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One-step conversion of fatty acids into their 2-alkenyl-4,4-dimethyloxazoline derivatives directly from total lipids

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Abstract

2-Alkenyl-4,4-dimethyloxazoline derivatives of fatty acids were obtained by direct reaction of 2-amino-2-methylpropanol with oils or total lipid extracts. The yields were similar to those obtained by the formation of oxazoline derivatives from fatty acid methyl esters after transesterification of the starting lipids. The procedure is especially useful in the analysis by gas chromatography–mass spectrometry of samples rich in lipids containing α-unsaturated ethers (alkenylglycerols), as it avoids the formation of the corresponding dimethylacetals that can complicate the chromatograms and the mass spectra of the fatty acid oxazoline derivatives.

1. Introduction

The location of the positions of the double bonds in the alkyl chain of fatty acids has been accomplished by gas chromatography–mass spectrometry (GC–MS) following two different strategies, both involving chemical modification prior to the analysis: derivatization at the double bonds ("on-site" modification) to give compounds with distinctive fragmentation patterns or, more commonly, derivatization of the terminal carboxilic acid group ("remote-site" modification) with reagents that enhance charge stabilization to minimize double bond migration. Various functional group modifiers have been successfully employed in the structure elucidation of complex mixtures of fatty acids, mainly N-acylpyrrolidine [1,2], picolinyl esters [3,4] and dimethyloxazoline [5–11].

4,4-Dimethyloxazoline derivatives of fatty acids have been found to show several advantages over other fragmentation-directing derivatives: they are readily prepared and purified, markedly stable towards most reagents and capable of hydrolytic decomposition on heating in acidic media to regenerate the starting material [5,6]. They show good chromatographic properties with volatilities comparable to those of simple esters, and have been successfully resolved by means of capillary columns of medium and low polarity. However, perhaps the most important advantage in the use of these derivatives is that they produce clear mass spectra, with abundant diagnostic peaks and regular fragmentation patterns [5–12].

2-Alkenyl-4,4-dimethyloxazoline (DMOX) derivatives are usually prepared, after saponification of the starting lipids, by heating the free acids with 2-amino-2-methylpropanol (AMP) or by reaction with dicyclohexylcarbodiimide followed by treatment with $SOCl_2$ [5–10]. Recently, Fay and Richli [12] described a procedure

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that, by means of prolonged heating with AMP at high temperature, allows the preparation of DMOX derivatives starting from the fatty acid methyl esters (routinely used for the analysis of lipids by GC) obtained from total lipid by acid-catalysed transmethylation. The DMOX derivatives so obtained are easily purified from the reaction mixture by extraction into dichloromethane.

However, when samples containing aliphatic aldehydes or alkenylglycerol-derived lipids are subjected to acidic methanolysis, dimethylacetals (compounds derived from aldehydes, either initially free or released from alkenylglycerides) are formed [13]. These products are subsequently extracted into the DMOX fraction in the purification step, contaminating the chromatograms and complicating the mass spectra.

In this paper, we describe a procedure for the direct conversion of fatty acids (either free or esterified) in oils or total lipid extracts into their DMOX derivatives. The procedure is rapid and simple as it eliminates the saponification and/or transesterification steps, and thus avoids the formation of the interfering dimethylacetals.

2. Experimental

2.1. Materials

2-Amino-2-methylpropanol (for synthesis) (AMP), acetyl chloride (for analysis), toluene (analytical-reagent grade) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Cod liver oil (CLO) was purchased from a local pharmacy. Mussels (*Mytillus edulis galloprivincialis*) were collected from the Ria of Arosa coast (Pontevedra, Spain) and the lipids were extracted by the method of Bligh and Dyer as described by Christie [14]. The total lipid amount was determined gravimetrically as described by Herbes and Allen [15].

2.2. Gas chromatography

A Perkin–Elmer Model 8700 gas chromatograph equipped with a flame ionization detector, a programmed-temperature vaporization injection system and an SP-2330 fused-silica capillary column (30 m × 0.25 mm I.D., 0.2 μm phase thickness) was used. Nitrogen was the carrier gas at a head pressure of 10 p.s.i. (1 p.s.i. = 6894.76 Pa). The oven temperature was raised from 150 to 210°C at 1°C/min and held at the final temperature for 10 min. The injector was operated in the solvent elimination mode, receiving the sample cold, eliminating the solvent at low temperature (45°C, splitting ratio 1:140), and then being programmed to 300°C at 15°C/s. The detector temperature was 265°C. The same conditions were used for fatty acid methyl esters and for DMOX derivatives.

2.3. Gas chromatography–mass spectrometry

A Hewlett-Packard Model 5890 gas chromatograph equipped with a split–splitless injection system in combination with a Hewlett-Packard Model 5971 mass-selective detector was used. The column was the same as for GC analysis. Helium was used as the carrier gas at a column head pressure of 5 p.s.i. The oven temperature was kept at 80°C for 2 min, then raised at 40°C/min to 150°C and then at 1°C/min to a final temperature of 210°C. The mass spectrometer was operated in the electron impact ionization mode (70 eV). The transfer line and ion source temperatures were both 280°C.

2.4. Derivatization

Lipid samples (CLO or mussel total lipid extract) were dissolved in hexane. Each sample was subjected to three different derivatization procedures, as follows.

Methylation

A 4-mg amount of total lipids was subjected to acidic transmethylation according to Lepage and Roy [16], and the resulting methyl esters were dissolved in 1 ml of toluene.

Methylation and oxazolination

Samples (4 mg) were subjected to the two-step derivatization procedure of Fay and Richli [12].

Direct oxazolination

Oil or total lipid extracts (4 mg) were dried under nitrogen at ambient temperature in PTFE-lined screw-capped tubes. To each tube, 500 μl of AMP (prewarmed at 40°C) were added and the tubes were throughly flushed with nitrogen, capped and heated in a heating block at 180°C for 18 h. The reaction mixtures were cooled and dissolved in 5 ml of CH_2Cl_2, then washed twice with 2 ml of distilled water. The organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated with nitrogen and the residue was dissolved in 100 μl of hexane. This hexane solution was injected directly into the gas chromatograph.

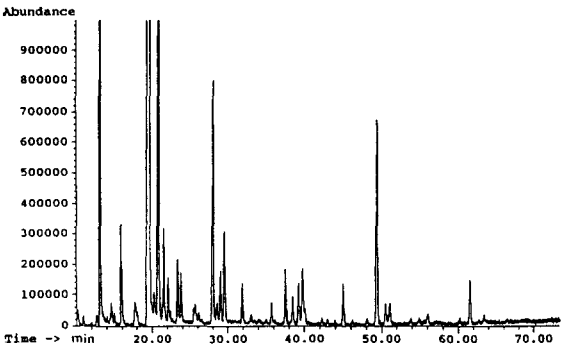

Fig. 1. Total ion current chromatogram of DMOX derivatives of fatty acids from mussel lipid extract on a fused-silica capillary column coated with a polar phenyl methyl silicone phase (SP-2330). For detailed analytical conditions, see Experimental.

3. Results and discussion

Most of the derivatives employed for the analysis of fatty acids by GC–MS show low volatilities, so high temperatures are needed to elute them within reasonable periods of time. This has limited their analysis to the use of low- and medium-polarity columns, operated at temperatures high enough to elute the components but low enough to avoid excessive column bleeding [17].

Taking advantage of the good chromatographic properties of oxazoline derivatives, the elution temperatures of which are around 10°C higher than those for their corresponding methyl esters [5,12], we applied a very polar column (SP-2330) to their analysis by GC–MS. Fig. 1 shows the total ion current chromatogram of mussel fatty acids as their DMOX derivatives (prepared by means of the acid-catalysed transmethylation and subsequent oxazolination procedure of Fay and Richli [12]), obtained with a temperature programme with a maximum temperature of 210°C, far below the recommended maximum column temperature (275°C). Good separation was achieved in a short analysis time, permitting good mass spectra to be obtained, even from certain minor peaks, for complicated fatty acid mixtures such as those coming from marine invertebrate lipids [18]. No column bleeding was observed under these conditions.

However, certain peaks in the chromatogram presented uncommon mass spectra which, below the molecular ion of the corresponding DMOX derivative, show odd-mass fragmentions. The suspected presence of co-eluting dimethylacetals that are frequent in marine samples subjected to acid transmethylation [13] and that show intense odd-mass peaks at M − 1 and M − 31 mass units, was confirmed when the chromatographic trace at m/z 75 (base peak in the dimethylacetal spectrum [13]) was extracted from the total ion current data. Fig. 2 shows the result of monitoring the same sample at two selected ions: m/z 113 (a typical fragment characteristic of DMOX derivatives [5]) and 75. As can be seen, peaks due to dimethylacetals co-eluted with those of certain DMOX derivatives.

To overcome this problem, several alternatives could be applied: the use of a base-catalysed transmethylation procedure that does not affect the vinyl ether linkage in alkenylglycerides, the purification of DMOX from dimethylacetals by thin-layer or column adsorption chromatography [19] or the improvement of the GC separation. All these approaches show different disadvantages such as an increased procedural time or, with basic methylation, the lack of esterification of free fatty acids [19]. From an operative point of view, the most convenient action seems to be to avoid the formation of these dimethylacetals. This alternative is possible if the oxazoline derivatization is carried out directly, without a prior transmethylation or saponification step. In

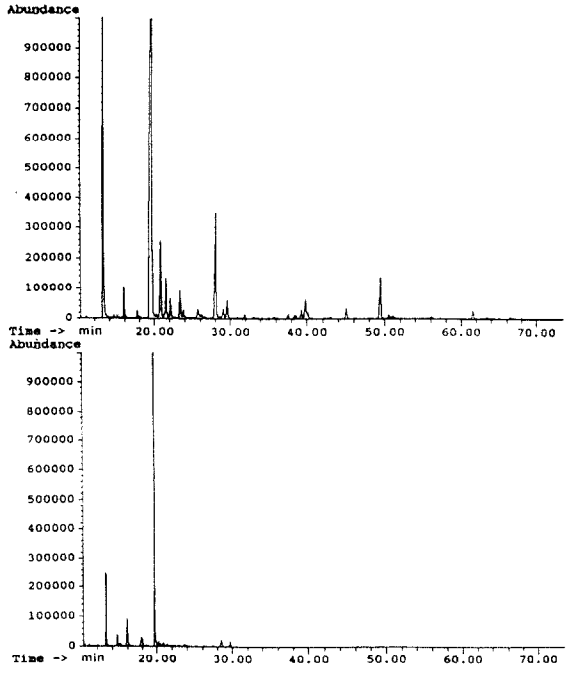

Fig. 2. Selected ion chromatograms at m/z 113 (top) and 75 (bottom) of DMOX derivatives of fatty acids from mussel lipid extract prepared by the two-step procedure (methylation plus DMOX formation).

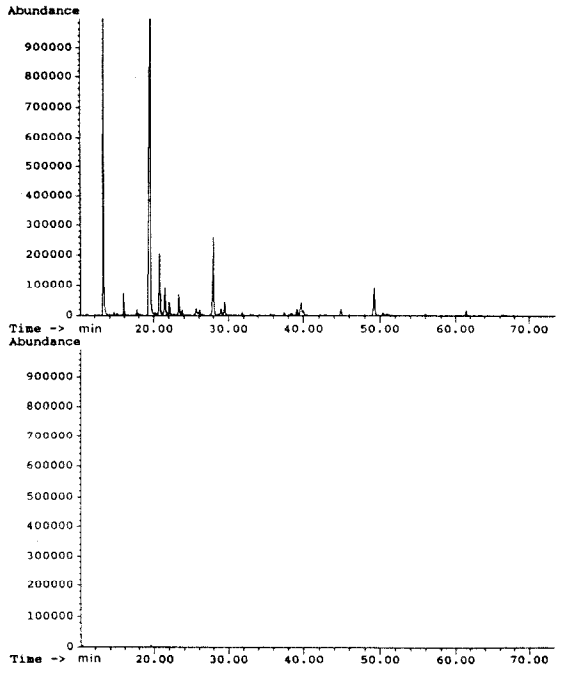

Fig. 3. Selected ion chromatograms at m/z 113 (top) and 75 (bottom) of DMOX derivatives of fatty acids from mussel lipid extract prepared by the direct procedure.

this way, the methodology proposed is rapid and direct, and reduces the time, cost and glassware needed for the analysis.

When an equivalent mussel total lipid extract was derivatized using the direct procedure described in this paper, and the corresponding chromatogram was monitored at m/z 75, no dimethylacetals could be detected (Fig. 3). On the other hand, it must be noted that the intensity of the peaks monitored at m/z 113 was approximately the same as in Fig. 2. This suggests that no significant losses occur when using the direct method compared with the methylation–oxazolination procedure.

To assess the yields of the direct oxazolination procedure, CLO was selected as the test sample as it contains fatty acids representative of different chain lengths and degrees of unsaturation and essentially lacks ether–lipids.

Equivalent CLO samples were subjected to acid-catalysed transmethylation, to acid-catalysed transmethylation plus DMOX derivative

Table 1
Peak area percentages of cod liver oil fatty acid derivatives

Fatty acid	Methyl ester	DMOX[a]	
		A	B
14:0	4.87	5.37	5.67
16:0	11.57	13.74	13.58
16:1ω7	10.00	11.99	11.93
18:0	2.78	3.06	2.93
18:1ω9	18.83	22.01	22.18
18:1ω7	5.12	6.24	6.42
18:2ω6	1.34	1.68	1.52
20:1ω9	15.10	16.39	16.58
18:4ω3	1.75	0.33	0.32
20:4ω6	0.46	0.25	0.27
22:1ω9	8.54	8.73	8.86
20:5ω3	9.40	5.50	5.57
22:5ω3	1.89	0.86	0.80
22:6ω3	9.07	3.83	3.38

[a] Peak-area distributions correspond to the following fatty acid derivatives: (A) DMOX obtained from oxazoline formation over methyl esters and (B) DMOX obtained from oxazoline formation directly from the oil.

formation or to direct oxazolination, and subsequently analysed by GC with flame ionization detection. The results are summarized in Table 1. As noted previously for picolinyl derivatives [17], when the peak-area distribution of the oxazoline derivatives was compared with that of the methyl esters, it was observed that polyunsaturated and late-eluting fatty acids were discriminated against to some extent. However, there is no sensible difference in the peak-area distribution of the DMOX derivatives obtained either from fatty acid methyl esters or directly from the oil.

4. References

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