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## Note

# Methylation of ω-oxocarboxylic acids with diazomethane: effect of solvent on by-product formation

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An essential prerequisite for the analysis of fatty acids by gas chromatography is their quantitative transformation into more volatile, less polar derivatives. Many methods for esterification have been published. Most are based on diazomethanolysis[1], acid-catalysed methanolysis with boron trifluoride–methanol[2] or hydrochloric acid–methanol[3]. Diazomethane is a particularly mild agent for methylation but its explosiveness and toxicity have discouraged the wider use it merits. However, when proper precautions are taken, the small amount involved minimizes these deterrents. Methylation is currently carried out by the introduction of gaseous diazomethane or by the addition of an ethereal solution of diazomethane to a solution of the sample[4]. The reaction proceeds at room temperature, or at 0°C, requires only a short period of time and the rate of methylation can be increased by adding various catalysts, such as methanol or water[5]. The main advantage of this method is, however, that the products do not need to be isolated.

The high reactivity of diazomethane, which is one of the reasons for its use as a derivatizing agent in organic acid analysis, can be at the same time its limitation. Diazomethane not only attacks the acidic hydrogens of acids, phenols or enols[6], but may also react with carbonyl compounds[7] and olefinic bonds[8].

ω-Oxocarboxylic acids theoretically can be converted by diazomethane into their homologous keto methyl esters. These by-products make the recognition of the original compounds rather difficult. In this paper we describe a study of the experimental conditions which might avoid undesired side reactions.

## EXPERIMENTAL

### *Reagents and materials*

All organic solvents were high-performance liquid chromatographic (HPLC) grade, from Romil Chemicals, N-methyl-N-nitroso-*p*-toluenesulphonamide (NTSA) and heptanal from Fluka, 2-(2-ethoxyethoxy)ethanol from Kodak, formic and acetic acids from Merck, boron trifluoride in methanol (14%) from Supelco, hexanal, octanal and decanal from Sigma and 10-undecenoic acid from Aldrich.

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*Synthesis*

ω-Oxononanoic and ω-oxodecanoic acids were synthesized from oleic and 10-undecenoic acids respectively, by hydroxylation with hydrogen peroxide–formic acid[9], followed by oxidation of the dihydroxy acid with potasium periodate, using aqueous ethanol as the solvent[10]. ω-Oxo-9-dodecenoic acid was prepared from 12,13-epoxy-9(*Z*)-octadecenoic acid (from Vernonia seed oil). The steps were: acetylation to open the epoxy group, saponification to obtain the diol[11], followed by oxidation with periodic acid[10]. ω-Oxocarboxylic acids were methylated with boron trifluoride–methanol, purified by silica gel thin-layer chromatography (TLC) and identities checked by IR and gas chromatography–mass spectrometry (GC–MS).

*Esterification of ω-oxocarboxylic acids and reaction of aldehydes with diazomethane*

As standard solutions, 3 mg of each ω-oxo acid or aldehyde were separately dissolved in 1 ml of an appropriate solvent. Diazomethane was prepared from NTSA according to the procedure of Cohen[12]; gaseous diazomethane was passed through each standard solution until a yellow tinge became visible against a white background. The reaction was allowed to continue at 0°C (cooling with ice–water) for 5 min, and then the excess of diazomethane was consumed by adding acetic acid diluted in diethyl ether.

*GC and GC–MS*

The gas chromatograph used was a Konik KNK-3000, with a flame ionization detector. A fused-silica column, 25 m × 0.22 mm, coated with OV-1/OV-101 BP, 0.25-μm film (SGE) was used; nitrogen was used as carrier gas. The column temperature was programmed from 80 (10 min) to 180°C (5 min) at 3°C/min; injector and detector temperatures, 250°C.

GC–MS analyses were conducted with a Konik KNK-2000 gas chromatograph interfaced, via an open coupling system, to an AEI-MS-30SB-VG mass spectrometer. The GC conditions were similar to those mentioned above, except that helium was used as carrier gas. The MS conditions were as follows: ionization by electron impact, 70 eV; accelerating voltage, 4 kV; emission current, 100 μA; ion source temperature, 220°C. The data were processed with a VG 11/250 data system.

## RESULTS AND DISCUSSION

Simple aliphatic aldehydes react with diazomethane, usually under very mild conditions, to yield the homologous ketone along with, in most cases, the epoxide[7]. Initial work was directed at determining solvent effects on the reaction of diazomethane with the aldehydes hexanal, heptanal, octanal and decanal. Diethyl ether, hexane, dichloromethane, diethyl ether–methanol (9:1) and methanol were used as the solvents. A typical gas chromatogram of the reaction products of heptanal, in diethyl ether containing 10% methanol, is shown in Fig. 1. The electron-impact mass spectra of these compounds are presented in Fig. 2.

Peak 1, scan 27, was heptanal, identified by comparison of the mass spectrum of the authentic compound. In the mass spectrum of peak 2, scan 43, the presence of the base peak at *m/z* 43, the intense rearrangement fragment at *m/z* 58[13] and the molecular weight at 128 are compatible with hexyl methyl ketone. In the mass spectrum of peak 3,

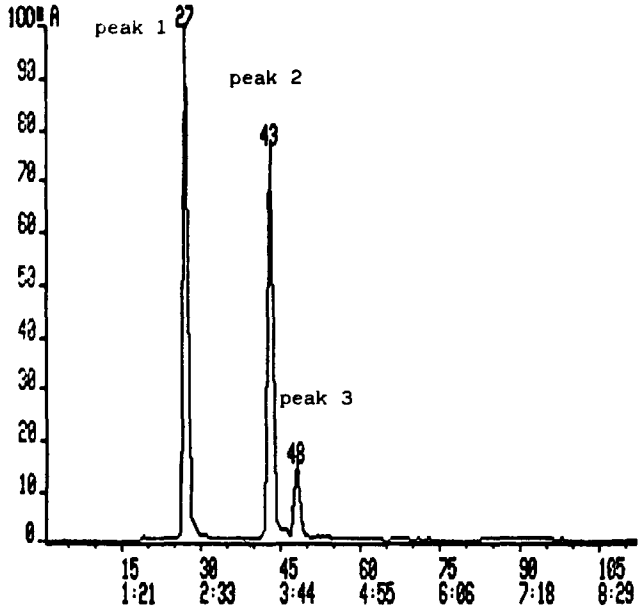


Fig. 1. Reconstructed gas chromatogram of the products of reaction between heptanal and diazomethane, in diethyl ether contaning 10% methanol. Conditions as described in the Experimental section. Total ion abundance plotted *vs.* scan number (upper line) and retention time (lower line).

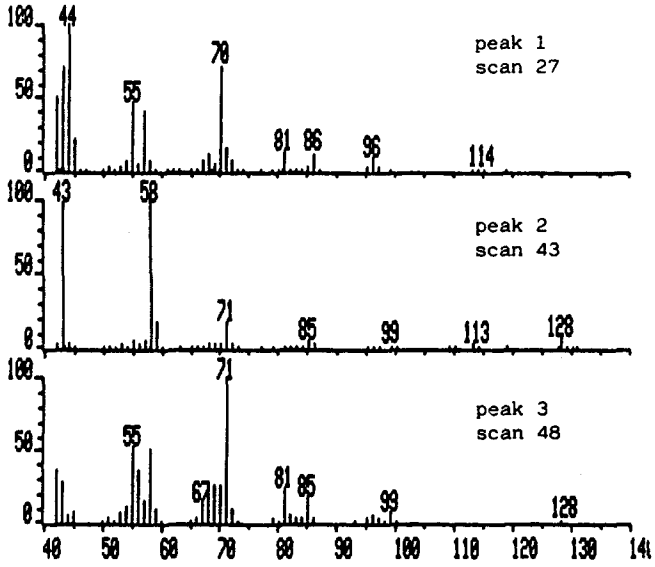


Fig. 2. Mass spectra of the compounds in peak 1, scan 27, peak 2, scan 43 and peak 3, scan 48 from the gas chromatogram shown in Fig. 1.

TABLE I
EFFECT OF SOLVENT ON THE REACTION OF HEPTANAL WITH DIAZOMETHANE AT 0°C FOR 5 min

| *Solvent* | *Heptanal (%)* | *Hexyl methyl ketone (%)* | *1,2-Epoxyoctane (%)* |
|---|---|---|---|
| Diethyl ether | 100 | – | – |
| Hexane | 100 | – | – |
| Dichloromethane | 100 | – | – |
| Diethyl ether–methanol (9:1) | 58.9[a] | 34.2 | 6.8 |
| Methanol | 26.8[a] | 49.2 | 23.9 |

[a] Average of three replicates of three determinations.

scan 48, the presence of the base peak at *m/z* 71 (hydrogen rearrangement with concomitant γ-scission), ions of *m/z* 58 and 42 (McLafferty rearrangement)[13] and the molecular weight at 128 suggest the structure of 1,2-epoxyoctane. When identical reactions were performed with hexanal, octanal or decanal, their respectives ketones and epoxides were obtained.

The relative percentages of products obtained with heptanal, in each solvent, are summarized in Table I. The results of these experiments demonstrate that inert solvents, under the assayed conditions, exert an inhibiting effect on the by-product formation, whereas methanol participates in the reaction. Similar results were obtained with hexanal, octanal and decanal. Therefore the production of ketone and

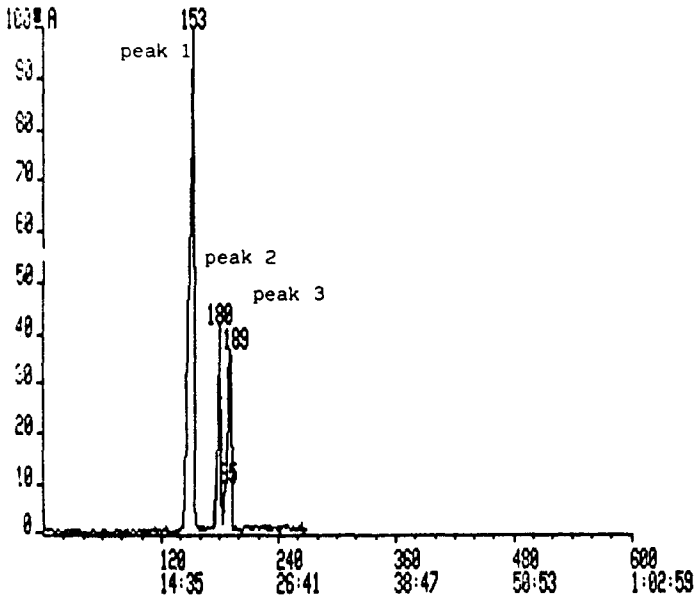


Fig. 3. Reconstructed gas chromatogram of the products of reaction between ω-oxodocecanoic acid and diazomethane, in diethyl ether containing 10% methanol. Conditions as described in the Experimental section. Total ion abundance plotted *vs.* scan number and retention time.

epoxide can be avoided by using diethyl ether, hexane or dichoromethane as the solvent.

Schlenk and Gellerman[5] have demonstrated that the esterification of long-chain fatty acids is instantaneous when gaseous diazomethane is introduced into a solution of the acids in diethyl ether containing 10% methanol. The reaction products of $\omega$-oxodecanoic acid with diazomethane, in this solvent, were investigated. GC–MS analysis shows the presence of three compounds (Fig. 3) whose electron-impact mass spectra are presented in Fig. 4.

The mass spectrum of peak 1, scan 153, shows fragment ions of *m/z* 74, 87 and 169 ($M^+ - CH_3O$) characteristic of a methyl ester. The molecular ion peak expected (*m/z* 200) is absent. Those of *m/z* 157 ($M^+ - CHOCH_2$) and 172 ($M^+ - CO$) are ascribed to the presence of a formyl group. Therefore MS ions were consistent with methyl $\omega$-oxodecanoate. The mass spectrum of peak 2, scan 180, presents characteristic fragments of a methyl ester, *m/z* 74, 87 and 183 ($M^+ - CH_3O$), and diagnostic peaks of an acetyl group at *m/z* 43, 58 and 157 ($M^+ - CH_3COCH_2$)[13]. The molecular ion is absent, but ions at *m/z* 183 and 157 suggest a molecular ion at *m/z* 214, therefore the compound was identified as methyl 10-oxoundecanoate. In the mass spectrum of peak 3, scan 189, fragments of a methyl ester (*m/z* 74 and 87) again appear and the fragments at *m/z* 71, 58 and 42[13] suggest the presence of a 1,2-epoxy group; the compound was tentatively identified as methyl 10,11-epoxyundecanoate.

The relative percentages of ketone and epoxide produced were 14 and 10%, respectively. In our esterification conducted in diethyl ether–methanol (9:1) with diazomethane the error in measuring the actual and desired ester product as accompanied by the by-products is greater than the errors commonly observed in measuring peak areas or in detector calibration for specific responses in GC itself.

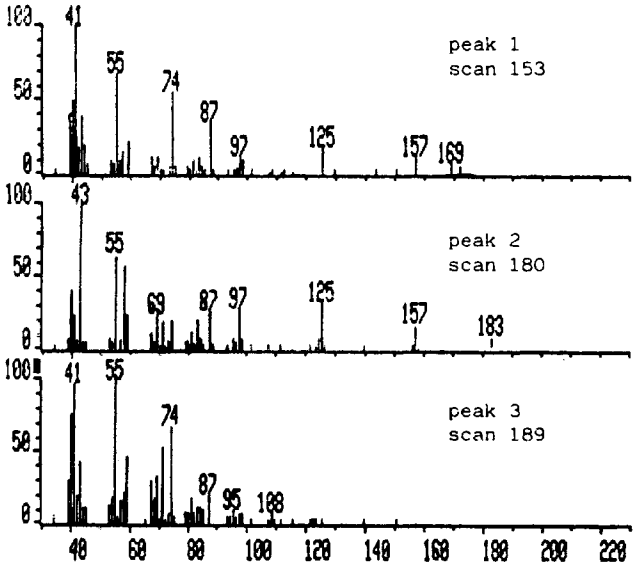


Fig. 4. Mass spectra of the compounds in peak 1, scan 153, peak 2, scan 180 and peak 3, scan 189 from the gas chromatogram shown in Fig. 3.

Therefore, we tried to see whether it was possible to stop the reaction of diazomethane with ω-oxocarboxylic acids at methyl ester. When esterification of ω-oxodecanoic acid was carried out in diethyl ether, hexane or dichloromethane, diazomethane gave quantitative recovery of the methyl ester and the formation of ketone and epoxide was negligible (less than 1%). Similar results were obtained with ω-oxononanoic acid and ω-oxo-9-dodecenoic acid.

Therefore, in the esterification of ω-oxocarboxylic acid with diazomethane, if the only desired product is the methyl ester the use of methanol must be avoided.

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