Determination of the stuctures of fatty acids

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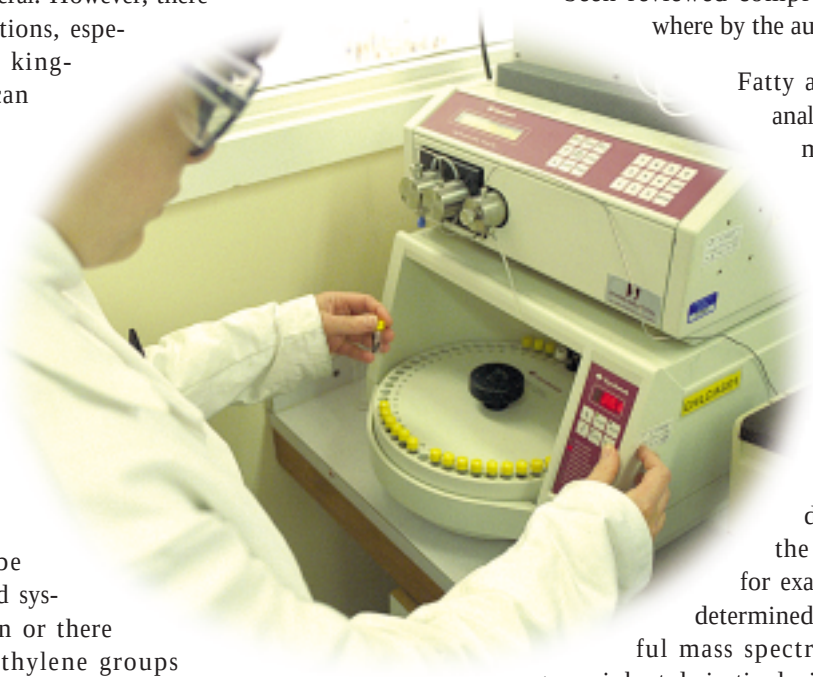
Fatty acids are the basic building blocks of all lipids. The text books state that the common fatty acids of animal and plant origin consist of even-numbered linear chains of 16 to 22 carbon atoms, with zero to six double bonds of the *cis* configuration; polyunsaturated fatty acids have methylene-interrupted double bond systems in general. However, there are countless exceptions, especially in the plant kingdom. Fatty acids can be both odd- and even-numbered, with two to almost a hundred carbon atoms. Double bonds can have either *cis* or *trans* geometry, and acetylenic and allenic bonds occur; these can be part of a conjugated system of unsaturation or there can be several methylene groups between them. Also, there can be a host of further structural features, including branch points, alicyclic or heterocyclic rings, oxygenated functions, and many more. The exact number of different fatty acids of natural origin has never been tabulated, but it must be well over a thousand, and innumerable that are man-made can be added to the list.



The fatty acid components of a lipid determine, to a large extent, its physical and often its biological properties. It must be assumed that nature does not behave randomly and must synthesise each of the distinctive fatty acids that may occur in organisms for good biological reasons. In addition, fatty acids formed as by-products in industrial or related processes may have biological effects on consumers. Therefore, it is important that we have rapid unequivocal methods for determination of fatty acid structure. When pure components can be isolated for study, a host of chemical degradative and spectroscopic methods are available. The real challenge is to identify minor components unequivocally among complex mixtures. Then, gas chromatography-mass spectrometry comes to the fore, and this, together with suitable derivatization methods and ancillary chromatographic techniques, has greatly simplified a complex task. The topic has been reviewed comprehensively elsewhere by the authors.[1,2]

Fatty acids are usually analysed by gas chromatography as methyl esters, but the mass spectra of such derivatives rarely contain ions indicative of structural features; the positions of double bonds in the aliphatic chain, for example, cannot be determined. To obtain useful mass spectra, the carboxyl group is best derivatized with a reagent containing a nitrogen atom. When the molecule is ionized in the mass spectrometer, the nitrogen atom rather than the alkyl chain carries the charge, and double bond ionization and migration is minimized.

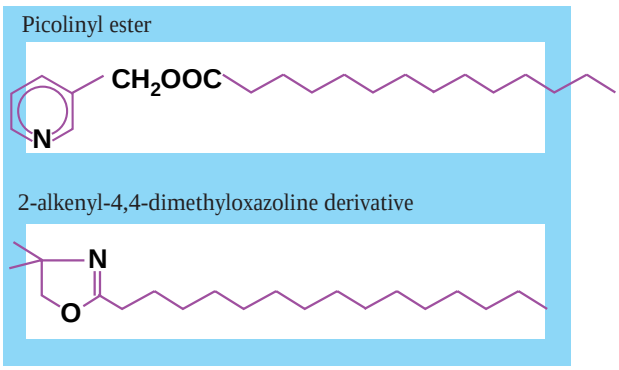


Figure 1 Chemical structures of picolinyl esters and 4,4-dimethyloxazoline (DMOX) derivatives.

Radical-induced cleavage occurs evenly along the chain and gives a series of relatively abundant ions of high mass from the cleavage of each carbon-carbon bond. When a double bond or other functional group is reached, diagnostic ions tend to occur. Most analysts now prefer either picolinyl (3-hydroxymethylpyridinyl) ester or 4,4-dimethyloxazoline (DMOX) derivatives (Fig. 1). Both have their merits in mass spectrometry terms, and each has advantages for particular types of fatty acid; they are best considered as complementary. In our experience, picolinyl esters are by far the best for branched-chain and cyclopropane fatty acids, while DMOX derivatives have advantages for conjugated double bonds and other cyclic fatty acids. In most other applications, they are similar.

As an example, the mass spectra of the picolinyl ester and DMOX derivatives of erucic (13-docosenoic) acid, a major component of rapeseed and other brassica seed oils, is illustrated in Figure 2. That of the picolinyl ester is typical in that it has prominent ions at *m/z* = 92, 108, 151 (the McLafferty ion) and 164, which are all fragments about the pyridine ring. The molecular ion (*m/z* = 429) is easily distinguished and it is always odd-numbered, because of the presence of the nitrogen atom, but most other ions are even numbered. In interpreting such spectra, the simplest approach is to start with the molecular ion and progress downwards, as if one were unzipping the molecule one methylene group at a time. Thus, there

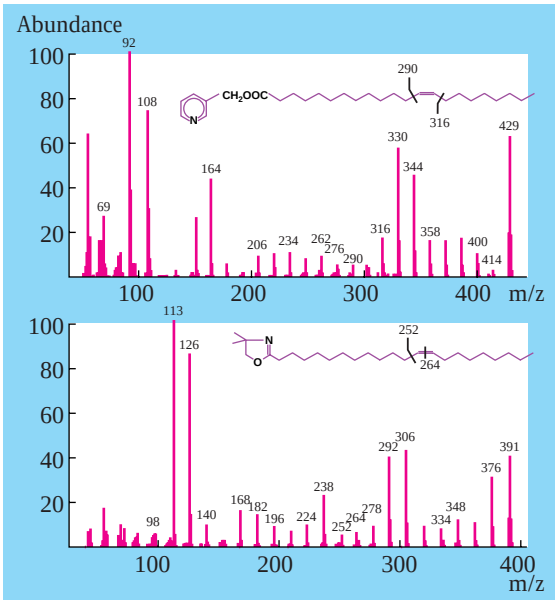


Figure 2 Mass spectra of picolinyl ester (upper) and dimethyloxazoline derivative (lower) of erucic acid.

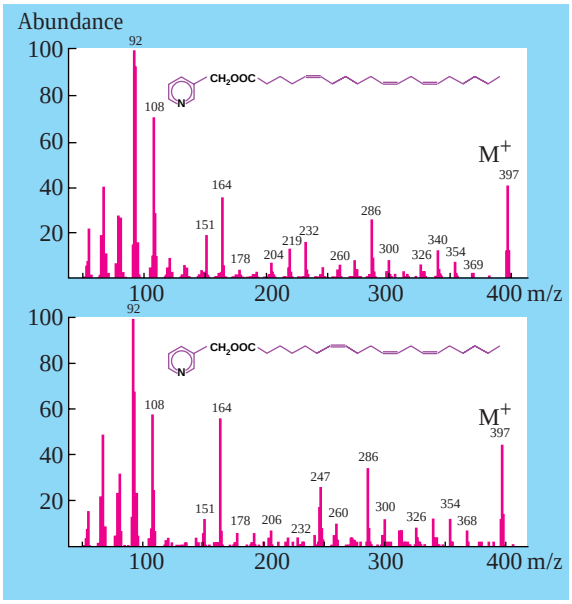


Figure 3 Mass spectra of picolinyl ester of 5,11,14-eicosatrienoic acid (upper) and of 7,11,14-eicosatrienoic acid (lower).

is loss of a methyl group to *m/z* = 414, a further methylene to *m/z* = 400, and so forth. When the double bond is reached, there is a gap of 26 amu between ions at *m/z* = 290 and 316, that, amongst other features, serves to locate the double bond.

The mass spectrum of the DMOX derivative of erucic acid and other fatty acids invariably has prominent ions at *m/z* = 113 and 126, the former representing cleavage between carbons 2 and 3 (the McLafferty ion). In this instance, the molecular ion is at *m/z* = 391. There are many similar features to the spectrum of the picolinyl esters, but in this instance the double bond is located by a gap of 12 amu between ions at *m/z* = 252 and 264 (carbons 12 and 13).

The efficacy of this methodology with closely related isomers is illustrated by Figure 3, which contains mass spectra of picolinyl ester derivatives of 5,11,14- and 7,11,14-eicosatrienoic acids, common components of Gymnosperm seed lipids (*Pinus contortus* in this instance). Both have the molecular ion at *m/z* = 397), and the spectra are virtually identical in the region from *m/z* = 260 to 397, where there are diagnostic features for the double bonds in positions 11 and 14. However, the ions at *m/z* = 219 and 232 in the mass spectrum of the 5,11,14-isomer help to locate the double bond in position 5, while in that of the 7,11,14-isomer, the distinctive ion at *m/z* = 247 points to a double bond in position 7 (odd-numbered ions are unusual).

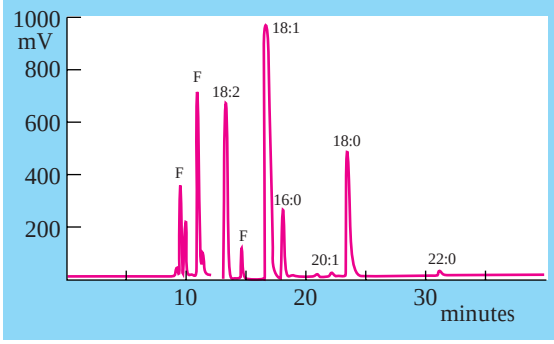


Figure 4 Separation of picolinyl ester derivatives prepared from the seed oil of *Dichapetalum toxicarium* by reversed-phase high-performance liquid chromatography. F = a fluorinated fatty acid.

When there is dubiety in the interpretation of a mass spectrum, the problem can usually be resolved by deuteration with Wilkinson's catalyst. With this method, deuterium adds to the double bonds in a simple way and is easily located by mass spectrometry. However, it is rarely possible to carry out this reaction with complex natural mixtures of fatty acids. Either the component of interest must be isolated in a pure state or in a simpler fraction in which other components do not interfere. We have developed two procedures for this purpose, silver-ion and reversed-phase high-performance liquid chromatography. The former is usually used with methyl ester derivatives and gives fractions which can differ in the position, geometry and number of double bonds in the molecules. Such methodology has proved extremely useful for the characterization of cyclic fatty acids formed when vegetable oils are heated to high temperatures, as in frying foods, for example.[3]

Reversed-phase HPLC is a mild method in that it is carried out at ambient temperature and involves only liquid-liquid interactions. With fatty acid derivatives, separation is based both on the chain-length and degree of unsaturation of components, each double bond reducing the retention time by the equivalent of about two methylene groups. Initially, it proved difficult to adapt the technique to picolinyl esters and DMOX derivatives, because of the basic nature of the molecules which resulted in tailing and poor resolution. However, new deactivated stationary phases of the ODS type are now available that permit elution of basic compounds as sharp peaks without addition of ionic species to the mobile phase. Figure 4 illustrates a separation of picolinyl ester derivatives prepared from the seed oil of *Dichapetalum toxicarium*, which is unusual in that it contains some fatty acids with fluorine atoms in the terminal methyl group. A column of Hichrom RPB^{TM} was utilized with acetonitrile as the mobile phase and a flow gradient of 0.5 to 1.5 ml/min. It was then a relatively simple matter to collect the fractions of interest for further characterization, including gas chromatography-mass spectrometry.

We now have a battery of techniques available to us that permits determination of most fatty acid structures with relative ease.

References

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[2] Dobson, G. & Christie, W.W. (1996). *Trends in Analytical Chemistry* 15, 130-137.

[3] Dobson, G. (1998). In: Hamilton, R.J. (ed.). *Lipid Analysis in Oils and Fats*. Blackie, London, 136-180.