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MASS SPECTRAL DETERMINATION OF ALDEHYDES, KETONES, AND CARBOXYLIC ACIDS USING 1,1-DIMETHYLHYDRAZINE¹

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Abstract—Analyses of nanogram to milligram quantities of aliphatic aldehydes, fatty acids, and unhindered aliphatic ketones such as those typically found in pheromonal blends have been effected by treating these mixtures with 1,1-dimethylhydrazine. The aldehydes and ketones form *N,N*-dimethylhydrazones, while the fatty acids form methyl esters. Structural elucidation of the reaction products was achieved using EI and CI gas chromatography-mass spectrometry.

Key Words—Semiocemicals, pheromones, methyl esters, *N,N*-dimethylhydrazones, *N,N*-dimethylhydrazine, mass spectrometry.

INTRODUCTION

Insects produce a variety of semiochemicals that govern many, if not most, of their behavioral activities. Such chemicals are typically produced in nanogram to microgram quantities as complex mixtures of functionally diverse components (Shorey and McKelvey, Jr., 1977; Ritter, 1979). Common to many of these semiochemical blends are aldehydes, ketones, and carboxylic acids (Brand et al., 1979; Silverstein, 1977; Tamaki, 1977). The interpretation of the mass spectra of these aliphatic aldehydes and ketones is not always straightforward,

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however. These compounds frequently possess low abundance or nonexistent molecular ions. Aldehydes can have prominent M-18 ions, making it difficult to distinguish them from unsaturated alcohols, and, of course, diunsaturated alcohols are not easily differentiated from monounsaturated aldehydes by either electron impact (EI) or chemical ionization (CI) mass spectrometry.

In the course of our investigation of the marking pheromone complexes of various *Philanthus* spp. (McDaniel et al., unpublished work), we encountered complex mixtures of aldehydes, ketones, and free acids. We therefore sought a derivatization technique that would not only allow chromatographic separation, but would also allow unequivocal mass spectral characterization of the products.

Reaction of a carbonyl moiety with 1,1-dimethylhydrazine (DMH) to form the *N,N*-dimethylhydrazone as synthetic intermediate has been previously reported (Smith and Walker, 1962; Newkome and Fishel, 1966; Corey and Enders, 1976). VandenHeuvel and Horning (1963) have used the hydrazone products of ketones for gas chromatographic analyses, relying solely on retention times for characterization. GC-MS analysis is a much more powerful technique that potentially offers a facile, unequivocal characterization of *N,N*-dimethylhydrazones derived from aldehydes and ketones.

We found that *N,N*-dimethylhydrazone formation proceeded smoothly and essentially quantitatively for aliphatic aldehydes. Good yields were obtained for aliphatic unhindered ketones, whereas hindered ketones produced only modest yields. We discuss the mass spectra of these derivatives and their application to structural elucidation of the parent carbonyl compound.

The DMH reagent surprisingly converted similar quantities of aliphatic carboxylic acids to their corresponding methyl esters. We were thus able, after the addition of a single reagent, to perform GC-MS analyses of mixtures of aldehydes, ketones, and carboxylic acids.

METHODS AND MATERIALS

Hydrazone formation was achieved by the addition of approximately 1 mmol of neat 1,1-dimethylhydrazine (Mallinckrodt, St. Louis, Missouri) to approximately 10^{-4} - 10^{-2} mmol of the aldehyde or ketone. Reactions were carried out in Wheaton 5-ml Reactivials® sealed with Teflon®-lined caps. The samples were held at 50° for approximately 1 hr in a Pierce Reacti-Therm®. Appropriate dilutions were made prior to analysis by the addition of *n*-hexane. Yields were estimated using FID gas chromatography by comparing peak areas of the starting material to those of the hydrazone product.

Esterification of fatty acids was examined using stearic acid as a model compound. One millimole stearic acid was dissolved in 100 ml THF; 100- μ l aliquots (1.0 μ mol stearic acid) were placed in the Reactivials, the solvent removed with N₂, and 100 μ l (1.3 mmol) neat 1,1-dimethylhydrazine was added.

The vials were sealed with Teflon-lined caps and allowed to react for 1 hr at 50°C. The 1,1-dimethylhydrazine was removed with a stream of nitrogen, and an appropriate volume of THF was added. Conversion was quantitated by gas chromatographic comparison to standard solutions of methyl stearate.

Gas chromatographic analyses were performed on a Hewlett-Packard 5710 gas chromatograph with a FID detector. The column employed was a 1.8-m × 2-mm ID glass column packed with 3% SP-2100 on 100/120 mesh Supelcoport.

Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a Hewlett-Packard 5710A gas chromatograph-5982 mass spectrometer interfaced to a 5933 data system. Compounds were separated on a glass 2-m × 2-mm ID column packed with 3% SP-2100 on 100/120 mesh Supelcoport. Electron impact spectra were generated with a source potential of 70 eV; chemical ionization spectra were generated using ultrapure methane (Airco, Incorporated) as both the carrier gas and the ionizing gas. The internal source pressure was 0.5 torr, with a source potential of 200 eV.

RESULTS AND DISCUSSION

While the aldehydes examined reacted essentially quantitatively with DMH under our experimental conditions, the reactivity of DMH with ketones varied dramatically, with yields appearing to be quite susceptible to steric hindrance. This observation is in agreement with previous reports (VandenHeuvel and Horning, 1963). Hydrazone yields are presented in Table 1.

The EI mass spectra of the *N,N*-dimethylhydrazones of the aldehydes were very definitive. All had a McLafferty rearrangement ion ($m/z = 86$) as the base

TABLE 1. CONVERSION OF ALDEHYDES AND KETONES TO THEIR *N,N*-DIMETHYLHYDRAZONES

Parent compound	Conversion (%)
11-Undecenal	100
Tetradecanal	100
Z-9-Tetradecenal	95
Z-11-Hexadecenal	100
2-Heptanone	61
3-Heptanone	86
4-Heptanone	15
4-Octanone	17
2-Methyl-4-octanone	2
3-Methyl-4-octanone	3
2,6-Dimethyl-4-heptanone	2
2-Undecanone	58

TABLE 2. MASS SPECTRAL DATA OF
N,N-DIMETHYLHYDRAZONES OF SELECTED ALDEHYDES

Parent compound	Diagnostic ions: <i>m/z</i> and (abundances)	
	<i>M</i> +	Base peak
11-Undecenal	210 (33%)	86
Tetradecanal	254 (22%)	86
<i>Z</i> -9-Tetradecenal	252 (23%)	86
<i>Z</i> -11-Hexadecenal	280 (21%)	86
<i>E</i> -14-Methyl-8-hexadecenal	294 (31%)	86
12-Oxoheptacosanal	464 (16%)	86

peak, with molecular ions of typically 20–30% relative abundance (Table 2). Chemical ionization mass spectra of these hydrazones had the $(M+1)^+$ ions as their base peaks with little other structural information. Typical EI and CI spectra are presented in Figure 1.

The EI mass spectral data of the *N,N*-dimethylhydrazones of some representative ketones are presented in Table 3. Usually, the base peak for these hydrazones was at $m/z = 44$, the dimethylamino cation. For the hydrazone of 2-ketones, the McLafferty ion at $m/z = 100$ was always prominent and was the base peak for the hydrazone of 2-pentadecanone. When the derivatized keto functionality was more internally positioned on unbranched ketones, less prominent but still diagnostically useful rearrangement ions were present. Other types of ketone hydrazones yielded either few or no McLafferty rearrangement ions. In these cases, preparation of the *N,N*-dimethylhydrazone provided little additional useful structural information. CI mass spectra of the ketone hydrazones, as in the case of the aldehyde hydrazones, had the $(M+1)^+$ ion as the base peak, and possessed no other ions which were diagnostically useful. Representative EI and CI mass spectra for the *N,N*-dimethylhydrazone of 2-pentadecanone are shown in Figure 2.

During our investigation of the reaction of DMH with aldehydes, we found that the aldehydes contained significant amounts of corresponding carboxylic acids. Unexpectedly, the addition of DMH to these aldehyde-carboxylic acid mixtures resulted not only in the expected hydrazone formation, but also in the formation of the methyl esters of the carboxylic acids. We examined this reaction using micromole quantities of stearic acid and obtained a conversion of $62 \pm 9\%$ ($\bar{X} \pm SD$) for eight replications. Earlier runs with nanomolar quantities of acid and millimolar quantities of DMH were essentially quantitative, presumably because of mass action.

The mass spectra of methyl esters are well known and will not be discussed (Ryhage and Stehagen, 1963; Heller and Milne, 1978).

The vials were sealed with Teflon-lined caps and allowed to react for 1 hr at 50°C. The 1,1-dimethylhydrazine was removed with a stream of nitrogen, and an appropriate volume of THF was added. Conversion was quantitated by gas chromatographic comparison to standard solutions of methyl stearate.

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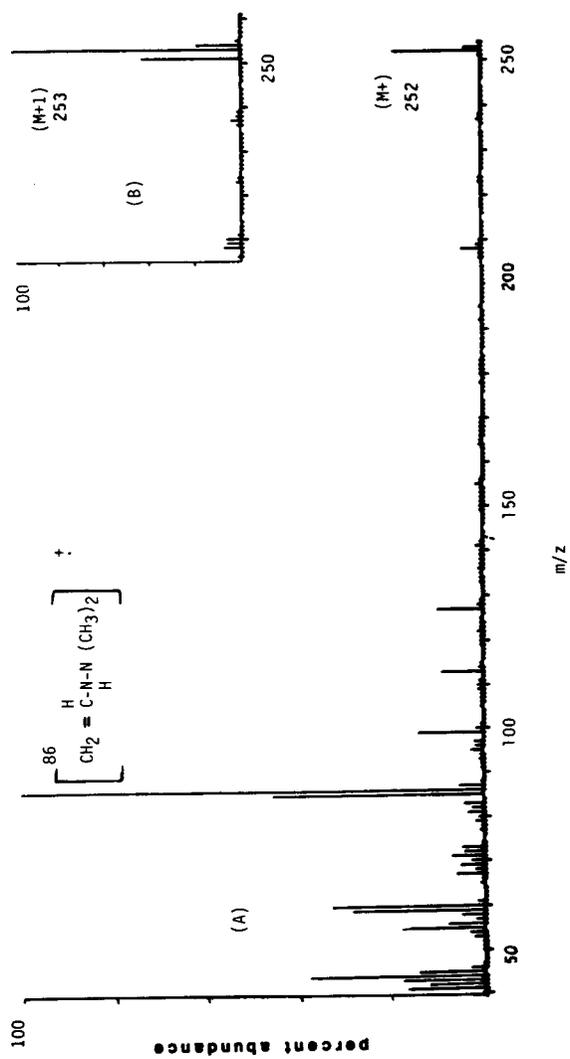
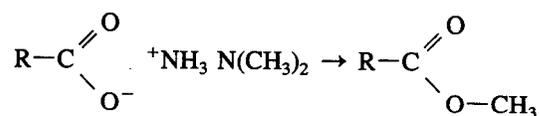
FIG. 1. Mass spectra of the *N,N*-dimethylhydrazone of *Z*-9-tetradecenal: (A) EI; (B) CI.

TABLE 3. DIAGNOSTIC MASS SPECTRAL FRAGMENT IONS OF *N,N*-DIMETHYLHYDRAZONES OF SELECTED KETONES

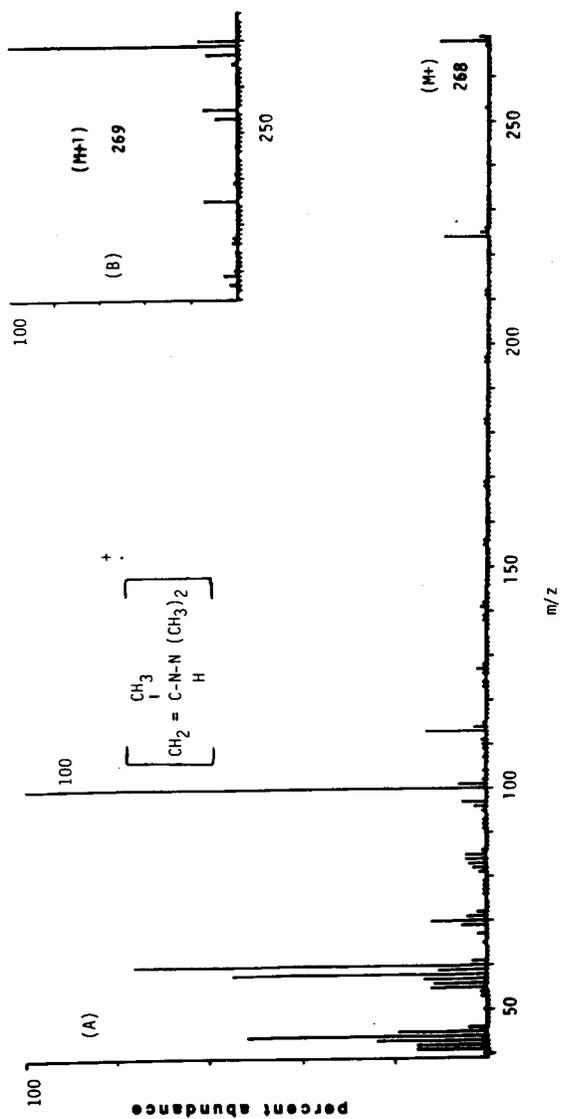
Parent compound	Diagnostic ions, <i>N,N</i> -dimethylhydrazones: <i>m/z</i> (relative abundance)		
	M+	Base peak	McLafferty rearrangement
2-Methyl-2-hexen-4-one	154 (11%)	44	---
3-Heptanone	156 (73%)	44	144 (25%)
2-Octanone	170 (45%)	44	128 (14%), 142 (0.2%)
2-Methyl-3-heptanone	170 (20%)	44	128 (2%)
4-Methyl-3-heptanone	170 (8%)	44	128 (2%)
2-Methyl-4-heptanone	170 (62%)	44	128 (0%), 142 (0%)
3-Methyl-4-heptanone	170 (25%)	44	142 (0%)
2,6-Dimethyl-4-heptanone	184 (15%)	44	142 (0%)
2-Methyl-4-octanone	184 (39%)	44	142 (1%)
2-Undecanone	212 (9%)	44	100 (67%)
2-Pentadecanone	268 (11%)	100	100 (100%)

The mechanism of the esterification reaction has not yet been established, but is currently being investigated. One possibility is that esterification of the carboxylic acids might be occurring in the injection port of the gas chromatograph via a thermolysis of the acid-DMH salt:



To examine this possibility, we removed excess DMH from the reaction mixture and partitioned the nonvolatile residue between hexane and 0.1 N H₂SO₄. The stronger mineral acid should release the weaker carboxylic acid from the salt form if it is present. Subsequent gas chromatographic and GC-MS analysis of the hexane layer showed no diminution of the amount of ester product. This hexane layer was also examined by infrared spectrophotometry. The IR spectrum clearly established that esterification was occurring in the DMH solution.

The use of 1,1-dimethylhydrazine to characterize aldehydes, simple alkyl ketones, and fatty acids thus appears to offer great potential in the analysis of insect semiochemicals. The method will frequently obviate the necessity to preparatively isolate individual components and will allow structural analysis of quantities of semiochemicals approximating that in a single insect. We are currently using this reagent in our laboratory in just such a manner for pheromone analyses.

FIG. 2. Mass spectra of the *N,N*-dimethylhydrazone of 2-pentadecanone: (A) EI; (B) CI.

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