

## Note

### Derivatization with 1,1-dimethylhydrazine for identification of carbonyl compounds resulting from ozonolysis

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Carbon-carbon double bonds are present in a variety of biologically important natural products. Biological activity is often directly related to the position of the double bond. A variety of methods are available for the location of double-bond positions<sup>1–3</sup>. Ozonolysis is one of the micro-methods which has been used extensively<sup>4,5</sup>. However, the carbonyl fragments arising from ozonolysis are often difficult to identify only by gas chromatographic (GC) retention times. Particularly, small carbonyl fragments, especially bifunctional carbonyls arising from polyunsaturated compounds, are difficult to identify. Moreover, the mass spectra of ozonolysis products are often featureless and rarely show observable molecular ions.

Derivatization with 1,1-dimethylhydrazine to form N,N-dimethylhydrazones has been used to characterize aldehydes by GC-mass spectrometry (GC-MS)<sup>6</sup>. In the present study, we describe the use of this derivatization reaction in the analysis of carbonyl products obtained from ozonolysis of unsaturated compounds.

## EXPERIMENTAL

### *Materials*

All chemicals including compounds identical to those found in some insect pheromones were purchased from Sigma (St. Louis, MO, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.) chemical companies. A mixture of (4*E*,6*Z*,10*Z*)-4,6,10-hexadecatrienyl acetate and (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate was a gift of Dr. A. Cork (Overseas Development Natural Resources Institute, Kent, U.K.).

### *Apparatus and chromatographic conditions*

Capillary GC was performed on a Hewlett-Packard 5890A instrument equipped with a flame ionization detector. A 25 m × 0.22 mm I.D. fused-silica column coated with methyl silicone gum OV-1 was used for analysis. The oven temperature was initially 60°C for 4 min then increased at a rate of 6°C/min to 280°C.

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### GC-MS

The samples were analysed on a Hewlett-Packard 5890 gas chromatograph linked to a 5970B mass selective detector. A 20 m × 0.22 mm I.D. fused-silica capillary column coated with OV-101 methyl silicone was directly coupled to the detector. The temperature programme was identical to that used for GC. The detector was set to monitor masses  $m/z$  35–350 and 70 eV EI spectra were recorded at about 1.5 scans/s.

### Ozonolysis

Micro-ozonolysis was performed as described by Beroza and Bierl<sup>4,5</sup>. Each test substance (50 ng) in hexane (5  $\mu$ l) was placed in the bottom of a cone-shaped micro-test tube and a slow stream of ozone (1–2 ml/min), generated by a micro-ozonizer (Supelco, Bellefonte, PA, U.S.A.), was passed for 1–2 min at room temperature. A solution of triphenylphosphane in hexane (1  $\mu$ l, 1 mg/ml) was added to reduce the ozonides.

### Derivatization with 1,1-dimethylhydrazine

1,1-Dimethylhydrazine (1  $\mu$ l) was added to the ozonolysed solution in hexane. After 5 min at room temperature the solution was analysed by GC of GC-MS.

## RESULTS AND DISCUSSION

The derivatization with 1,1-dimethylhydrazine proceeds smoothly under mild conditions. Aldehydes react essentially quantitatively and the reaction is complete in a few minutes. Presence of triphenylphosphane, used as a reducing agent to cleave ozonides, does not interfere with the reaction. The reaction mixture can be examined directly by GC or GC-MS.

The test compounds used were selected to represent different classes of compounds, with different degrees of unsaturation, encountered as pheromones. Table I summarizes the results of the present study. The mass spectra of N,N-dimethylhydrazones of aldehydes are very characteristic. A strong molecular ion is always present; therefore, it was possible to determine the molecular weights of the resulting aldehydes of ozonolysis readily. Even low-molecular-weight aldehydes, which are usually difficult to recognize by GC because of their high volatility, were identified without difficulty. Acetoxy aldehydes are particularly difficult to identify because authentic compounds are not usually available and their mass spectra seldom show molecular ions. All the N,N-dimethylhydrazone derivatives of acetoxy aldehydes identified in this study showed very prominent molecular ions (Table I).

In the case of (7Z,11Z)-7,11-hexadecadienyl acetate, we expected to see a peak for the mono- or di-hydrazone of succinic dialdehyde. However, we were unable to record a peak for this compound.

During our investigations, we found that it was possible to ozonolyse polyunsaturated compounds partially, one double bond at a time, by using a slow stream of ozone for a short time. The flow of ozone and duration of the reaction were controlled in such a manner that the polyunsaturated test compound peak did not disappear completely after ozonolysis. In fact, results obtained from a partial ozonolysis followed by derivatization with 1,1-dimethylhydrazine are more easily interpretable, and yield more information than those from a total ozonolysis. Fig. 1 shows the results of a partial ozonolysis of a mixture of (4E,6Z,10Z)-4,6,10-

TABLE I  
N,N-DIMETHYLHYDRAZONES IDENTIFIED AFTER DERIVATIZATION OF OZONOLYSIS PRODUCTS

Ozonolysed compound	Products identified	Mass spectral data, m/z (%)
(7Z,11Z)-Hexadecadienyl acetate	$\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	128( $\text{M}^+$ , 38), 113(8), 99(13), 86(59), 85(52), 71(13), 59(15), 44(100), 43(53)
	$\text{CH}_3\text{CO}_2(\text{CH}_2)_6\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	214( $\text{M}^+$ , 34), 170(4), 155(5), 127(8), 113(10), 99(21), 86(100), 85(58), 59(37), 44(90), 43(82)
(Z)-5-Decenyl acetate	$\text{CH}_3\text{CO}_2(\text{CH}_2)_4\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	186( $\text{M}^+$ , 17), 127(8), 111(6), 99(11), 86(18), 85(30), 59(20), 44(57), 43(100)
	$\text{CH}_3(\text{CH}_2)_3\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	128( $\text{M}^+$ , 37), 113(6), 99(11), 86(56), 85(48), 71(12), 59(12), 44(100), 43(49), 42(46)
$\text{C}_6\text{H}_5\text{CH}=\text{CHCH}_2\text{OCOCH}_3$	$\text{CH}_3\text{CO}_2\text{CH}_2\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	144( $\text{M}^+$ , 12), 102(3), 101(4), 85(12), 72(7), 57(8), 45(33), 44(37), 43(100), 42(63)
	$\text{C}_6\text{H}_5-\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	148( $\text{M}^+$ , 100), 133(24), 118(27), 106(11), 104(13), 92(17), 90(22), 77(34), 63(8), 51(26), 44(35)
EZZ + EEZ <sup>a</sup>	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	142( $\text{M}^+$ , 16), 127(4), 113(5), 99(8), 86(44), 85(37), 71(10), 59(24), 45(30), 44(100), 43(58), 42(52)
	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	196( $\text{M}^+$ , 6), 154(4), 86(7), 85(100), 44(40), 43(13), 42(21)
	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{CH}-\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	222( $\text{M}^+$ , 8), 112(8), 111(100), 68(13), 44(28), 43(13), 42(18)
	$\text{CH}_3\text{CO}_2(\text{CH}_2)_3\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	172( $\text{M}^+$ , 14), 112(9), 99(9), 97(28), 86(18), 85(19), 68(13), 45(19), 44(83), 43(100), 42(47)
	$\text{CH}_3\text{CO}_2(\text{CH}_2)_3\text{CH}=\text{CH}-\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	198( $\text{M}^+$ , 16), 138(6), 123(12), 111(18), 95(15), 94(18), 82(12), 80(12), 68(13), 67(14), 59(18), 44(54), 43(100), 42(40)
	$\text{CH}_3\text{CO}_2(\text{CH}_2)_3\text{CH}=\text{CH}-\text{CH}=\text{CH}(\text{CH}_2)_2\text{CH}=\text{N}-\text{N}(\text{CH}_3)_2$	252( $\text{M}^+$ , 9), 85(100), 44(32), 43(25), 42(15)

<sup>a</sup> (4E,6Z,10Z)-4,6,10-hexadecatrienyl acetate and (4E,6E,10Z)-4,6,10-hexadecatrienyl acetate.

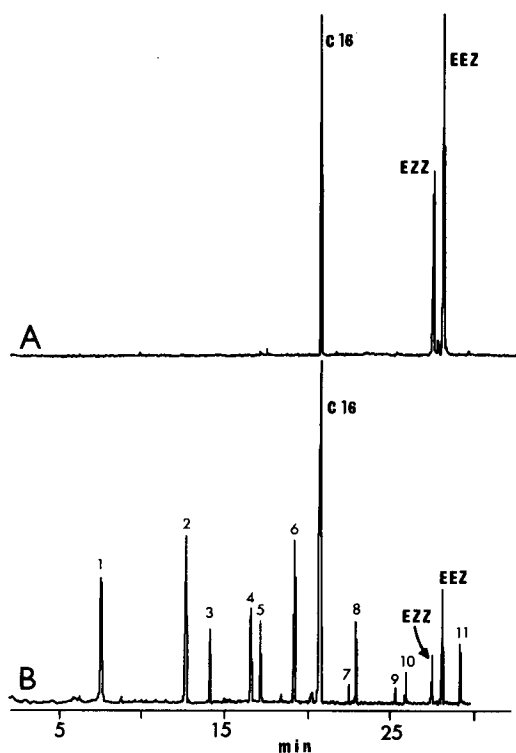


Fig. 1. Reconstructed ion chromatograms obtained on a methylsilicone column (20 m  $\times$  0.22 mm I.D., 4 min at 60°C, 6°C/min to 280°C, splitless injection). (A) (4*E*,6*Z*,10*Z*)-4,6,10-hexadecatrienyl acetate [EZZ] and (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate [EEZ] together with hexadecane [C16] as an internal standard. (B) After partial ozonolysis, followed by derivatization with 1,1-dimethylhydrazine. Peaks 1–10 are 1,1-dimethylhydrazones of the following: 1 = hexanal; 2 = 4-acetoxybutanal; 3 = unidentified; 4 = unidentified; 5 = (*Z*)-4-decenal; 6 = (*E*)-6-acetoxy-2-hexenal; 7 = (2*Z*,6*Z*)-2,6-dodecadienal; 8 = (2*E*,6*Z*)-2,6-dodecadienal; 9 = (4*E*,6*Z*)-10-acetoxy-4,6-decadienal; 10 = (4*E*,6*E*)-10-acetoxy-4,6-decadienal. Peak 11 is triphenylphosphane.

hexadecatrienyl acetate and (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate. The latter is the major component of the sex pheromone of cocoa pod borer moth, *Conopomorpha cramerella*<sup>7</sup>. If partial ozonolysis breaks only one double bond at a time, six aldehyde fragments are expected from each geometric isomer of 4,6,10-hexadecatrienyl acetate. The masses of expected carbonyl compounds after derivatization with 1,1-dimethylhydrazine is illustrated in Fig. 2. All the expected carbonyl compounds were detected

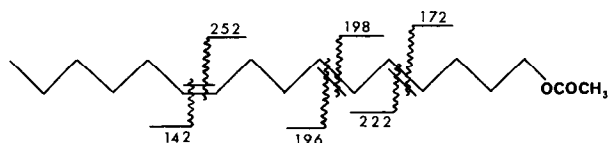


Fig. 2. Molecular masses of N,N-dimethylhydrazones expected from partial ozonolysis fragments of (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate.

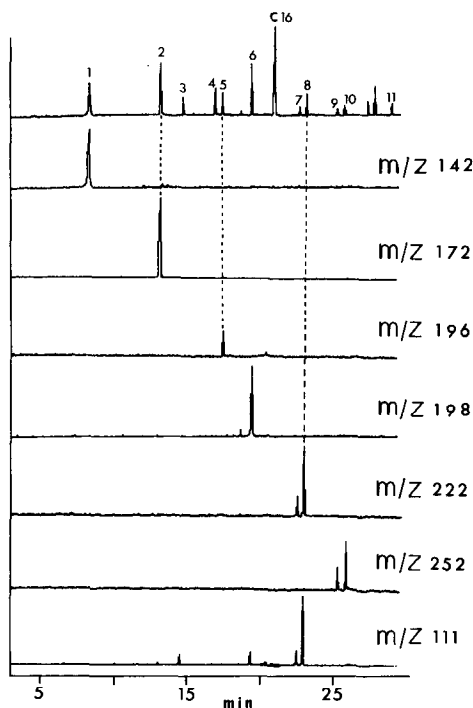


Fig. 3. A reconstructed gas chromatogram and seven mass chromatograms for the ions  $m/z$  142, 172, 196, 198, 222, 252, and 111, obtained from GC-MS analysis of derivatized partial ozonolysis products of (4*E*,6*Z*,10*Z*)-4,6,10-hexadecatrienyl acetate and (4*E*,6*E*,10*Z*)-4,6,10-hexadecatrienyl acetate. Refer to Fig. 1 for peak assignments and chromatographic conditions.

in the ozonolyzed mixture (Fig. 1). The peaks 1, 2, 5 and 6 originated from both (*E,Z,Z*)- and (*E,E,Z*)-isomers, whereas peaks 7 and 9 were fragments of the (*E,Z,Z*)-isomer, and peaks 8 and 10 were those from (*E,E,Z*)-isomer.

Although 50 ng of each polyunsaturated compound was used customarily in the present study, MS analysis (Fig. 3) and single-ion monitoring investigations revealed that only a few nanograms of the starting material was sufficient for a complete analysis. Peaks 3 [ $m/z$  44(100), 60(80), 85(64), 111(19)] and 4 [ $m/z$  44(100), 83(17), 86(27), 125(50)] were two unidentified products. They appeared in all chromatograms after derivatization, independent of which compound was ozonolysed.

*N,N*-Dimethylhydrazone spectra are not only useful for mass determination of aldehyde fragments; they also yield structural information. The hydrazones of saturated aldehydes show a significant fragment ion at  $m/z$  86 resulting from a McLafferty rearrangement<sup>8</sup>. This ion is also seen in the spectra of unsaturated aldehydes if the first double bond is not in the vicinity of the carbonyl group<sup>6</sup>.  $\alpha,\beta$ -Unsaturated aldehydes are unable to yield this McLafferty ion; they undergo an allylic fission to give a significant ion at  $m/z$  111 due to the ion  $\text{CH}_2=\text{CH}-\text{CH}=\text{CH}-\text{N}=\text{N}^+(\text{CH}_3)_2$  (ref. 9). MS search for the ion  $m/z$  111 indicated the peaks 6, 7, and 8 are  $\alpha,\beta$ -unsaturated aldehydes (Fig. 3). A McLafferty ion is also not possible when a double bond is present at C-4. Such unsaturated aldehydes give

a very significant ion at  $m/z$  85 due to the ion  $\text{CH}_2=\text{CH}-\text{N}=\text{N}^+(\text{CH}_3)_2$  (ref. 8). In fact this ion is the base peak of the spectra of the GC peaks 5, 9 and 10 of Fig. 3.

The partial ozonolysis procedure appears to offer great potential in the structure elucidation of polyunsaturated insect pheromones<sup>3</sup>. It is currently being applied in our laboratory for the structure elucidation of a number of unknown polyunsaturated pheromones. The results will be reported elsewhere.

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