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GAS–LIQUID CHROMATOGRAPHY OF SELECTED BENZIMIDAZOLE FUNGICIDES BY FLASH-HEATER METHYLATION WITH TRIMETHYL-ANILINIUM HYDROXIDE

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SUMMARY

A new method of methylation of benomyl, thiabendazole and fuberidazole (systemic benzimidazole fungicides) was established, based upon the flash-heater reaction with trimethylanilinium hydroxide. The chromatographic response was observed to be linear with the concentration of synthetic standards and the flash-heater products of the examined fungicides. The methyl derivatives were identified spectroscopically.

INTRODUCTION

Benzimidazole pesticides have high systemic fungicidal activity and a wide application. They are commonly used in plant protection. A number of chromatographic methods have been employed in the determination of the active substances in these formulations and of the residues from the plant material. Quantitative thin-layer chromatography (TLC) was used to evaluate the residue of methyl 2-benzimidazole carbamate (carbendazim) and methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate (bemomyl), applying a biological method of detection, *i.e.*, bioautography[1–5]. For the analysis of residues of benomyl[6–9] and 2-(4′-thiazolyl)benzimidazole (thiabendazole)[9] one can also employ high-performance liquid chromatography (HPLC). However, there are inherent difficulties in the analysis of these compounds, especially when using gas–liquid chromatography (GLC). Nevertheless, these fungicides have been analysed by GLC after transforming them into methyl[10] or pentafluorobenzoyl[11,12] derivatives. The methods of identification and determination employed have been described both for the compounds and for their residues.

Derivatization by flash-heater reaction with trimethylanilinium hydroxide (TMAH) has recently been developed for the analysis of carbendazim[13]. This paper describes the application of this methylation technique to the GLC analysis of selected benzimidazole fungicides.

EXPERIMENTAL

Materials

Pesticide standards were obtained from commercial sources and purified by recrystallization from *n*-hexane–dimethylformamide: 2-(2'-furyl)benzimidazole (fuberidazole) (Bayer, Leverkusen, G.F.R.), thiabendazole (Celamerck, Ingelheim, G.F.R.) and benomyl (DuPont, Wilmington, DE, U.S.A.). Trimethylanilinium hydroxide (Methelute®) was purchased as a 0.02 *M* methanolic solution from Pierce (Rockford, IL, U.S.A.).

Preparation of methyl derivatives of benzimidazole fungicides. Methyl fuberidazole [1-methyl-2-(2'-furyl)benzimidazole] and methyl thiabendazole [1-methyl-2-(4'-thiazolyl)benzimidazole] were obtained in a conventional way from fuberidazole and thiabendazole, using a 0.1 *M* methanolic solution of sodium hydroxide and methyl iodide. Dimethyl carbendazim (2-methoxycarbonyl-N-methylamino-1-methylbenzimidazole) was synthesized from carbendazim by a previously described sodium hydride–methyl iodide procedure[14].

Methyl derivatives of benzimidazole fungicides were purified by preparative TLC on glass plates (E. Merck, Darmstadt, G.F.R.) covered with silica gel 60 F_{254}. The mobile phase was *n*-hexane–benzene–acetone (1:1:1).

The melting points of the methyl derivatives were: methyl thiabendazole, 152°C; methyl fuberidazole, 74°C; dimethyl carbendazim, 65°C.

Apparatus

For identification of the methyl derivatives, several types of equipment were used. For the m.p. measurements, an electrothermal capillary apparatus was employed. The UV and IR spectra were run on Pye Unicam SP-1800 and SP-200 spectrophotometers, respectively. The mass spectra were recorded with a LKB-9000 s mass spectrometer, and the nuclear magnetic resonance (NMR) spectra were measured at 60 Hz with a Jeol spectrometer (tetramethylsilane as internal standard).

Gas–liquid chromatography

A Perkin-Elmer 900 chromatograph with a flame ionization detector was used, equipped with a glass column (6 ft. × 3 mm I.D.). The stationary phase was 3 % OV-25 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, PA, U.S.A.). Gas chromatographic analysis of benzimidazole fungicides after flash-heater methylation was performed at a detector temperature of 280°C and an injection chamber temperature of 260°C; hydrogen flow-rate, 40 ml/min; air flow-rate, 360 ml/min; nitrogen flow-rate, 40 ml/min. The isothermal analysis was performed at 240°C.

Optimization of methylation reaction

Samples containing 0.02 mmole of thiabendazole and fuberidazole in 10 ml of methanol, and 0.02 mmole of benomyl in 10 ml of dimethylformamide, were treated with TMAH, at molar ratios (THAH: fungicide) ranging from 1:1 to 8:1. For each determination, 2 μl (0.04 μmole) of the fungicide–TMAH solution were injected into the gas chromatograph. The quantities of TMAH required for the maximum product yields were estimated by the peak area method, employing a standard curve prepared from authentic samples of the methyl derivative of a pesticide.

Standard methanol solutions of the fungicide methyl derivatives ranging in concentration from 10 ng/μl to 100 ng/μl were prepared to determine the linear behaviour of the response. The linearity of the response with respect to the flash-heater methylation was then estimated by use of methanol or dimethylformamide solutions containing 5.0, 10.0, 20.0, 40.0 and 80.0 ng/μl of a pesticide with an approximate molar ratio TMAH:fungicide of 4:1.

RESULTS AND DISCUSSION

Methylation of the examined fungicides by TMAH at the injection chamber temperature of 260°C takes place at the position 1 of the imidazole ring. In the methylation of thiabendazole and fuberidazole, a molar ratio of TMAH to fungicide of 2:1 gives the maximum yield of the methyl derivatives, 98.2% and *ca.* 99%, respectively.

Thiabendazole   Methyl thiabendazole

Fuberidazole   Methyl fuberidazole
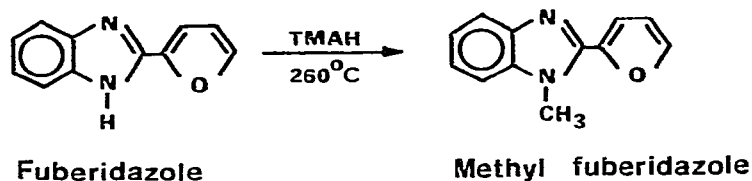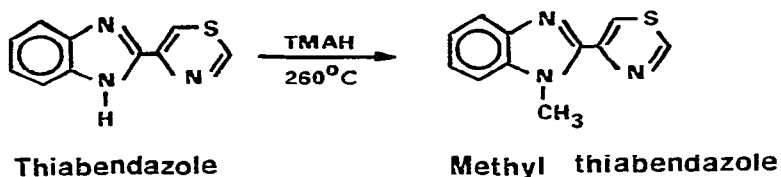
Methylation of benomyl occurs both at the position 1 of the imidazole ring and at the carbamate chain. It is accompanied by transformation of benomyl to carbendazim and by activation of the nitrogen atom at position 1 of the imidazole ring. Eventually the dimethyl carbendazim derivative is obtained. The fungicidal activity of benomyl depends on its hydrolysis to carbendazim, *i.e.*, the biologically active metabolite[15]. Activation of the nitrogen atom in the imidazole ring of benomyl during the flash-heater reaction with TMAH gives a 98% yield of the dimethyl derivative at a molar ratio of TMAH to fungicide of 4:1. In the case of flash-heater methylation of carbendazim the minimum molar ratio is 8:1[13].

Fig. 1 shows the detector response curves (calibration graphs) for the benzimidazole fungicide methyl derivatives prepared from standards. The flash-heater

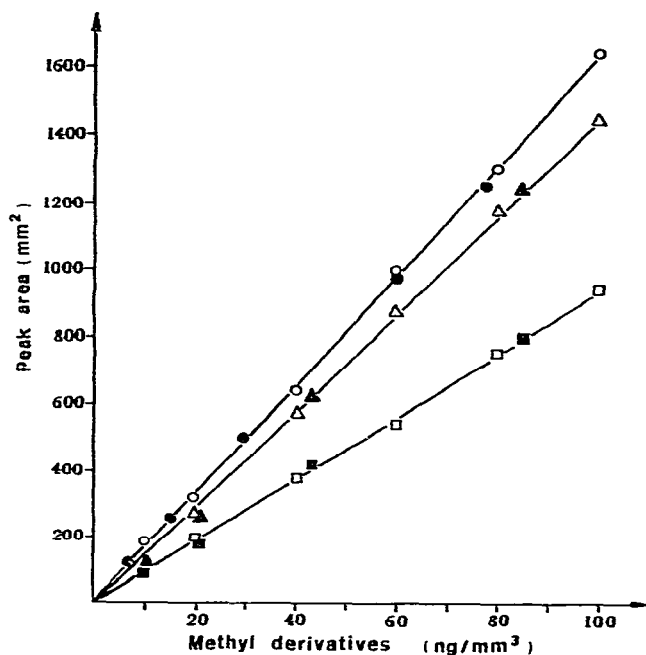Benomyl   Dimethyl carbendazim
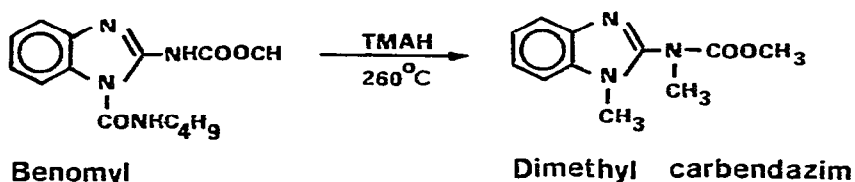
Fig. 1. Calibration graphs of the methyl derivatives of benzimidazole fungicides: ○, dimethyl carbendazim; ●, TMAH + benomyl (4:1); □, methyl fuberidazole; ▨, TMAH + fuberidazole (2:1); △, methyl thiabendazole; ▲, TMAH + thiabendazole (2:1).
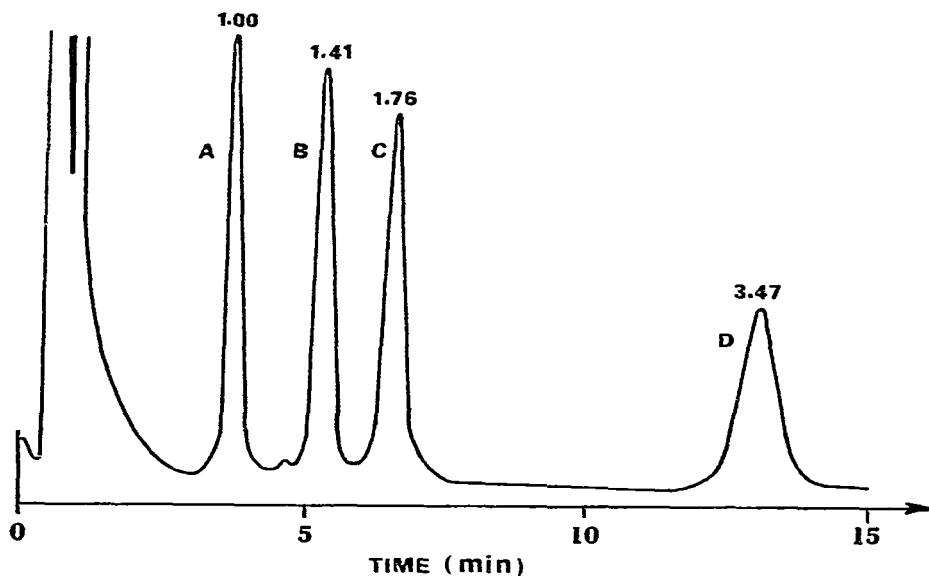

Fig. 2. Separation of a mixture of the flash-heater products of benzimidazole fungicides using a 3% OV-25 column. Peaks: A = Internal standard (9-fluorenone); B = dimethyl carbendazim; C = methyl fuberidazole; D = methyl thiabendazole.

method was then tested to determine whether a linear response could also be obtained with increased concentrations of the methyl derivative. The chromatographic response of the compounds was observed to be linear with concentration of the methyl benzimidazole derivatives. The curves also passed through zero, indicating that no measurable degradation took place on the column.

The chromatographic separation and the retention times, relative to the internal standard 9-fluorenone, for the flash-heater methylated products of benzimidazole fungicides are given in Fig. 2.

Identification of the methyl derivatives

The obtained standards, *i.e.*, the dimethyl derivative of carbendazim and the methyl derivatives of thiabendazole and fuberidazole, were identified by ultraviolet (UV) and infrared (IR) spectroscopy, and also by means of nuclear magnetic resonance (NMR) spectroscopy and mass spectral (MS) analysis.

TABLE I

CHARACTERISTICS OF THE UV AND IR SPECTRA OF THE METHYL DERIVATIVES

Derivative	Spectroscopic characteristics			
	UV (nm)		IR (cm^{-1})	
Dimethyl carbendazim	$\lambda_{max.}$	250	ν_{N-CH_3}	2960
		254	$\nu_{C=O}$	1715
		276		
		284		
Methyl thiabendazole	$\lambda_{max.}$	236	ν_{N-CH_3}	2940
		244		
		272		
		298		
Methyl fuberidazole	$\lambda_{max.}$	246	ν_{N-CH_3}	2920
		250		
		308		
		322		

As shown in Table I, a methanol solution (10 ppm) of the dimethyl derivative of carbendazim has a characteristic UV spectrum. The UV spectrum of benomyl is identical with that of carbendazim, having absorption maxima at 244, 280 and 286 nm. Introduction of a methyl group into the imidazole ring and also into the carbamate chain results in both red and blue shifts. The wavelengths of maximum absorption for the methyl derivatives of thiabendazole and fuberidazole are also given in Table I, together with the IR spectra (KBr technique). None of the analyzed methyl derivatives exhibited the N–H imine group absorption from the imidazole ring (at 3500–3300 cm^{-1}). The intense absorption band at 1715 cm^{-1}, characteristic of C=O vibration of the dimethyl carbendazim derivative, was observed. This demonstrates the preservation of the carbamate chain in this derivative.

In the NMR spectrum of the dimethyl carbendazim derivative dissolved in deuterochloroform (Fig. 3), signals appear at $\delta = 3.45$, 3.75 and 3.84 ppm (singlets due to the N–CH$_3$ group of the carbamate chain, to the ester group and to the methyl
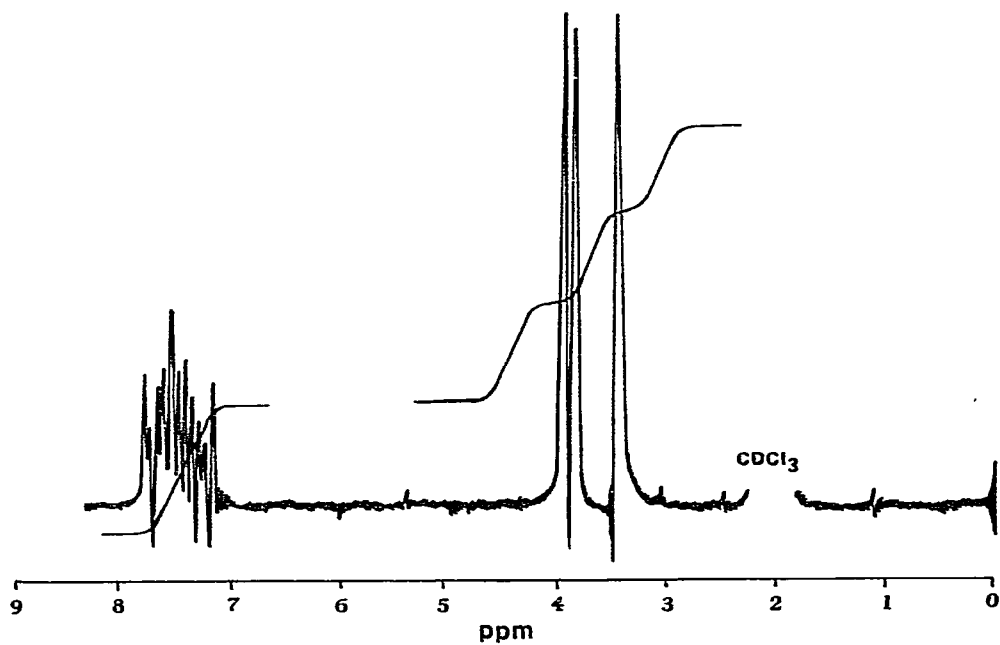
Fig. 3. NMR spectrum of the dimethyl carbendazim derivative at 60 Hz.
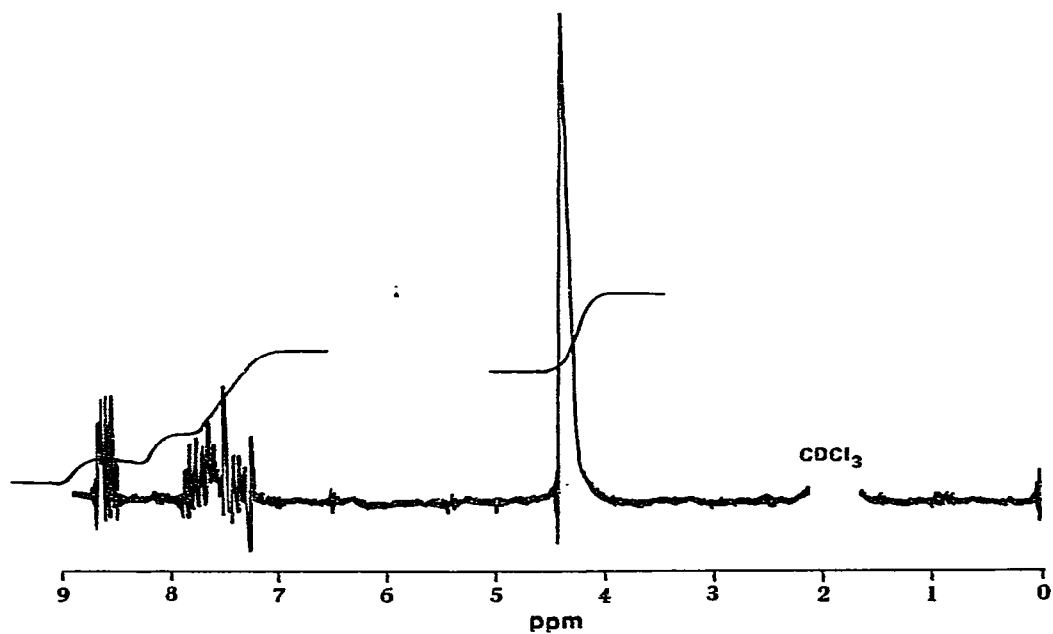

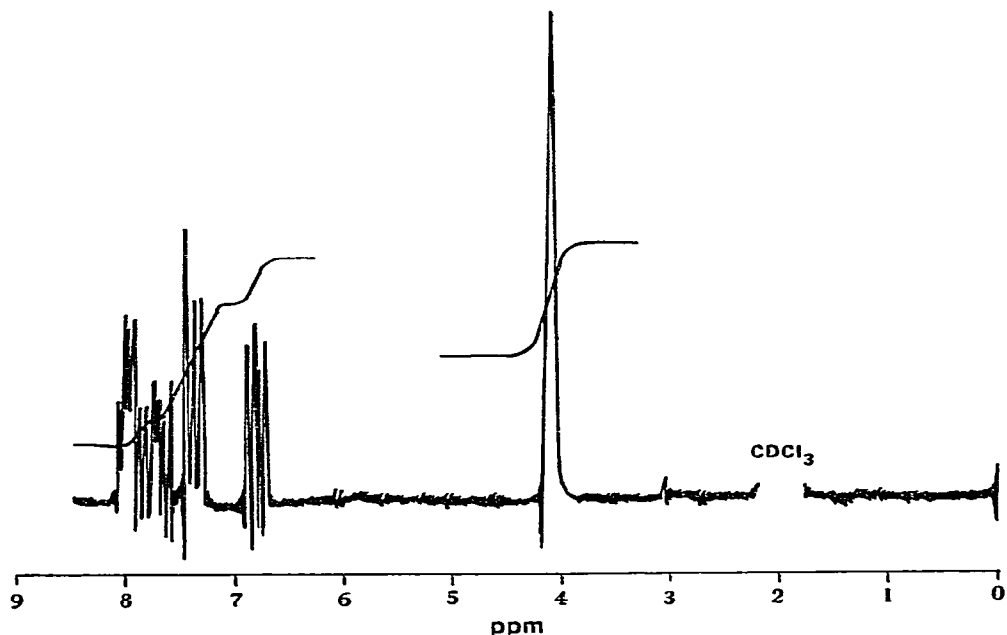Fig. 4. NMR spectrum of the methyl thiabendazole derivative at 60 Hz.

Fig. 5. NMR spectrum of the methyl fuberidazole derivative at 60 Hz.

group in the imidazole ring, respectively). As shown in Figs. 4 and 5, the NMR spectra of the methyl derivatives of thiabendazole and fuberidazole, dissolved in the same solvent, show singlets at $\delta = 4.32$ ppm (3H) and $\delta = 4.15$ ppm (3H), respectively, most probably due to the methyl group of the imidazole ring.

The mass spectrum of benomyl (Fig. 6) lacks the molecular peak at m/e 290 (M^+). However, a molecular ion peak is observed for (m/e 191). The fragmentation pattern of benomyl is as follows: m/e 191 (M^+), 159 ($M^+ - CH_3OH$), 131 ($-CO$),
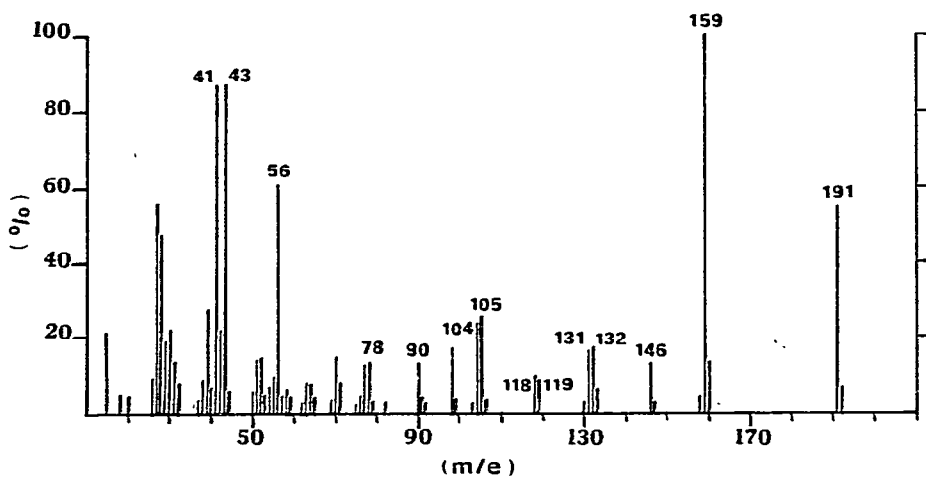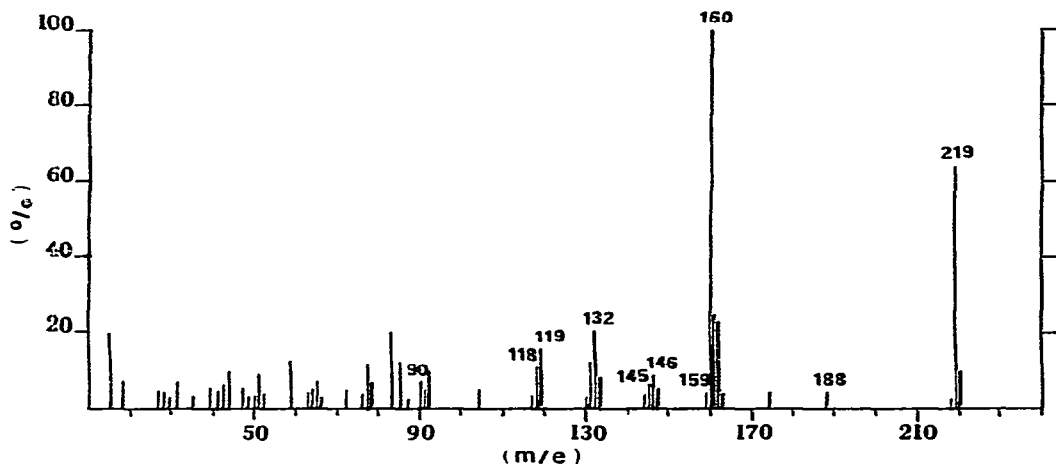

Fig. 6. Mass spectrum of benomyl at 250°C.

Fig. 7. Mass spectrum of the dimethyl carbendazim derivative at 250°C.

104 (− HCN), 78 (− CN); m/e 191 (M$^+$), 132 (− CH$_3$COO), 105 (− HCN); m/e 191 (M$^+$), 146 (− H − CO$_2$), 119 (− HCN), 118 (− H) and 90 (− H − HCN). The peak at m/e 56, 43, 41 are connected with fragmentation of butyl isocyanate, which is a product of the transformation of benomyl to carbendazim.

The mass spectrum of the dimethyl carbendazim derivative (Fig. 7) exhibited ion peaks at m/e 219 (M$^+$), 188 (− CH$_3$O), 160 (− CO), 145 (− CH$_3$) and m/e 219 (M$^+$), 160 (− CH$_3$COO), 159 (− H), 132 (− HCN), 118 (− CH$_2$) and 90 (− H − HCN). Three fragmentation paths are observed in the benomyl mass spectrum (Fig. 6) and two for its dimethyl derivative (Fig. 7).

The fragmentation patterns of the methyl derivatives of thiabendazole and fuberidazole are shown in Figs. 8 and 9. The mass spectrum of methyl thiabendazole exhibited ion peaks at m/e 215 (M$^+$), 188 (− HCN), 155 (− SH), 143 (− C), 131 (−
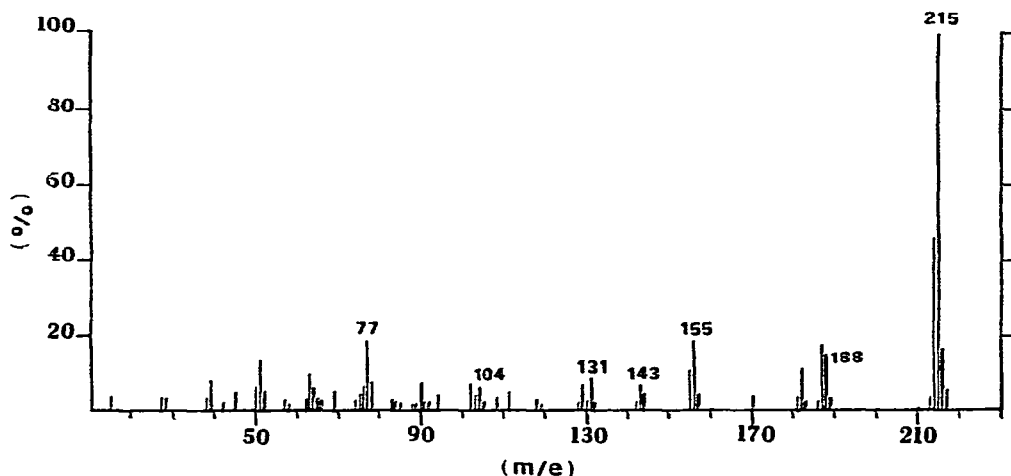

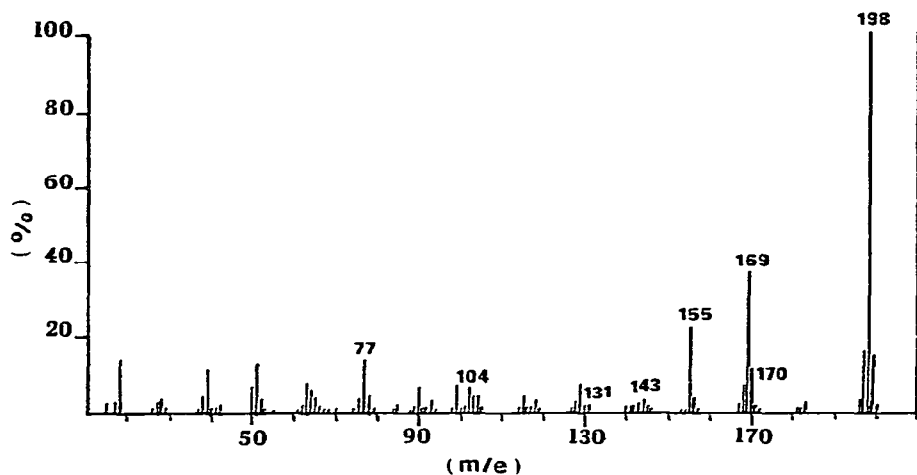Fig. 8. Mass spectrum of the methyl thiabendazole derivative at 250°C.

Fig. 9. Mass spectrum of the methyl fuberidazole derivative at 250°C.

C), 104 ($-$ H $-$ C$_2$H$_2$) and 77 ($-$ HCN). The mass spectrum of methyl fuberidazole exhibited ion peaks at m/e 198 (M$^+$), 170 ($-$ CO), 169 ($-$ H), 155 ($-$ CH$_2$), 143 ($-$ C), 131 ($-$ C), 104 ($-$ H $-$ C$_2$H$_2$) and 77 ($-$ HCN).

The parent peaks for dimethyl carbendazim (m/e 219) methyl thiabendazole (m/e 215) and methyl fuberidazole (m/e 198) correspond with the molecular weight of each compound. Shift of the peak at m/e 219 to 132 for dimethyl carbendazim (Fig. 7) can be attributed to initial degradation of the carbamate chain, and the subsequent shift from m/e 132 to 90 for all the methyl benzimidazole derivatives can be ascribed to N-demethylation and to degradation of the imidazole ring. Shift of the peak at m/e 215 to 132 for methyl thiabendazole and that at m/e 198 to 132 for methyl fuberidazole can be attributed to initial degradation of the thiazolyl and furyl rings.

The results concerning the identification of dimethyl carbendazim and methyl thiabendazole supplement those from previous work[10,13].

CONCLUSION

The possibility of application of the flash-heater methylation with trimethylanilinium hydroxide to the selected benzimidazole fungicides and their qualitative identification and GLC determination has been established. The selectivity of this reaction is preserved when the molar ratio of TMAH to fungicide is 2:1 (fuberidazole and thiabendazole) or 4:1 (benomyl), and the injection chamber temperature is 260°C, which gives almost quantitative yields of the methyl derivatives. The linear behaviour of the detector (FID) response for nanogram amounts of benzimidazole fungicides with TMAH in the flash-heater reaction allows one to apply this method to the GLC determination of the residues of these fungicides.

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