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Note

Separation and double-bond determination on nanogram quantities of aliphatic monounsaturated alcohols, aldehydes and carboxylic acid methyl esters

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The structure identification of unknown natural products (such as fatty acid derivatives, insect pheromones, etc.) often involves the determination of the position of a carbon–carbon double bond in the molecule. Most procedures for locating the position of unsaturation involve derivatization followed by mass spectrometry (MS) and/or gas chromatography (GC). Typical procedures include: ozonolysis[1], methoxymercuration[2–4], silylation[5,6], epoxidation[7–10], oxyselenation[11], reaction with vinyl methyl ether[12,13], and mass spectral fragmentation without derivatization[14–17]. Recently, the stereospecific addition of dimethyl disulfide (DMDS) across the double bond has been used to derivatize 100–1000-ng quantities of monounsaturated acetates found in insect pheromones[18]; this reaction technique had been reported earlier for analysis of 1-mmole amounts of *trans*-alkenes[19].

We describe here an application of the DMDS reaction to mixtures of monounsaturated alcohols, aldehydes, and methyl esters and a modification of the procedure to give increased sensitivity to 10 ng of compound. Analysis is demonstrated for mixtures of monounsaturated compounds which differ only in the position of the double bond or in *cis vs. trans* geometry; these mixtures are not resolved prior to derivatization.

EXPERIMENTAL*

Apparatus

A Finnigan Corp. Model 4000/6000 gas chromatograph–mass spectrometer–computer system was used with a 60 m × 0.25 mm I.D. fused-silica, DB-1 capillary column fitted directly into the ion source of the spectrometer. The spectra were recorded in the electron impact mode at 70 eV, using 1-sec scans of the m/z 40–400 range. Reconstructed gas chromatograms were plotted for total ion abundance *vs.* scan number.

* Mention of a commercial product does not constitute endorsement of that product by the United States Department of Agriculture.

Procedure

A heptane solution (1–20 μl) containing 50–500 ng of the monounsaturated compounds was treated with 50 μl of DMDS and 5 μl of 0.06% iodine in diethyl ether; following overnight reaction at 40°C, the addition of 10–50 μl of heptane and 25 μl of an aqueous solution of sodium thiosulfate (5%) stopped the reaction. A stream of nitrogen was used to concentrate the heptane layer as needed for splitless injection. For reaction with 10–50 ng of compound, only 10 μl of DMDS, 2 μl of iodine solution, and 10 μl of sodium thiosulfate solution were used. The adducts were analyzed by GC–MS soon after preparation to avoid decomposition on storage.

RESULTS AND DISCUSSION

The addition of DMDS across the double bond of a monounsaturated compound is stereospecific[18] and thus the *Z*- and *E*-isomers of a single compound give derivatives which are separable by GC but have the same mass spectrum. Buser *et al.*[18] suggest that the reaction mechanism involves *trans*-addition to give the *erythro*- and *threo*-isomers from the *E*- and *Z*-acetates, respectively. The mass spectrum of each DMDS derivative shows not only the molecular ion (an increase of 94 mass units), but also intense ions corresponding to cleavage between the two methylthio groups, as shown in Fig. 1. The masses of these fragments give the position of the double bond along the chain, as demonstrated by the ions *m/z* 117 and 215 in the spectrum of (*Z*)-11-hexadecenal-DMDS (Fig. 2A), *m/z* 187 and 91 in the spectrum of (*E*)-2-dodecen-1-ol (Fig. 2B), and *m/z* 217 and 145 in the spectrum of methyl (*Z*)-9-hexadecenoate (Fig. 2C). Methyl esters with a C-2 double bond failed to react with DMDS; otherwise derivatization was generally complete for all compounds tested, including those with a C-3 or terminal double bond.

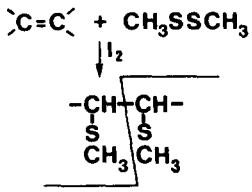
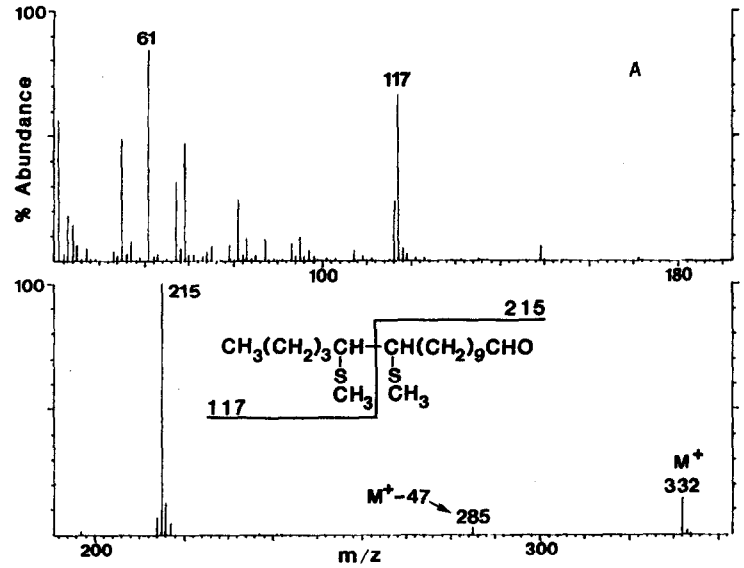


Fig. 1. Addition of dimethyl disulfide (DMDS) to a double bond.

The DMDS adducts gave an increase in the Kováts retention index[20] on the non-polar column of 580–750 units (5.8–7.5 methylene units) as compared to the parent compound, primarily due to the increase of 94 in the molecular weight. For example, the index of 11-dodecen-1-ol acetate increased from 1610 to 2350 (equivalent to 7.4 CH_2 groups) upon derivatization with DMDS. On the other hand, the adducts of compounds with an internal double bond had indexes which were only 580–640 units higher than those of the underivatized structures. The indexes of *cis*-adducts were 15–40 units lower than those of comparable *trans*-compounds, which is consistent with the 1–2.5°C differences in elution temperatures previously reported[18].


100
61
117
A
% Abundance
100
180
100
215
215
CH3(CH2)3CH–CH(CH2)9CHO
S
CH3
S
CH3
117
M+
332
M+-47
285
200
m/z
300

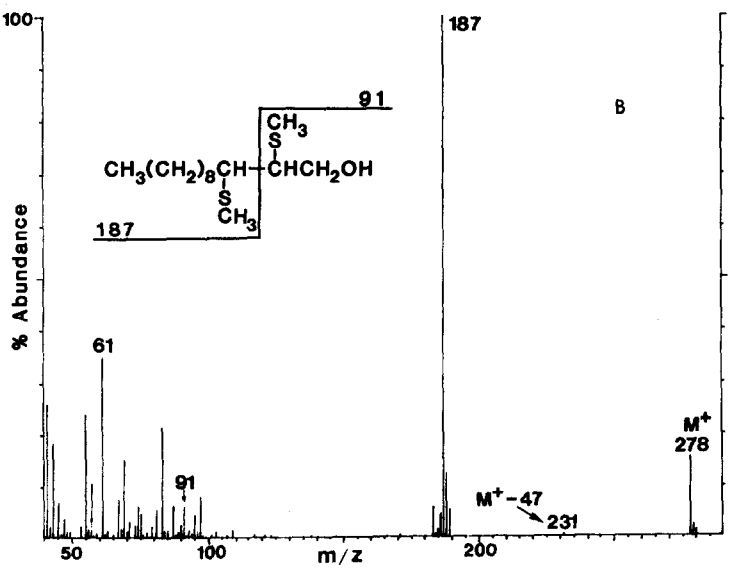

100
187
91
CH3
S
CH3(CH2)8CH–CHCH2OH
S
CH3
187
B
% Abundance
61
M+
278
91
M+-47
231
50
100
m/z
200

Fig. 2.

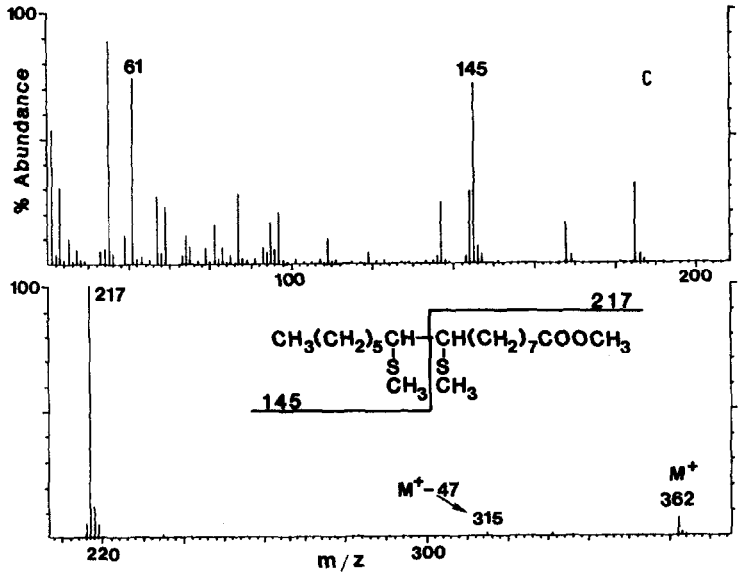


Fig. 2. Mass spectra of DMDS adducts: (A), (*Z*)-11-hexadecenal; (B), (*E*)-2-dodecen-1-ol; (C), methyl (*Z*)-9-hexadecenoate.

Mixtures of monounsaturated compounds are often not resolved on non-polar capillary columns. For example, (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-hexadecen-1-ols were incompletely resolved on the 60 m DB-1 (non-polar) column, but were well separated as their DMDS derivatives (Fig. 3). Similarly, (*Z*)-7- and (*Z*)-9-hexadecenal co-eluted

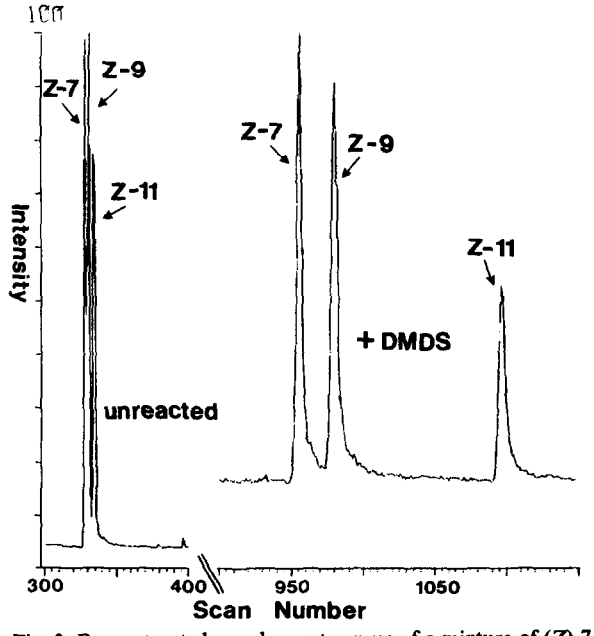


Fig. 3. Reconstructed gas chromatograms of a mixture of (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-hexadecen-1-ols and their DMDS addition products.

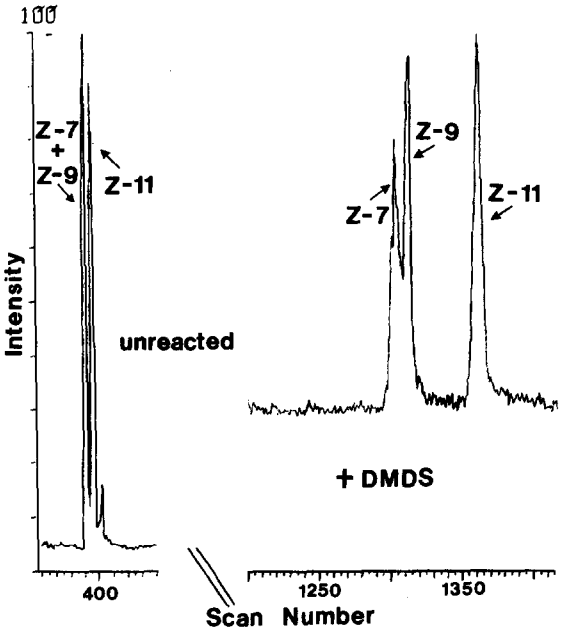


Fig. 4. Reconstructed gas chromatograms of a mixture of (*Z*)-7-, (*Z*)-9-, and (*Z*)-11-hexadecenals and their DMDS addition products.

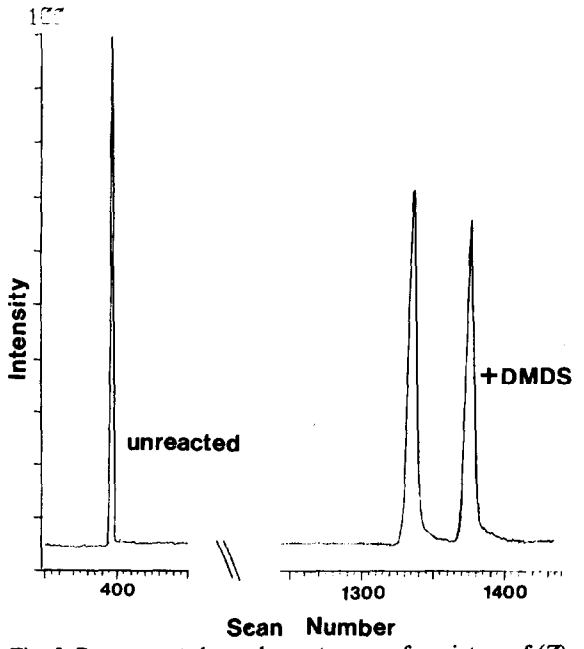


Fig. 5. Reconstructed gas chromatogram of a mixture of (*Z*)- and (*E*)-11-hexadecenal and their DMDS addition products.

while (*Z*)-11-hexadecenal was separated by only 5 scans; the derivatives, however, were sufficiently separated for GC–MS identification of all three isomers (Fig. 4). Also, no separation was apparent for underivatized (*Z*)- and (*E*)-11-hexadecenal, yet the DMDS adducts were resolved by approximately 40 scans (Fig. 5).

The analysis of fatty acids, as found in many natural products, offers another application of this derivatization technique. The addition of DMDS to mixtures of saturated and unsaturated methyl esters of such fatty acids simplifies the analysis by eliminating the peaks normally given by the unsaturated components. In addition,

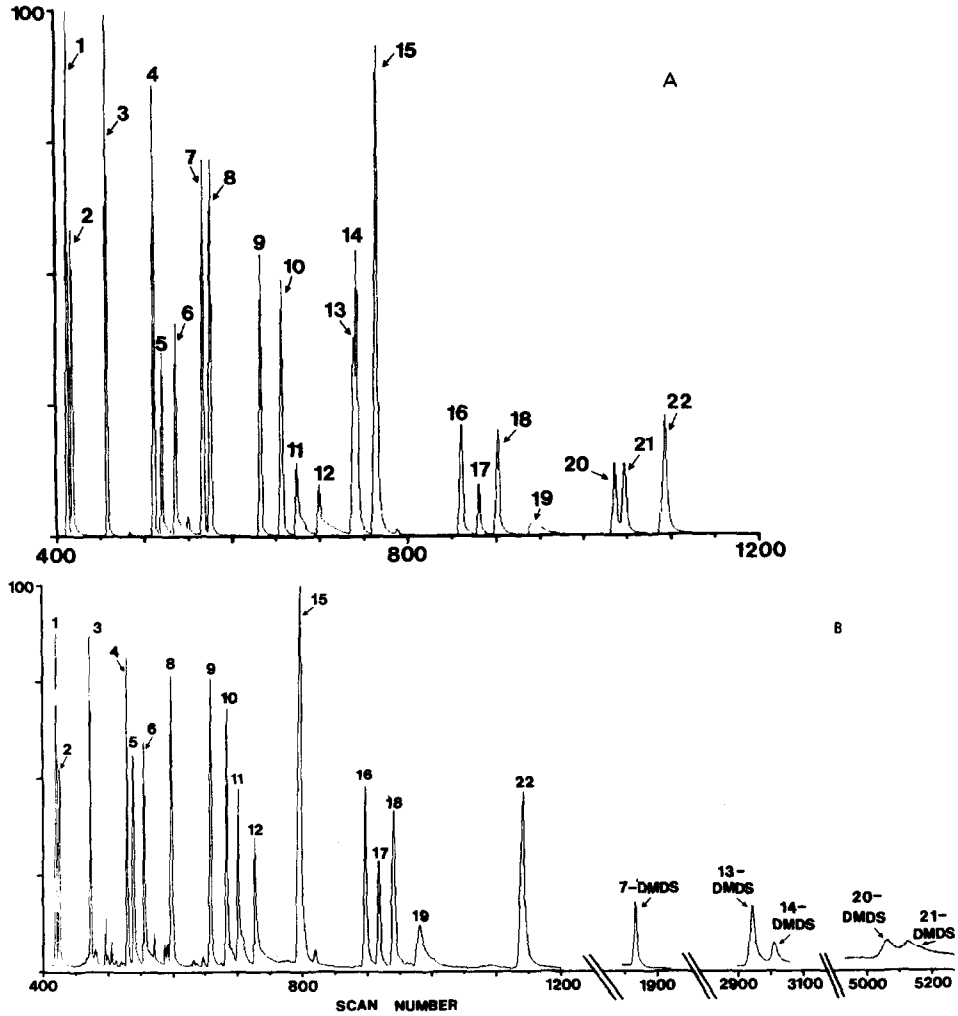


Fig. 6. Reconstructed gas chromatograms of a mixture of methyl esters of bacterial acids. Peaks: 1 = undecanoic; 2 = 2-hydroxydecanoic; 3 = dodecanoic; 4 = tridecanoic; 5 = 2-hydroxydodecanoic; 6 = 3-hydroxydodecanoic; 7 = (*Z*)-9-tetradecenoic; 8 = tetradecanoic; 9 = 12-methyltetradecanoic; 10 = pentadecanoic; 11 = 2-hydroxytetradecanoic; 12 = 3-hydroxytetradecanoic; 13 = (*Z*)-9-hexadecenoic; 14 = (*E*)-9-hexadecenoic; 15 = hexadecanoic; 16 = 14-methylhexadecanoic; 17 = *cis*-9,10-methylenehexadecanoic; 18 = heptadecanoic; 19 = 2-hydroxyhexadecanoic; 20 = (*Z*)-9-octadecenoic; 21 = (*E*)-9-octadecenoic; 22 = octadecanoic. (A), Before derivatization; (B) after reaction to give DMDS derivatives.

the geometric and positional isomers of the derivatized monounsaturated components can be conveniently characterized, as their retention times are much longer than those of the usual saturated components. This is illustrated witha the analysis of a mixture of methyl esters derived from bacterial acids. The mixture includes six hydroxy and five monounsaturated acids as shown in Fig. 6A. Reaction with DMDS displaces the peaks of the monounsaturated components to longer retention times depending on the position and geometry of the CH=CH groups (Fig. 6B). Polyunsaturated esters react with DMDS but their less volatile derivatives were not eluted under the GC conditions used.

The characteristic fragment ions in the spectra of the DMDS adducts were generally found to be so intense (30–100% relative abundance) that about 10 ng of compound could still be detected. Single-ion searches of the total reconstructed gas chromatograms for suspected fragments revealed very low quantities of components in extracts of insect pheromones, where the supply of material was severely restricted. However, the sensitivity can be enhanced to the low nanogram level by a specific ion search for m/z 61 $(CH_3SCH_2)^+$ which is present at 20–50% abundance in the spectra of all DMDS adducts (it must be remembered, that an m/z 61 ion $(CH_3COOH_2)^+$ is also given by underivatized acetates, but generally in less than 10% abundance in the case of unsaturated compounds).

The derivatization of monounsaturated compounds with DMDS offers the chemist another tool for the structure elucidation of natural products in nanogram amounts.

REFERENCES

1 M. Beroza and B. A. Bierl, *Anal. Chem.*, 39 (1967) 1131.
2 D. E. Minnikin, *Lipids*, 10 (1975) 55.
3 G. J. Blomquist, R. W. Howard, C. A. McDaniel, S. Remaley, L. A. Dwyer and D. R. Nelson, *J. Chem. Ecol.*, 6 (1980) 257.
4 R. D. Plattner, G. F. Spencer and R. Kleiman, *Lipids*, 11 (1976) 222.
5 T. Ariga, E. Araki and T. Murata, *Anal. Biochem.*, 83 (1977) 474.
6 T. Murata, T. Ariga and E. Araki, *J. Lipid Res.*, 19 (1978) 172.
7 B. A. Bierl-Leonhardt, E. D. DeVilbiss and J. R. Plimmer, *J. Chromatogr. Sci.*, 18 (1980) 364.
8 M. J. Vacheron, G. Michel and R. Guilluy, *Bull. Soc. Chim. Biol.*, 51 (1969) 177.
9 J. H. Tumlinson, R. R. Heath and R. E. Doolittle, *Anal. Chem.*, 46 (1974) 1309.
10 M. Cervilla and G. Puzo, *Anal. Chem.*, 55 (1983) 2100.
11 G. W. Francis and T. Tande, *J. Chromatogr.*, 150 (1978) 139.
12 R. Chai and A. G. Harrison, *Anal. Chem.*, 53 (1981) 34.
13 R. J. Greathead and K. R. Jennings, *Org. Mass Spectrom.*, 15 (1980) 431.
14 M. Horiike, N. Miyata and C. Hirano, *Biomed. Mass Spectrom.*, 8 (1981) 41.
15 M. Horiike and C. Hirano, *Biomed. Mass Spectrom.*, 11 (1984) 145.
16 M. Horiike and C. Hirano, *Agr. Biol. Chem.*, 46 (1982) 2667.
17 B. A. Leonhardt, E. D. DeVilbiss and J. A. Klun, *Org. Mass Spectrom.*, 18 (1983) 9.
18 H. R. Buser, H. Arn, P. Guerin and S. Rauscher, *Anal. Chem.*, 55 (1983) 818.
19 G. W. Francis and K. Veland, *J. Chromatogr.*, 219 (1981) 379.
20 E. Kováts, *Advan. Chromatogr.*, 1 (1966) 229.