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The Identification of Capsaicinoids in Tear-Gas Spray

REFERENCES: Fung, T., Jeffery, W., and Beveridge, A. D., **"The Identification of Capsaicinoids in Tear-Gas Spray,"** *Journal of Forensic Sciences,* JFSCA, Vol. 27, No. 4, Oct. 1982, pp. 812-821.

ABSTRACT: "Natural" capsaicin has been identified in "Halt!" sprays by thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), infrared spectrophotometry (IR), and gas chromatography/mass spectrometry (GC/MS). Individual capsaicinoids have been identified as capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. The recommended analytical procedure for small samples is HPLC followed by GC/MS. The alternative procedure of solvent extraction and preparation TLC followed by IR is recommended for large samples.

KEYWORDS: toxicology, tear gases, chemical analysis

Tear gas sprays marketed for personal protection or as animal repellents typically contain either chloroacetophenone (CN), *o*-chlorobenzalmalononitrile (CS), or capsaicin (oleoresin *Capsicum*) as the active lacrimator ingredient. Unauthorized possession of such devices is prohibited in Canada.[2]

Analytical methods are well documented for the separation and identification of CN and CS in tear gas sprays [*1-3*]. Separation and identification of capsaicin in tear gas sprays, however, has been less successful. Similarities in retention times of tear gas spray extracts and capsaicin standards have been reported for a thin-layer chromatographic (TLC) system [*2*] and for two different gas chromatography (GC) columns [*3*], but neither has specifically identified the components.

Tear gas sprays containing capsaicin generally contain $<1\%$ oleoresin *Capsicum*. This is the crude extract of *Capsicum* fruits (chilies) and is a complex mixture of oils, waxes, colored materials, and several vanillyl amides called capsaicinoids. Five capsaicinoids have been identified in naturally occurring *Capsicum* [*4-6*]. Two of these, capsaicin and dihydrocapsaicin, constitute 80 to 95% of the total capsaicinoids in *Capsicum* extracts. The other capsaicinoids are nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. The systematic names are listed in Table 1 and the structural formulas are given in Fig. 1. Methods to analyze capsaicinoids have been developed in the field of food science. These include high performance liquid chromatography (HPLC), TLC, and gas chromatography/mass spectrometry (GC/MS) [*4,5*].

Received for publication 11 Jan. 1982; accepted for publication 23 Feb. 1982.

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[2]Prohibited Weapons Order, Reg. No. SOR-74, 297 (1974) 108, Canada Gazette (part 2) 1625, May 22, 1974.

TABLE 1—*Systematic names of the five capsaicinoids studied.*

Familiar Name	Systematic Name
Capsaicin	*trans*-8-methyl-*N*-vanillyl-6-nonenamide
Dihydrocapsaicin	8-methyl-*N*-vanillyl-nonamide
Nordihydrocapsaicin	7-methyl-*N*-vanillyl-octamide
Homodihydrocapsaicin	9-methyl-*N*-vanillyl-decamide
Homocapsaicin	*trans*-9-methyl-*N*-vanillyl-7-decenamide

This study was undertaken to determine whether capsaicin and dihydrocapsaicin could be identified in tear gas sprays containing oleoresin *Capsicum*. Methods used were those routinely available in this laboratory including TLC, HPLC, GC/MS, and infrared spectroscopy (IR).

Experimental Procedure

Reagents and Materials

Two capsaicin standards, one labeled synthetic (Lot 33101) and one natural (Lot 35337-A), were purchased from ICN Pharmaceuticals Inc., Plainview, NY. A second source of capsaicinoids was prepared by extracting 5 g of red chili, purchased from a local spice shop, with 30 mL of chloroform for 4 h in a Soxhlet apparatus. The chloroform was removed in a rotary evaporator leaving a reddish, oily residue. The capsaicinoids in the residue were redissolved in 20 mL of acetonitrile. The acetonitrile solution was concentrated to 5 mL and then filtered to removed the precipitated waxes.

N,O-Bis(trimethylsilyl)-acetamide (TMS-BA) was purchased from Pierce Chemical Co., Rockford, IL.

"Halt!" dog repellent sprays, manufactured by Ari Inc., Griffin, GA, were obtained from Canada Post.

Procedure

Thin-Layer Chromatography

Precoated silica gel plates (Type 60, 0.25-mm layer thickness, E. Merck, Darmstadt, Germany) loaded with capsaicinoid samples from "synthetic" capsaicin, "natural capsaicin, chili extract, and "Halt!" were developed separately in two solvent systems: (1) chloroform/ethanol (98:2) [*7*] and (2) benzene/ethanol/ethyl acetate (5:1:1) [*2*]. The developed plates were visualized by spraying with a 1:1 mixture of freshly prepared solutions of 2% ferric chloride and 1% potassium ferricyanide followed by overspraying with 2*N* hydrochloric acid to enhance the blue color of the spots.

This procedure was repeated with TLC plates that had been impregnated with 5% silver chloride solution.

High Performance Liquid Chromatography

A Waters Associates (Milford, MA) liquid chromatographic unit was used, including a Model 6000A solvent delivery system, a Model 450 variable wavelength detector, and a data module. A reverse-phase (RP-8) stainless steel column (4.6 mm inside diameter by 250 mm) was purchased from Brownlee Lab (Santa Clara, CA). Elution was carried out at room tem-

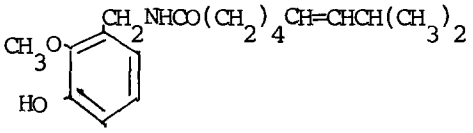
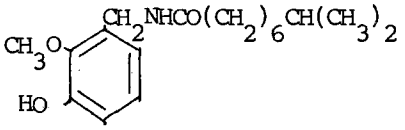
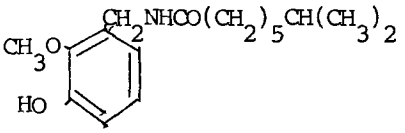
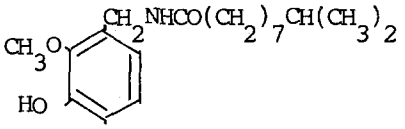
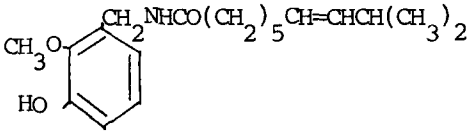
Familiar Name	Structural Formulas
capsaicin	
dihydrocapsaicin	
nordihydrocapsaicin	
homodihydrocapsaicin	
homocapsaicin	

FIG. 1—*Structural formulas of the five capsaicinoids studied.*

perature with a mobile phase composed of acetonitrile and 1% acetic acid in water (55:45) pumped at a rate of 2 mL/min.

Standard capsaicin solutions (5 μL) containing approximately 5 μg of capsaicinoids dissolved in acetonitrile were applied to the column. The signals were monitored at 281 nm.

Capsaicinoids were separated from the oily content of "Halt!" on a preparative scale by HPLC. A 10-mL aliquot of "Halt!" content was applied to the column. Eluants corresponding to the two capsaicinoid signals (Fig. 2) were separately collected. This procedure was repeated five times. The accumulated eluant fractions were extracted twice with 10-mL aliquots of chloroform in a separatory funnel. The chloroform phase was dried with anhydrous sodium sulfate and then evaporated to dryness under nitrogen.

Infrared Spectrophotometry

Capsaicinoids were isolated and purified from "Halt!" content for IR analysis in two steps. The first step followed a slightly modified solvent extraction method proposed by Spanyar and Blazovich [7]. Approximately 1 mL of the discharge from "Halt!" was dissolved in 30 mL of hexane. This hexane solution, together with 0.5 g of sodium chloride, was placed in a 100-mL separatory funnel and extracted with two successive 20-mL aliquots of 57% v/v

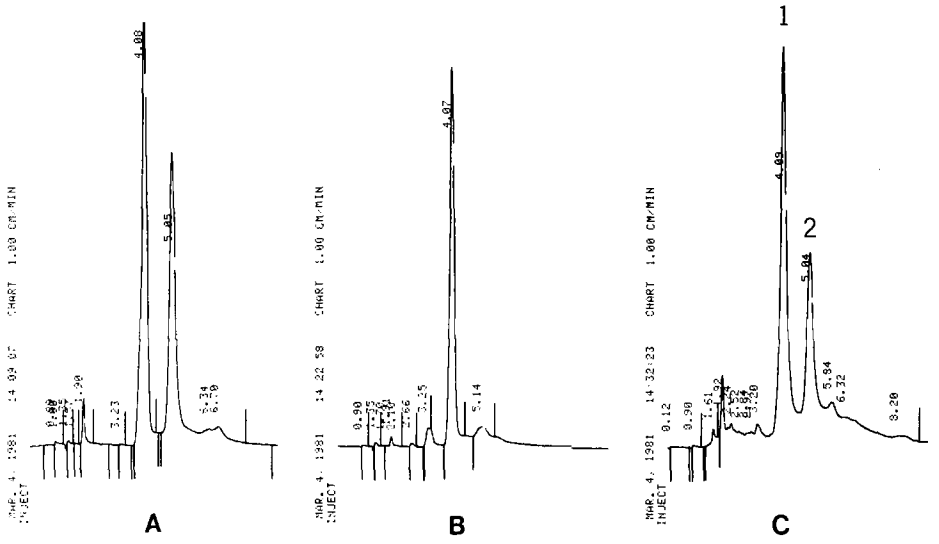


FIG. 2—*High performance liquid chromatograms of capsaicinoids in "natural" capsaicin* (A), *"synthetic" capsaicin* (B), *and "Halt!"* (C).

ethanol. The lower ethanolic layers were allowed to separate and then collected. The ethanol from the combined ethanolic solution was removed in a rotary evaporator. The remaining aqueous solution was transferred to a 60-mL separatory funnel and extracted with two 20-mL aliquots of diethyl ether. The ether layers were dried with anhydrous sodium sulfate and then evaporated to dryness under a stream of nitrogen.

The second step involved preparative TLC to further purify the capsaicinoids from traces of interfering coloring matters. Residue from the first step was redissolved in 0.5 mL of chloroform. This chloroform solution was applied as a streak onto a 20- by 20-cm silica gel plate and subsequently developed in a chloroform/ethanol (98:2) solvent system. The zone corresponding to the capsaicinoids was located by spraying the periphery of the plate with the visualizing reagent. This zone was scraped off (in the fume hood!) and extracted with anhydrous diethyl ether. After being dried with anhydrous sodium sulfate, the ether solution was evaporated to dryness to yield the purified capsaicinoids in semisolid form for IR analysis.

Infrared spectra were obtained with a Beckman 4260 spectrophotometer equipped with a 4× beam condenser. Capsaicinoids from standards and purified from "Halt!" were prepared as 1.5 mm KBr micropellets for analysis.

Mass Spectrometry

Purified capsaicinoids from "Halt!" (by solvent extraction followed by preparative TLC or preparative HPLC) and capsaicin standards were derivatized with TMS-BA at room temperature for several hours in a desiccator. The reaction mixture was evaporated to a small volume under nitrogen prior to analysis. Mass spectra of the trimethylsilyl derivatives of capsaicin and its analogues were obtained with a Finnigan 3100 quadrupole mass spectrometer interfaced to a Finnigan 9500 gas chromatograph. The mass spectrometer was operated at a potential of 70 eV. The gas chromatograph contained a 1.8 m by 6.35-mm inside diameter (6 ft by ¼ in.) glass column packed with 3% OV-1 on 80-100 mesh Chromasorb W (HP). Helium was used as the carrier gas. The oven temperature in the gas chromatograph was programmed from 230 to 270°C at 10°C/min.

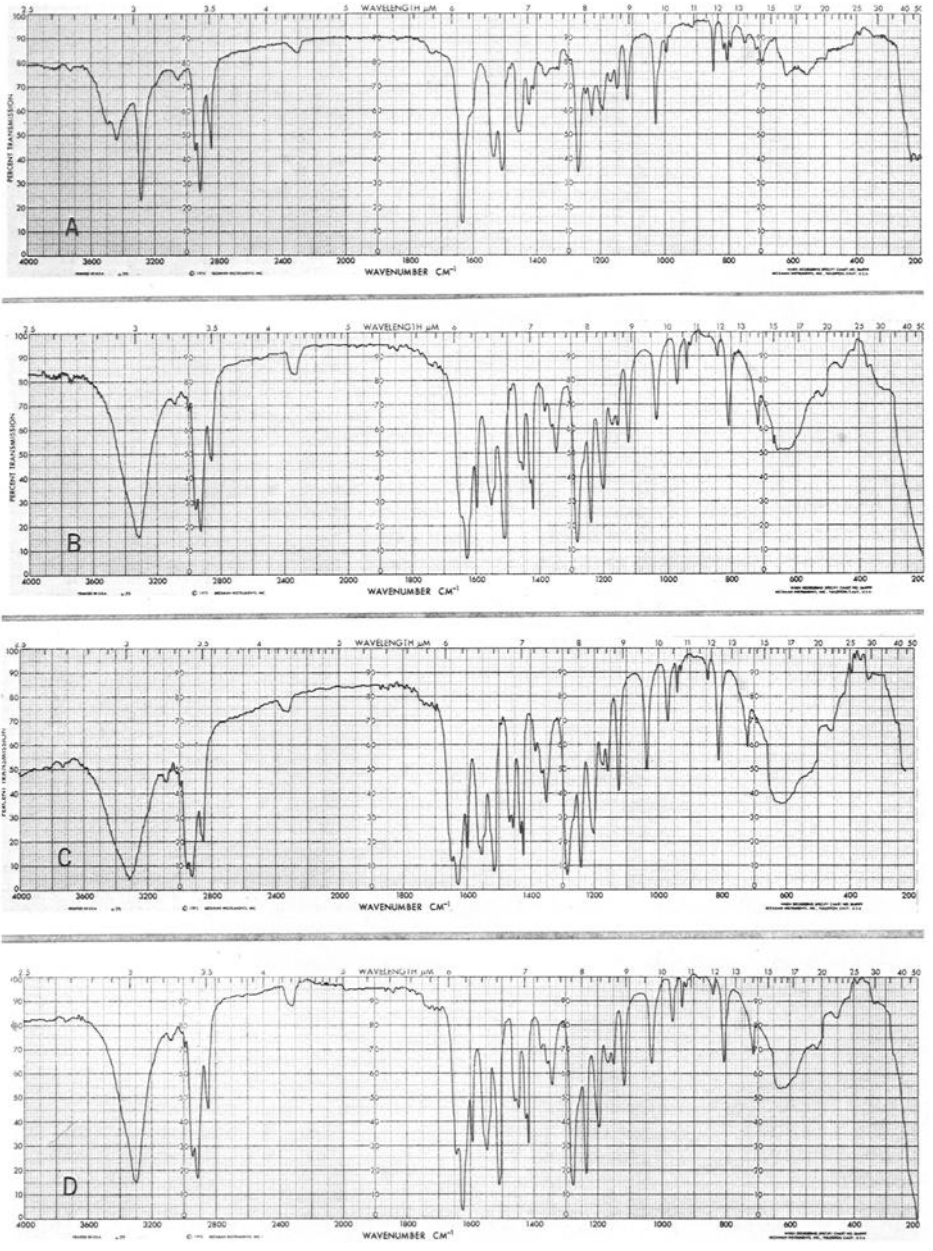


FIG. 3—*Infrared spectra of "synthetic" capsaicin* (A), *"natural" capsaicin* (B), *total capsaicinoids from red chili* (C), *and total capsaicinoids from "Halt!"* (D).

TABLE 2—R_f *values of capsaicinoids on silica gel plates.*

Solvent System	R_f	
	Capsaicin	Dihydrocapsaicin
Chloroform/ethanol (98:2)	0.10[a]	0.21[a]
	0.22	0.22
Benzene/ethanol/ethyl acetate (5:1:1)	0.41[a]	0.50[a]
	0.55	0.55

[a]Values obtained with plates impregnated with 5% silver nitrate.

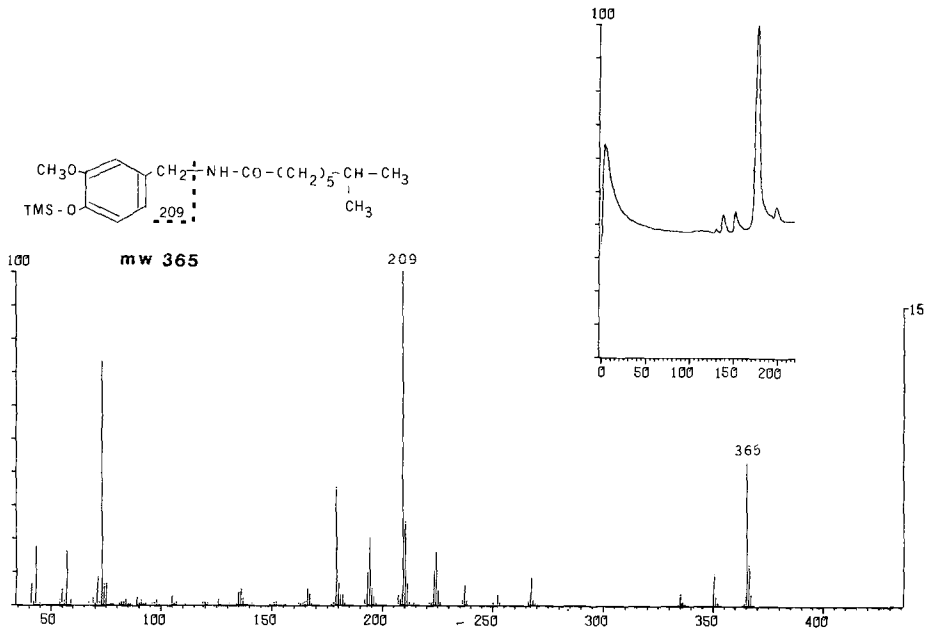


FIG. 4—*Mass spectrum of TMS-nordihydrocapsaicin from the TMS derivative of "synthetic" capsaicin. Insert shows the GC tracing.*

Results and Discussion

When TLC was carried out with silica gel plates, capsaicin and dihydrocapsaicin co-chromatographed to yield a single spot for each of the two solvent systems investigated. However, when plates impregnated with 5% silver nitrate were used, the two capsaicinoids were separated. This improved separation is due to complex formation between the Ag^+ ion and the π-electrons of the extra double bond [8] found in capsaicin causing it to migrate at a slower rate relative to dihydrocapsaicin. The R_f values are shown in Table 2. "Synthetic" capsaicin, which was shown by mass spectral data to be nordihydrocapsaicin, showed R_f values very similar to those of dihydrocapsaicin in all instances.

Figure 2 shows the high performance liquid chromatograms of "natural" capsaicin (A), "synethetic" capsaicin (B), and "Halt!" (C). It is obvious from the chromatograms that "Halt!" is manufactured from natural and not synthetic capsaicin. Eluants corresponding to the two fractions labeled *1* and *2* in Fig. 2C were collected separately for GC/MS analysis.

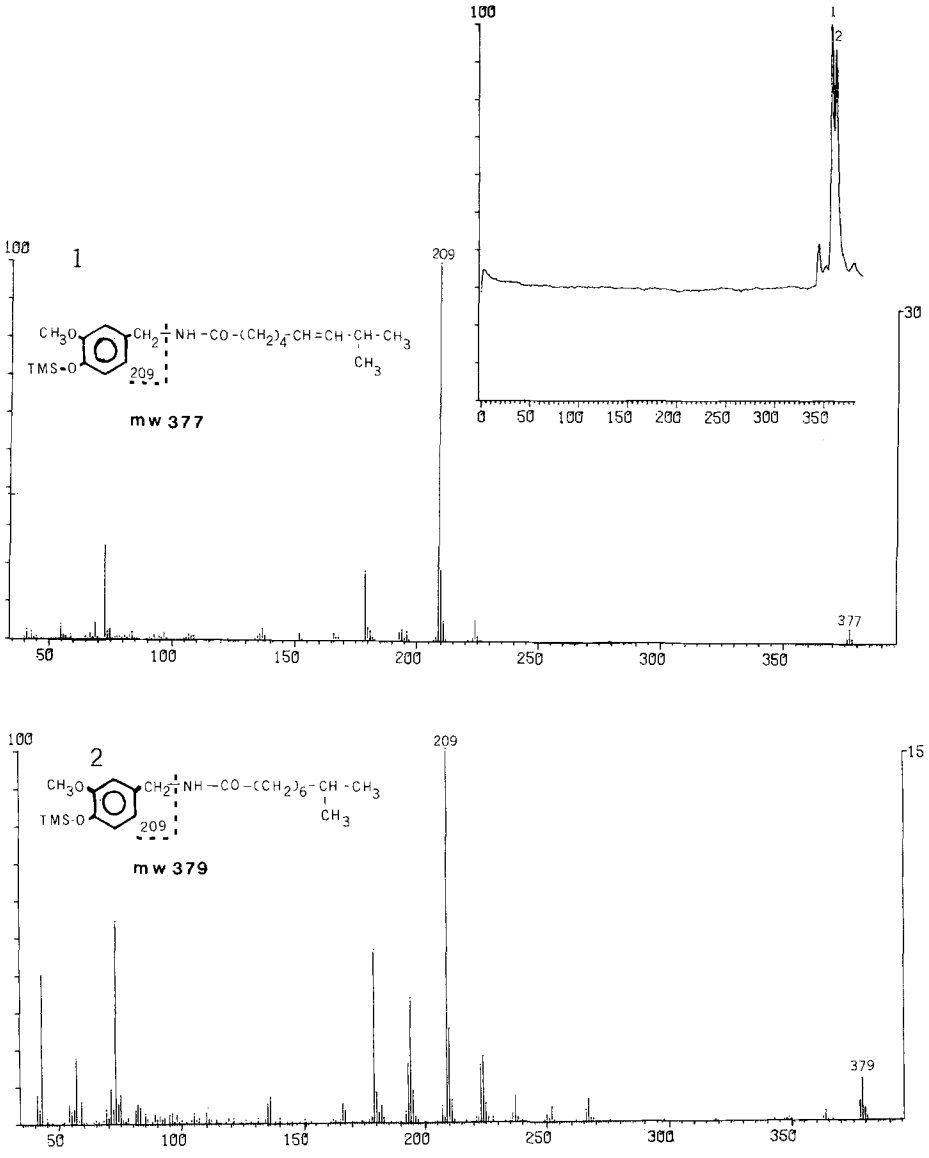


FIG. 5—*Mass spectra for the derivative of "natural" capsaicin. TMS-capsaicin* (top) *and TMS-dihydrocapsaicin* (bottom) *corresponding to Peaks 1 and 2, respectively, in the GC tracing.*

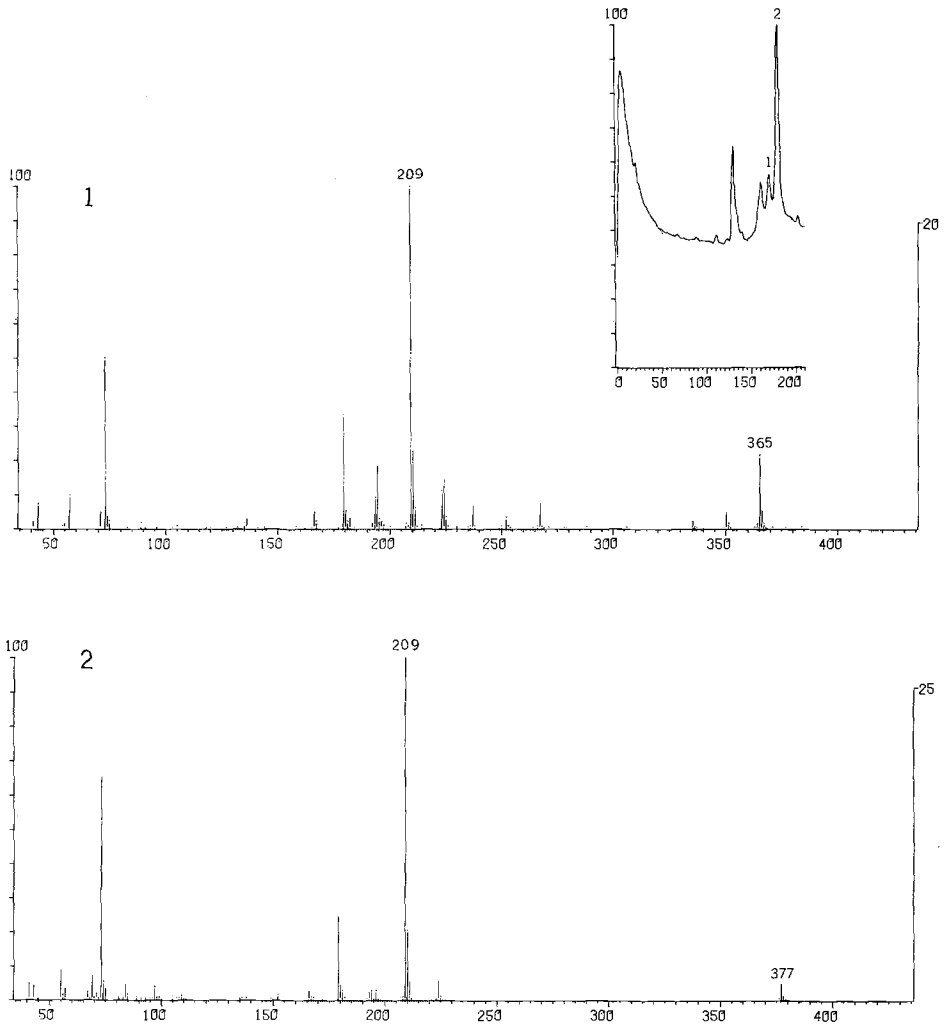


FIG. 6—*Mass spectra of TMS-nordihydrocapsaicin* (top) *and TMS-capsaicin* (bottom) *from the TMS derivatives of Fraction 1 capsaicinoids from "Halt!" by preparative HPLC.*

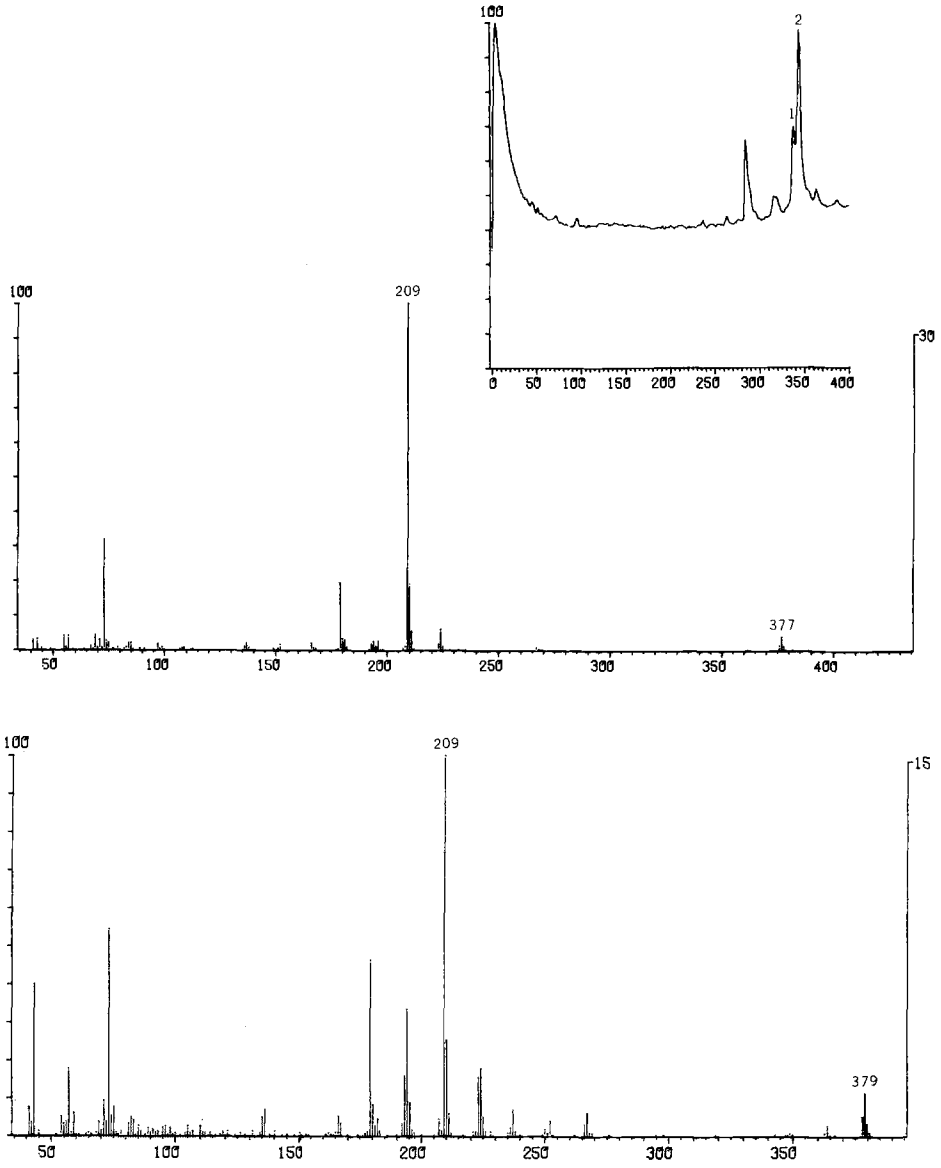


FIG. 7—*Mass spectra of TMS-capsaicin* (top) *and TMS-dihydrocapsaicin* (bottom) *from the TMS derivatives of Fraction 2 capsaicinoids from "Halt!" by preparative HPLC.*

Peak 1 was identified as capsaicin and Peak 2 as dihydrocapsaicin (see mass spectra discussion). The use of a preparative HPLC column should eliminate the need for repeated sample injection and eluant collection and thus greatly reduce analysis time. It is interesting to note that while "synthetic" capsaicin has R_f values similar to dihydrocapsaicin in TLC systems, its retention time in our HPLC system is identical to that of capsaicin.

Figure 3 gives the IR spectra of "synthetic" capsaicin (A), "natural" capsaicin (B), total capsaicinoids from red *Capsicum* fruits (C), and total capsaicinoids in "Halt!" (D). The presence of "natural" capsaicin in "Halt!" is obvious as B, C, and D are almost identical. Attempts to obtain IR of capsaicinoids from HPLC eluant were not satisfactory, probably because of impurities that co-eluted with Fractions 1 and 2.

The mass spectrum of the TMS derivative of "synthetic" capsaicin (Fig. 4) was found to be that of nordihydrocapsaicin with the molecular ion at $m/e = 365$. This provides an answer to the question raised by Gag and Merck [*3*] concerning the nature of "synthetic" capsaicin standard. Figure 5 shows the "natural" capsaicin standard to be composed mainly of capsaicin and dihydrocapsaicin. Figure 6 shows HPLC Fraction 1 from "Halt!" to consist of capsaicin and traces of nordihydrocapsaicin, and Fig. 7 shows Fraction 2 to be a mixture of capsaicin and dihydrocapsaicin. The presence of capsaicin in Fraction 2 suggests an overlapping of the tail end of Fraction 1 and the beginning of Fraction 2 during eluant collection.

Acknowledgment

The authors wish to thank H. D. Bowyer, A/Inspector in Charge-Security, Western Postal Region, Canada Post, for providing the "Halt!" sprays; and Heather Cleland of the Toxicology Section for obtaining the mass spectra.

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