FATTY ACIDS AND MASS SPECTROMETRY


In the first contribution dealing with this topic, I used an analogy in which mass spectrometry was compared to demolishing and re-assembling a brick wall. If we take it apart a few bricks at a time, it is possible to reassemble it easily. We will know the correct dimensions and where any door or window should be placed. With a fatty acid derivative, in comparison, we need to confirm that it is indeed a fatty acid, determine the molecular weight and then locate any double bonds or other functional groups. Methyl esters are only suitable in a few circumstances, for example in simple samples where no unusual fatty acids are expected. However, there are invaluable alternative derivatives, all of which contain nitrogen atoms in close proximity to the carboxyl group. With such compounds the nitrogen, rather than the aliphatic chain (as with methyl esters), carries the charge when the molecule is ionized in the mass spectrometer. Rather uniform fragmentation then occurs along the aliphatic chain, and with a little experience it is easy to pick out functional groups such as double bonds, methyl branch points or cyclic structures.

Three types of fatty acid derivative have been used most often for structure determination: pyrrolidides, 3-pyridylcarbinol (incorrectly termed ‘picoliny1’ in most publications) esters and 4,4-dimethyl-oxazoline (DMOX) derivatives (Figure 1). Pyrrolidides were the first derivative of this type to be described in 1971, but they have been overtaken by alternatives, presumably because their chromatographic properties are less than ideal. 3-Pyridylcarbinol esters have better mass spectrometric properties than pyrrolidides, and DMOX derivatives are better for analysis by gas chromatography (GC), so these two are now favoured in most laboratories. However, pyrrolidides derivatives should not be forgotten. Alternative derivatives have occasionally been proposed, but they are unlikely to be accepted without a substantial body of sample spectra for comparison or reference purposes.

In addition to the review articles cited in the first part of this topic, definitive reviews on the use of pyrrolidides [1], 3-pyridylcarbinol esters [2] and DMOX derivatives [3] have been published. Detailed protocols for the preparation of these derivatives are available elsewhere on this site.

Please note that I have not considered the use of mass spectrometry for quantitative analysis of fatty acids here. Then quite different problems arise and methyl ester derivatives may be as good as any.
other for the purpose. I will leave that topic for someone else to discuss, but readers should be aware that GC with flame-ionization detection is by far the simplest approach to quantitative analysis.

3-Pyridylcarbinol ('picolinyl') Esters

3-Pyridylcarbinol esters can be prepared best from free fatty acids so it is often necessary to first hydrolyse an intact lipid sample or methyl ester. The original method involved dissolving the fatty acid in an excess of thionyl chloride to form the acid chloride, which was then reacted with a 1% solution of 3-hydroxymethylpyridine in acetonitrile to form the 3-pyridylcarbinol ester for direct analysis by GC-MS [4]. Alternatively, a mild quantitative method may be preferred that involves the formation of an imidazolide by reacting the fatty acid with 1,1'-carbonyldiimidazole in dichloromethane prior to reaction with the 3-pyridylcarbinol reagent in triethylamine in the presence of 4-pyrrolidinopyridine as a catalyst [5]. A newer method no permits direct preparation from methyl esters or intact lipids [6].

As an example, the mass spectrum of the 3-pyridylcarbinol ester derivative of γ-linolenic acid (6,9,12-18:3) is illustrated in Figure 2. There are large ions at \( m/z = 92, 108, 151 \) and 164, which contain the pyridine ring and various elements of the carboxyl group and serve mainly to indicate that the compound is indeed a 3-pyridylcarbinol ester. It is more instructive to consider the high molecular weight part of the spectrum. There is an abundant molecular ion (\( m/z = 369 \)), useful confirmation that we have a C_{18} fatty acid with three double bonds. Then there is a uniform series of ions 14 atomic mass units (amu) apart, representing loss of each successive methyl and methylene group from the terminal end of the molecule, until we reach the ion at \( m/z = 298 \). There is a gap of 26 amu for the carbons constituting the terminal double bond to \( m/z = 272 \), a further gap of 14 amu for the methylene group at carbon-11, then another gap of 26 amu between \( m/z = 234 \) and 258, a gap of 14 amu for the methylene group at carbon-8, and so forth. The double bond nearest to the carboxyl group is not always easily spotted from first principles, but with a little experience it is not too difficult to define. Of course, it always helps to have access to spectra of standards for comparison purposes, as in the Archive pages of this website, for example.

![Figure 2. Mass spectrum of 3-pyridylcarbinyl 6,9,12-octadecatrienoate.](image-url)
4,4-Dimethyloxazoline (DMOX) Derivatives

DMOX derivatives can be prepared simply by reacting the free fatty acid (or the methyl ester or even an intact lipid) with 2-amino-2-methyl-1-propanol (AMP) in a micro-reaction vial at 180°C for 16 hours in a nitrogen atmosphere [7]. However, we have observed that the product must be stored under strictly anhydrous conditions otherwise partial hydrolysis can occur.

The mass spectrum of the DMOX derivative of oleic acid is illustrated in Figure 3. In this instance the ions at \( m/z = 113 \) and 126 confirm that we have indeed formed the DMOX derivative. Again, there is a clear molecular ion at \( m/z = 335 \), followed by gaps of 14 amu for the loss of each successive methylene group (\( m/z = 320, 306, 292, 278, \text{ etc} \)), until we find a gap of 12 amu which is indicative of the presence of the double bond, between \( m/z = 196 \) and 208. To locate this precisely, we must use the “12 mass rule”, *i.e.*

> “if a there is an interval of 12 amu between the most intense peaks of clusters of ions containing n and n-1 carbon atoms, there is a double bond between carbon n and n+1 in the molecule”.

This may seem rather convoluted, but works remarkably well in practise. Other functional groups, such as branch points or ring structures, are located as with 3'-pyridylcarbinol esters. However, problems can arise when functional groups are near either end of the molecule, when it helps to have access to spectra of standards.

**Which is Best?**

How do we decide when to use 3-pyridylcarbinol esters and when DMOX derivatives for structural analysis of fatty acids? DMOX derivatives have excellent properties for gas chromatography so can be easily resolved on all the common polar stationary phases used in GC analysis. In my opinion, the mass spectral characteristics in the high mass range are not as good as with 3-pyridylcarbinol esters in general, although DMOX derivatives do appear to be especially useful with some fatty acids with internal ring structures and cyclic and conjugated double bonds. 3-Pyridylcarbinol esters require much higher temperatures than the equivalent DMOX or methyl ester derivatives to elute them from GC columns, and at first they could only be analysed on non-polar stationary phases. However, we have used the thermally stable polar BPX-70™ and Supelcowax 10™ columns with some success with 3-pyridylcarbinol esters of fatty acids with up to 22 carbon atoms.
Pyrrolidides should not be forgotten. In spite of the marked differences in structure, pyrrolidides have exactly the same molecular weight as the corresponding DMOX derivatives and they give very similar fragmentation patterns in mass spectrometry, although the diagnostic ions are often of lower abundance. They can be preferable to DMOX derivatives for fatty acids with terminal functional groups. Their GC properties are intermediate between those of 3-pyridylcarbinol esters and DMOX derivatives.

Newer MS methods, for example those involving acetonitrile-chemical ionization, look interesting as they permit both the location of double bonds and their geometry (cis/trans), but they are more limited in their potential range of applications than the derivatives described above.

Finally in answering the question of which is best, I prefer not to take a rigid stance. 3-Pyridylcarbinol esters have often been my first choice when I know that I am facing samples containing novel fatty acid structures, although I almost always prepare DMOX derivatives for confirmation or for rapid screening of straightforward samples. Indeed, I often prepare and analyse methyl esters and pyrrolidides also, partly as this may permit better chromatographic resolution of some components, and partly to obtain reference spectra. My firm belief is that the various types of derivative should be considered as complementary to each other - not simply as alternatives.

References


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