaicin	1.267	68.6	1.334	69.7
Dihydrocapsaicin	0.426	23.0	0.425	22.2
Nordihydrocapsaicin	0.155	8.4	0.155	8.1
Total	1.848	100	1.914	100

a) mg/g of dry weight.
b) % of each capsaicinoid with respect to total amount.

recovery tests were repeated five times. The results are shown in Table I. Based on these experiments, this new procedure was applied to capsicum fruit. The results are shown in Table II.

Comparison with MC

To investigate the reliablity of the data (Table II) obtained by the GC method, the determination of individual capsaicinoids was performed by the MC method. The sample solution used was the same as that used in GC. Recently, Iwai *et al.*[4] reported the determination of five different capasicinoids in capsicum fruit by MC; in their report, the trimethylsilyl derivatives of capsaicinoids were prepared and analyzed. In our experiments by the MC method, the separation of individual capsaicinoids was carried out using the same column conditions as in the present GC method, and the mass chromatograph used was the same as that of Iwai *et al.*[4] Calibration plots for the molecular ion peak of each capsaicinoid were obtained from 0.4 to 1.6 μg for CP, 0.24 to 0.72 μg for DCP and 0.063 to 0.252 μg for NDCP. The equations were as follows: $y=-0.83409\ x^2+3.9301\ x+0.27141$ for CP, $y=-0.15206\ x^2+1.7661\ x+0.077439$ for DCP and $y=-1.8351\ x^2+2.8251\ x+0.020628$ for NDCP, where y is the ratio of the weight of each capsaicinoid to that of methyl arachidate and x is the ratio of the peak area of the molecular ion of each capsaicinoid to that of the base ion of methyl arachidate (m/e: 74). The analytical results for each capsaicinoid in capsicum fruit obtained by MC are shown in Table II.

The data obtained by GC corresponded well to those obtained by MC. Therefore, the present GC method appears to be suitable for the simultaneous determination of major pungent components in capsicum fruit; this method is simpler and more accurate than previous methods.

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Studies on Digitalis Glycosides. XXXVI.[1] Diels-Alder Type Reaction of 16,17-Dehydrodigitoxigenin 3-Acetate. (1). Dimerization (2)[2]

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Solvent effects on the dimerization of 16,17-dehydrodigitoxigenin 3-acetate (I) were investigated. Heating of I in less polar solvents, such as toluene, xylene, dioxane or tetrahydrofuran, gave the dimer III as the main product, in similar yields. The reaction in slightly basic media, such as toluene containing sodium acetate or dimethylformamide, afforded an isomeric dimer IV preferentially. The structure of IV was established on the basis of its spectral data and anhydration reaction in contrast to those of III. A mechanism is proposed for the formation of IV from I.

Keywords——16,17-dehydrodigitoxigenin 3-acetate; solvent effect in dimerization; Diels-Alder type reaction; enol-lactone; ^{13}C-NMR

In the previous paper,[1] we reported that a dimerization of 16,17-dehydrodigitoxigenin 3-acetate (I) took place on heating I in toluene to give a dimer III as the main product by a Diels–Alder type reaction. Later, we investigated the solvent effects in this reaction and observed the formation of an isomeric dimer IV as the main product in the reaction in slightly basic media. This paper deals with these results.

16,17-Dehydrodigitoxigenin 3-acetate (I) was heated in less polar solvents, such as toluene, xylene, dioxane, and tetrahydrofuran (THF), in a closed test tube under nitrogen, and the reaction products were separated by multiple development TLC (SiO_2, benzene: Et_2O: $CHCl_3$ = 2: 2: 1). From the less polar fraction (main), the dimer III[1], mp 179—182°, $C_{50}H_{68}O_{10}\cdot 2H_2O$, was isolated, and unchanged material was recovered from the more polar fraction (minor). The yields of III were similar in all the solvents.

When the reaction was carried out in slightly basic media, such as toluene containing a small amount of anhydrous sodium acetate (toluene–AcONa) or dimethylformamide (DMF), compound IV, mp 296—298°, $C_{50}H_{68}O_{10}\cdot 2H_2O$, was formed as the main product.

The reaction conditions and the yields of products are summarized in Table I. The molecular weight and integration values of hydrogen in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed that this product is a dimer of I, *i.e.*, an isomer of III. Spectral

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[2] A part of this work was reported at the meeting of the Chugoku-Shikoku Branch of the Pharmaceutical Society of Japan, October, 28, 1979.

[3] Location: *Yamashiro-cho, Tokushima, 770, Japan.*