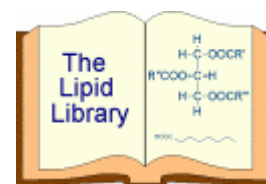


DIMETHYL DISULPHIDE ADDUCTS FOR MASS SPECTROMETRY



Abstract: *Dimethyl disulphide derivatives of fatty acids (methyl esters) are easy to prepare and a number of interesting applications have been described for the analysis of several types of fatty acid by mass spectrometry.*

In another article in this series, I described the use of dimethyl disulphide (DMS) derivatives for the location of double bonds by mass spectrometry. It involves simple addition of dimethyl disulphide across a double bond, a reaction catalysed by iodine, as shown in Figure 1 [1].

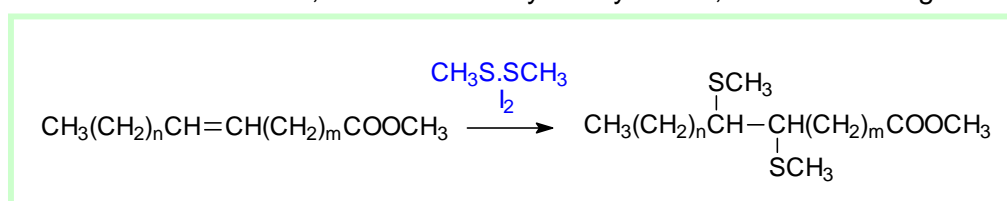


Fig. 1. Reaction of methyl oleate with dimethyl disulphide.

It has become evident that the reaction may have much more to it than I had at first thought, and a number of interesting publications describing its use have appeared. I described these in a substantial review elsewhere, and this publication should be consulted for many of the relevant references [2]. Indeed, there have been more than 70 publications using aspects of this method for fatty acids alone, and many more for pheromones, hydrocarbons, terpenes, *etc.*

Preparation and Application to Monoenes

A single reagent and a one-step reaction is required for the preparation, which is carried out simply by dissolving the fatty acid derivative (usually the methyl ester) in dimethyl disulphide (sometimes with an additional solvent) with a trace of iodine; after 24 hours at room temperature, the required products are obtained by a simple washing procedure. Some residual unchanged fatty acid may remain, but this elutes well ahead of the product when this is subjected to gas chromatographic (GC) analysis. Indeed, the adduct adds substantially to the molecular weight of the original ester, and tends to elute at a temperature about 40°C higher than the latter from a GC column containing a non-polar silicone phase.

As I explained elsewhere on this site in more detail (with sample mass spectra), the reaction is especially useful for monoenoic fatty acids. The mass spectrum of the dimethyl disulphide (DMS) adduct of methyl oleate gives an abundant molecular ion, then cleavage occurs between the carbons that originally constituted the double bond to yield two substantial fragment ions that serve to locate it. The procedure has been used to identify a large number of monoenoic fatty acids from natural sources.

Adduct formation has been shown to be entirely stereospecific, presumably by *trans* addition, so that *threo*- and *erythro*- derivatives are formed from *cis*- and *trans*-isomers respectively. Although the different geometrical isomers have indistinguishable mass spectra, they are eluted separately from GC columns containing either polar or non-polar phases, with that derived from the *cis*-isomer eluting first. There may be potential, therefore, to use this as an additional

method for determining *trans* fatty acids. Indeed, it has been used to advantage in conjunction with hydrazine reduction (see below) to determine the configurations of particular double bonds in polyunsaturated fatty acids.

Also, DMDS adducts can be resolved better than the unchanged esters on GC columns, and this property has been used to separate and quantify petroselinic, oleic and vaccenic acids in seed oils [3]. While simpler alternatives may be available for this specific purpose, it may be worth keeping it mind for confirmation or for other difficult analyses.

Applications to Diunsaturated Fatty Acids

Dienoic fatty acids present more of a problem than monoenes for the technique. The considerable increase in molecular weight means that rather high temperatures are required for GC analysis. Also, when the two double bonds are in close proximity, complications can arise in the reaction with dimethyl disulphide. There is no problem when double bonds are separated by more than four carbon atoms, but this is a relatively rare occurrence in nature. However, 9,15-octadecadienoic acid (with four methylene groups between the double bonds) from mango pulp was characterized simply as the *bis*-DMDS derivative [4]. Similar types of dienoic fatty acids have been characterized from sponges in Carballeira's laboratory in the same way.

When the double bonds are closer together, a variety of products is possible. Most natural dienoic fatty acids have methylene-interrupted double bonds. When dimethyl disulphide was reacted under mild conditions (30 minutes reaction, 35°C) with methyl linoleate, only one double bond reacted [5]. Thus, an equimolar mixture of methyl 9,10-*bis*(methylthio)octadec-12-enoate and methyl 12,13-*bis*(methylthio)octadec-9-enoate was formed, and again distinctive mass spectra were obtained which permitted location of the double bonds. This technique has also been used with ether lipids.

When higher temperatures (up to 60°C) and longer reaction times (40 hours) were employed, a second mole of dimethyl disulphide was added, and cyclization occurred giving heterocyclic compounds with thietane, tetrahydrothiophene and tetrahydrothiopyran structures (4-, 5- and 6-membered rings), as illustrated in Figure 2 [6,7].

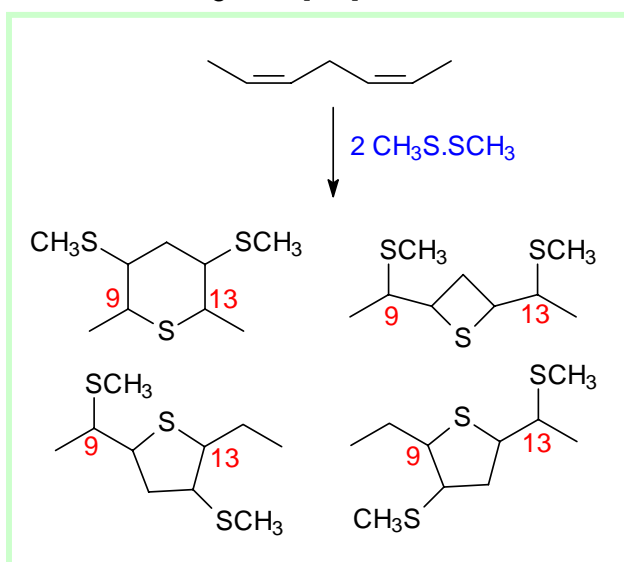


Fig. 2. Part structures of reaction of linoleate with two moles of dimethyl disulphide.

The reaction has been studied in a number of laboratories, but mainly in that of Carballeira. These heterocyclic compounds can give characteristic and diagnostic spectra also, so that the

technique continues to have some practical value. In one report, only thetane formation was observed, but in more systematic studies four distinct products were obtained in proportions that varied according to the reaction conditions [6]. Di-*cis* and di-*trans* forms of linoleate gave products with distinct stereochemistry and different chromatographic properties, suggesting again that the technique might have value for determining the geometry of double bonds in such fatty acids [7].

Long-chain fatty acids with 5,9-diene systems are common constituents of marine sponges. Reaction of these with dimethyl disulphide under appropriate conditions gives a 5-membered cyclic thioether substituted with two alkyl chains, each containing a methylthio group on the carbons immediately adjacent to the ring as illustrated in Figure 3 [5,8].

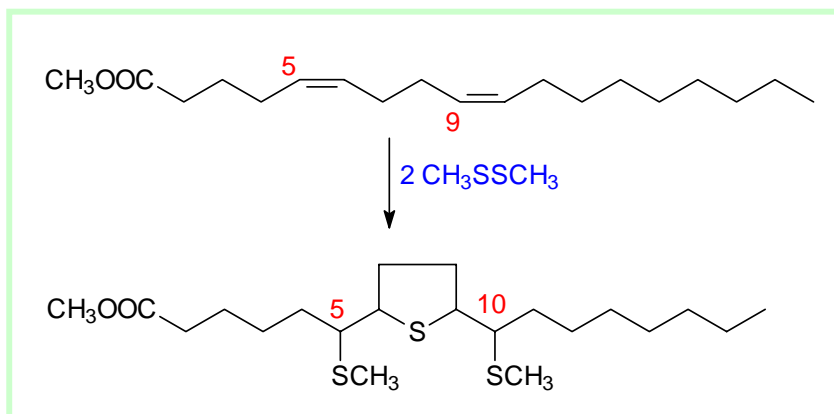


Fig. 3. Reaction of 5,9-18:2 with two moles of dimethyl disulphide.

These compounds also give characteristic mass spectra permitting location of the double bonds, and many different demospongiac acids of this type have been identified in this way, including some containing bromine atoms and methyl branches in addition to the 5,9-double bond system.

Applications to Polyunsaturated Fatty Acids

It is also possible to generate monoenes from polyunsaturated fatty acids in order to use the reaction. Thus, heneicosapentaenoic acid (21:5(n-3)) was from eel lipids was first isolated by silver ion TLC, before being subjected to partial hydrogenation with hydrazine to a mixture of monoenes; these were then converted to DMDS derivatives for analysis by GC-MS [9]. 5,9,12-18:3 and 5,9,12-17:3 fatty acids have been identified by this means from such odd natural sources as mites and slime moulds. Some fatty acids in seed oils of pine species also have this distinctive structure, and they are presently the subject of some interest to nutritionists. Again, the technique may be of some value here.

Limitations

I did not find DMDS derivatives suitable for determining the position of the double bond in a cyclopentene ring, although confirmation of the positions of double bonds in the aliphatic chain of natural fatty acids with this structural feature was obtained. It has been used successfully for hydroxy, methoxy, branched-chain and benzene ring-containing fatty acids with one double bond.

While conjugated fatty acids dienes and acetylenic fatty acids cannot be analysed as dimethyl disulfide adducts, a related reaction with ethanethiol can be used.

Thus, the reaction of dimethyl disulphide with double bonds may be a simple one, but it is keeping lipid analysts gainfully employed in a number of interesting ways. While it can be employed with care to polyenes, there is no doubt that it works best with monoenes.

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This article was first published in *Lipid Technology*, **9**, 17-19 (1997), and it has now been substantially re-written. For more detailed information, go to our web pages on mass spectrometry of fatty acids.

W.W. Christie

Scottish Crop Research Institute (and Mylnfield Research Services
Lipid Analysis Unit), Invergowrie, Dundee (DD2 5DA), Scotland

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