Journal of Chromatography, 562 (1991) 659–672
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5547

Gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry of cyclic fatty acid monomers isolated from heated fats

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ABSTRACT

The analysis of hydrogenated cyclic fatty acid monomers isolated from heated linseed and sunflower oils is achieved by gas chromatography–tandem mass spectrometry of their pentafluorobenzyl esters. Collisionally activated dissociation of the carboxylate anions produced by electron-capture ionization shows remote charge-site fragmentation that allows location of cyclopentane and cyclohexane rings by examining the resulting mass-analysed ion kinetic energy spectra. Oxidative ozonolysis of the methyl esters of the unsaturated cyclic fatty acid monomers allows location of some double bonds. However, preliminary results obtained with remote charge fragmentation of synthetic unsaturated models make this approach an alternative for double bond location in the cyclic fatty acid monomers isolated from heated fats.

INTRODUCTION

Among the deterioration products formed in fats and oils during deep fat frying operations, cyclic fatty acid monomers (CFAMs) [1–3] have been shown to exhibit a possible toxic effect [4–6]. Moreover, these CFAMs are not only formed in laboratory experimental conditions, but have also been found in domestic or industrial frying oil and in food fried in it [7,8].

These cyclic monomeric compounds originate from the intramolecular cyclization of the C_{18} polyunsaturated fatty acids, mainly linoleic and linolenic. Because they occur as complex mixtures of isomeric C_{18} unsaturated cyclic acids, much work, including organic synthesis of model and reference compounds, has been

0378-4347/91/$03.50

done in order to establish their structures [1,9–17]. Direct characterization of individual compounds was carried out by gas chromatography–mass spectrometry (GC–MS). The main structures were characterized as 1,2-disubstituted six-membered and five-membered carbon rings, using the mass spectra of the unsaturated and, chiefly, the hydrogenated compounds.

The rings were located by observing four characteristic fragment ions corresponding to cleavages of the substituents in the α-position [1,9]. However, the abundance of these fragments was very low, especially for the unsaturated five-membered ring compounds, and many components were not identified [11,13,17]. Moreover, electron-impact (EI) mass spectrometry does not allow the carbon–carbon double bond, to be located.

The present study was undertaken to establish the complete structures of the unsaturated CFAMs isolated from heated linseed and sunflower oils, including the positions of the double bonds. The remote charge fragmentation process of carboxylate anions by collisional activation [18] was recently demonstrated to be succesful in locating the three-membered rings in cyclopropane and cyclopropene fatty acids [19]. Recently, an improvement of this technique was described [20]. It consisted in coupling GC separation of fatty acids as pentafluorobenzyl esters to MS–MS analysis of the carboxylate anions generated in high yield by dissociative electron-capture ionization [20] (GC–MS–MS analysis). Therefore, the hydrogenated CFAMs were converted into their pentafluorobenzyl esters and submitted to GC–MS–MS in order to investigate the remote charge fragmentation process for confirming the basic skeleton of the molecules. The double bonds were tentatively located by GC–MS of the mixtures obtained after oxidative ozonolysis of the CFAM fractions. Finally, model unsaturated CFAMs were studied by remote charge fragmentation in order to investigate the efficiency of this process for locating rings and double bonds in CFAM fractions in a single GC–MS–MS run.

EXPERIMENTAL

Unless otherwise indicated, all the chemicals were obtained from Aldrich (Strasbourg, France). The cyclic fatty acid monomers were isolated as described previously [12,17]. Saturated and unsaturated cyclic model compounds have been prepared earlier by total synthesis [14]. The pentafluorobenzyl esters were prepared by dissolving *ca.* 1 mg of a CFAM mixture in dry methanol (10 μl) and acetonitrile (50 μl). Pentafluorobenzyl bromide (20 μl) and diisopropylamine (20 μl) were added, and after 1 h at room temperature, the volatiles were removed and the residue was dissolved in hexane prior to injection into the capillary GC column.

Oxidative ozonolysis was performed by bubbling ozone in oxygen (12 min) through appropriate amounts of CFAMs (*ca.* 1 mg) dissolved in a 7% BF_3–methanol medium (1–2 ml, Interchim, Montluçon, France) contained in a 10-ml

PTFE-lined screw-capped centrifuge tube. The tube was promptly capped and heated at 100°C for 1 h. After cooling, distilled water (6 ml) was added and the products were extracted with chloroform. If required, the extract was concentrated just before thhe GC–MS analysis.

The GC–MS analyses were conducted with a Nermag R10-10C quadrupole mass spectrometer coupled to a Girdel 31 gas chromatograph (Delsi-Nermag Instruments, Argenteuil, France) fitted with a split/splitless injector and a DB5 capillary column (60 m × 0.32 mm I.D., film thickness 1 μm, J and W Scientific, Folsom, CA, U.S.A.). Helium was used as the carrier gas with a linear velocity of 35 cm/s, and the oven temperature was programmed from 40 to 220°C at 3°C/min. Mass spectra were generated at 70 eV with a source temperature of 150°C.

The MS–MS analyses were done on a reverse geometry VG Analytical ZAB-2F (VG Analytical, Manchester, U.K.) instrument coupled to a Dani 3800 gas chromatograph (Dani, Milan, Italy) fitted with an HT5 capillary column (12 m × 0.33 mm I.D., film thickness 0.1 μm, SGE, Villeneuve, St. Georges, France). The instrument was fitted with either a fast-atom bombardment (FAB) or a chemical ionization (CI) source, and with a collision cell in the second field-free region. The negative-ion FAB spectra were produced using a 8-keV xenon gun. Electron-capture spectra were obtained in negative CI conditions using methane as reagent gas. The source temperature was 200°C. The collisionally activated dissociation mass-analysed ion kinetic energy (CAD-MIKE) spectra were recorded using helium as the collision gas at a scan speed of 5000 eV/s.

RESULTS AND DISCUSSION

The CAD-MIKE spectra of the carboxylate anions of cyclopropane and cyclopropene fatty acids, desorbed by FAB, showed remote-charge-site fragmentations [19]. This behaviour, analogous to the allylic cleavages observed for unsaturated acids [21], allowed location of the rings, as well as distinction between *cis* and *trans* isomers [19]. Using the same fragmentation process, we investigated first the behaviour of 1,2-disubstituted cyclopentane rings on model compounds [14] of the hydrogenated CFAMs.

Cyclopentane disubstituted acids

The CAD-MIKE spectra of the carboxylate anions from 9-(2′-butylcyclopentyl)nonanoic, 7-(2′-hexylcyclopentyl)heptanoic and 5-(2′-octylcyclopentyl)pentanoic acids, desorbed by FAB, are presented in Fig. 1 A–C. All fragment ions contain the carboxylate group linked to a part of the hydrocarbon chain. Remote-charge-site fragmentation of the hydrocarbon chain occurs, with easier cleavage at points a and b (see Fig. 1) corresponding to fragmentation α to the cyclopentane ring. The cleavage of the C–C bond of the hydrocarbon moiety is substantially enhanced, and counting of the number of regularly spaced signals

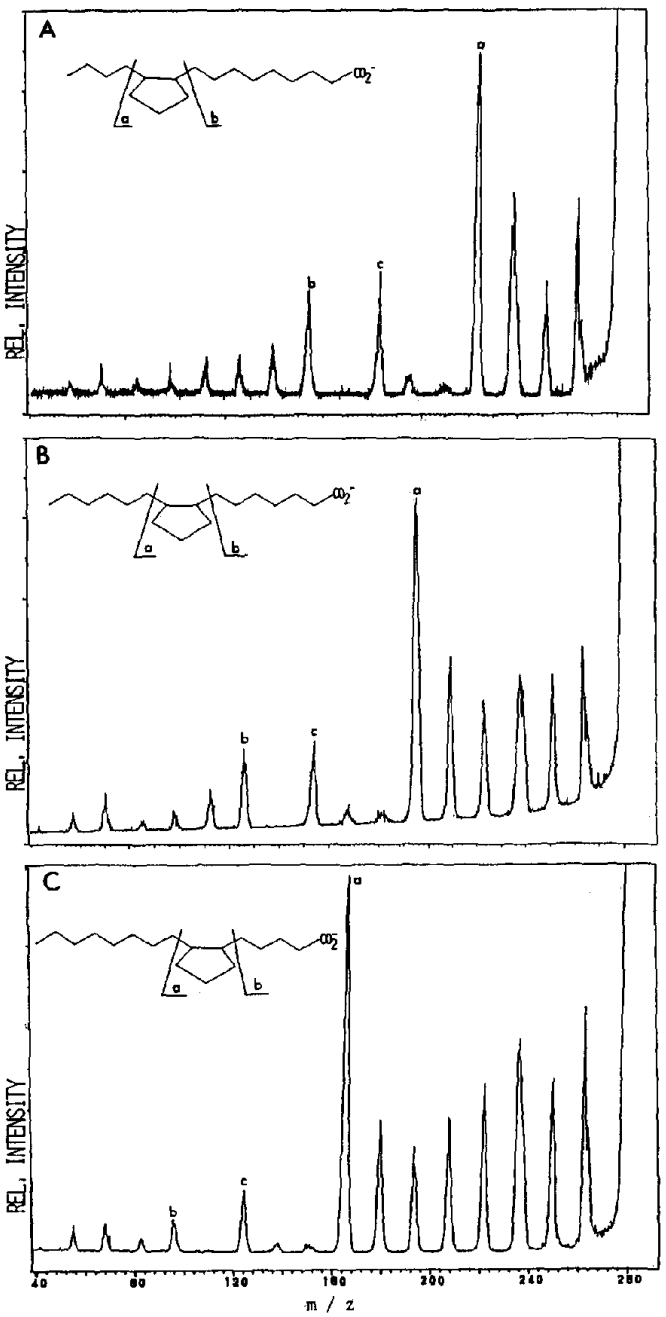

Fig. 1. CAD-MIKE spectra of carboxylate anions (*m/z* 281) of 1,2-disubstituted cyclopentyl acids, measured from negative FAB-generated parent ions.

from the main beam to this characteristic fragment gives the number of carbon atoms of the *n*-alkyl substituent. The fragmentation of the chain between the carboxylate group and the ring is also enhanced, but to a lesser extent. Another fragment (labelled c in Fig. 1) of enhanced intensity is also found in the spectra between the characteristic fragment ions a and b. Its origin is not yet understood, but it probably involves peculiar and characteristic cleavage of the cyclopentane ring. It is noteworthy that no signal appears between fragment ions b and c. This pattern of three enhanced signals a, b and c, with two minor peaks between a and c, and no fragment between c and b, appears to be characteristic of cyclopentane-disubstituted acids. The main fragmentations at the position α to the ring correspond to the known weak characteristic fragmentation of the disubstituted cyclopentyl acids in electron ionization MS [14,17]. However, the characteristic peaks obtained with remote charge fragmentation are of considerably enhanced intensity. This fragmentation appears to be different from that of cyclopropane acids, where cleavage β to the ring occurs [19]. Preliminary results on prostaglandins, however, showed remote charge fragmentation α to the five-membered ring [22].

Hydrogenated cyclic fatty acid monomers

In vegetable oils, the unsaturated C_{18} CFAMs are most likely formed from linoleic and linolenic acids. So, two types of oil were selected, one rich in linolenic acid, linseed oil, and one rich in linoleic acid, sunflower oil. After heat treatment, the CFAMs were isolated as their methyl esters and characterized by GC–MS and GC with Fourier transform infrared spectroscopy [12]. This study showed that the CFAMs isolated from linseed oil were mostly dienoic cyclic C_{18} isomers (molecular mass 292), whereas those isolated from sunflower oil were monoenoic cyclic C_{18} fatty acids (molecular mass 294). Some diunsaturated cyclic esters were characterized as cyclohexenyl compounds, but the generally accepted fragmentation pattern for saturated cyclic fatty acids, which corresponds to cleavage α to the ring [1,9], was absent or gave very weak fragment ions. However, other six-membered ring compounds were suspected, as well as five-membered ring components [12]. As expected, double bond locations could not be determined.

As carbon skeletons are generally more easily deduced from the mass spectra of saturated species, the CFAM fractions were hydrogenated, and the resulting saturated CFAM methyl esters were submitted to GC–MS [17]. Cyclohexyl derivatives, mainly methyl 9-(2′-propylcyclohexyl)nonanoate, were unambiguously characterized on the basis of a well-documented literature [1,9,10]. Very complex spectra were also observed, and it was proposed that these spectra were those of CFAMs with a five-membered ring. However, characteristic fragments ions were hardly distinguished from other important fragments [17], and tedious steps of synthesis were necessary to confirm some cyclopentyl structures [14,16].

In the analysis of fatty acids using the remote charge fragmentation process, it was demonstrated that carboxylate anions could be advantageously generated by

the dissociative electron capture of pentafluorobenzyl esters of fatty acids [20,23]. This improvement allowed GC–MS–MS to be performed. Therefore, the pentafluorobenzyl esters of the hydrogenated CFAM mixtures were submitted to GC–MS–MS.

Despite poor chromatographic separations, which precluded a complete *trans* and *cis* isomer differentiation, the CAD-MIKE spectra of the main hydrogenated CFAM carboxylates (*m/z* 281) allowed location of the rings. Figs. 2 and 3 show the GC–MS–MS data for some disubstituted cyclopentyl carboxylates isolated from heated linseed and sunflower oils, respectively. In particular, the remote charge-site fragmentation spectra allowed structural confirmation of one peak (Fig. 3), tentatively identified previously as methyl 4-(2′-nonylcyclopentyl)butanoate [17].

Characteristic CAD-MIKE spectra of cyclohexane-substituted carboxylates were also obtained. Remote charge-site fragmentations occured with characteristic patterns giving rise to enhanced fragment ions (a and b), corresponding to cleavages α to the cyclohexane ring (Fig. 4). Here again, cleavage of the C–C bond of the hydrocarbon moiety is substantially enhanced, except for the methylcyclohexyl acids, in which the fragmentation of the carboxylate side is increased to a greater extent (Fig. 4). These favoured α-cleavages correspond to the known fragmentation of the cyclohexyl derivatives in EI-MS [9,10], but here again the remote site fragmentation spectra are much clearer. The main hydrogenated CFAM identified in heated linseed and sunflower oils are indicated in Figs. 5 and

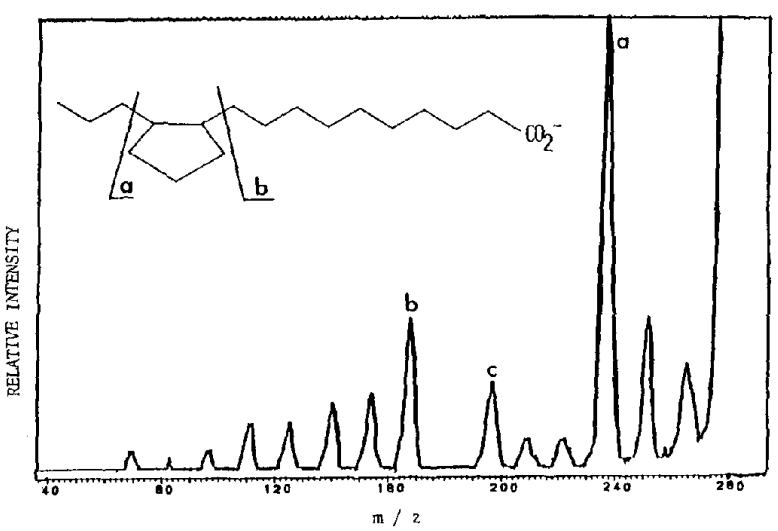

Fig. 2. CAD-MIKE spectrum of the carboxylate anion (*m/z* 281) of a 1,2-disubstituted cyclopentyl acid isolated from heated linseed oil, obtained from dissociative electron capture in a GC–MS–MS analysis of its pentafluorobenzyl ester.

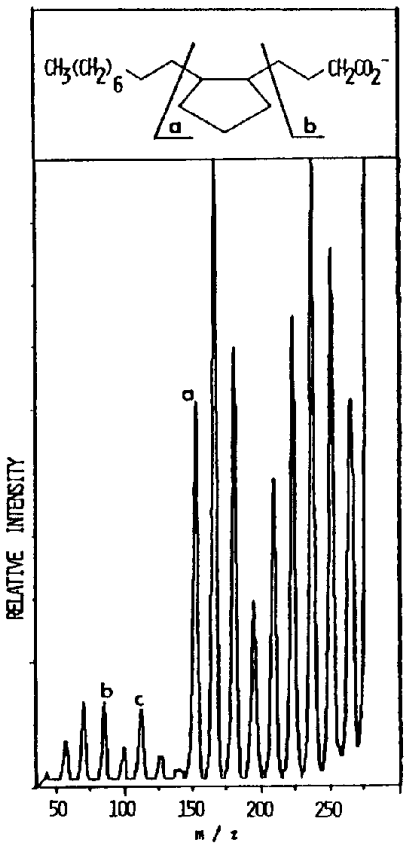

Fig. 3. CAD-MIKE spectrum of the carboxylate anion (m/z 281) of a 1,2-disubstituted cyclopentyl acid isolated from heated sunflower oil, obtained in a GC–MS–MS analysis of its pentafluorobenzyl ester.

6. This study confirms our earlier work conducted with GC–EI-MS of the CFAM methyl esters [17], and extends the number of identified compounds. In particular, the CFAMs with a very short alkyl moiety (*i.e.* Fig. 4 for methylcyclohexyl and Fig. 7 for methylcyclopentyl acids) were more easily characterized.

Oxidative ozonolysis of the CFAMs

In general, the locations of double bonds in aliphatic chains of unsaturated species are determined by GC–MS, after chemical derivatization of the double bonds [24]. Most of the methods are suitable for unsaturated fatty acids, and the subject has been reviewed recently [25]. The main, and probably most reliable method [26] involves oxidation of the double bonds to the vicinal diols, followed by GC–MS analysis of the corresponding trimethylsilyl ethers. However, this method becomes beset with difficulties when complex isomeric mixtures are to be

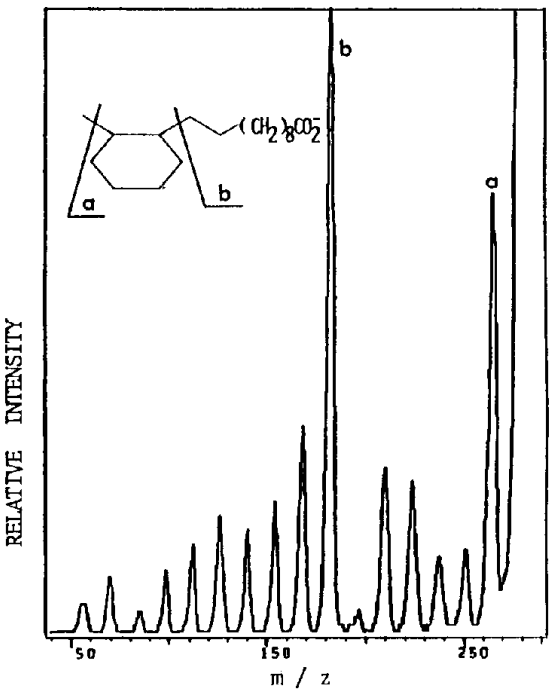

Fig. 4. CAD-MIKE spectrum of the carboxylate anion (*m/z* 281) of a 1,2-disubstituted cyclohexyl acid isolated from heated sunflower oil, obtained in a GC–MS–MS analysis of its pentafluorobenzyl ester.

analysed. Moreover, it was completely unsuccessful when applied to the unsaturated CFAM methyl esters [27].

Oxidative fission of double bonds, and particularly ozonolysis, is an attractive alternative to chemical derivatization [28]. Ozonolysis procedures are quite simple, and the products are easily analysed by GC. Oxidative ozonolysis in BF_3–methanol [29] is particularly attractive, as the methyl mono- and diesters reaction products are easily separated and characterized by GC–MS.

9-(2′-Butylcyclopentyl)nonanoic acid (n = 3)
10-(2′-Propylcyclopentyl)decanoic acid (n = 2)
9-(2′-Propylcyclohexyl)nonanoic acid (m = 2)

Fig. 5. Main hydrogenated cyclic fatty acid monomers identified in heated linseed oil. Identifications made from the CAD-MIKE spectra of the carboxylate anions (*m/z* 281) obtained by GC–MS–MS of the pentafluorobenzyl esters.

4-(2′-Nonylcyclopentyl)butanoic acid (n = 8)
7-(2′-Hexylcyclopentyl)heptanoic acid (n = 5)
9-(2′-Butylcyclopentyl)nonanoic acid (n = 3)
12-(2′-Methylcyclopentyl)dodecanoic acid (n = 0)
9-(2′-Propylcyclohexyl)nonanoic acid (m = 3)
10-(2′-Ethylcyclohexyl)decanoic acid (m = 1)
11-(2′-Methylcyclohexyl)undecanoic acid (m = 0)

Fig. 6. Main hydrogenated cyclic fatty acid monomers identified in heated sunflower oil. Identifications made from the CAD-MIKE spectra of the carboxylate anions (m/z 281) obtained by GC–MS MS of the pentafluorobenzyl esters.

Oxidative ozonolysis of a mixture of cyclic monomer methyl esters isolated from heated linseed oil gave essentially seven products which were easily separated by GC (Fig. 8). The two major products (DMC8 and DCM9) were identified as the octanedioate and nonanedioate dimethyl esters. The other peaks, labelled A to E, were tentatively identified as dimethyl and trimethyl esters of di- and tricarboxylic acids. Formation of dimethyloctanedioate and nonanedioate fixes

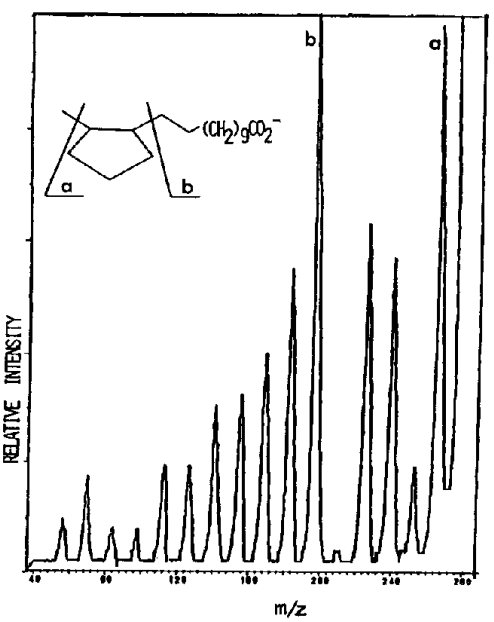

Fig. 7. CAD-MIKE spectrum of the carboxylate anion (m/z 281) of an acid isolated from heated sunflower oil and identified as 12-(2′-methylcyclopentyl)dodecanoic acid.

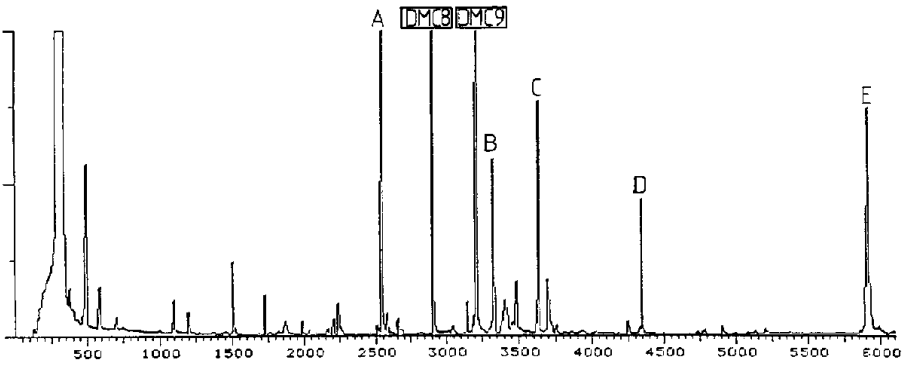

Fig. 8. Total ion current chromatogram of the mixture obtained after oxidative ozonolysis of a CFAM fraction isolated from heated linseed oil (DB5 capillary column, 60 m × 0.32 mm I.D., film thickness 1 μm, programmed from 40°C to 220°C at 3°C/min).

the position of one double bond on the carboxylate side, on either carbon-8 or carbon-9. The mass spectrum of peak B (Fig. 9) displayed fragment ions characteristic of a di- or trimethyl ester of a di- or triacid: M − 31, M − 43, M − 63, M − 73, M − 105 (the molecular mass of 260 was confirmed by ammonia CI-MS).

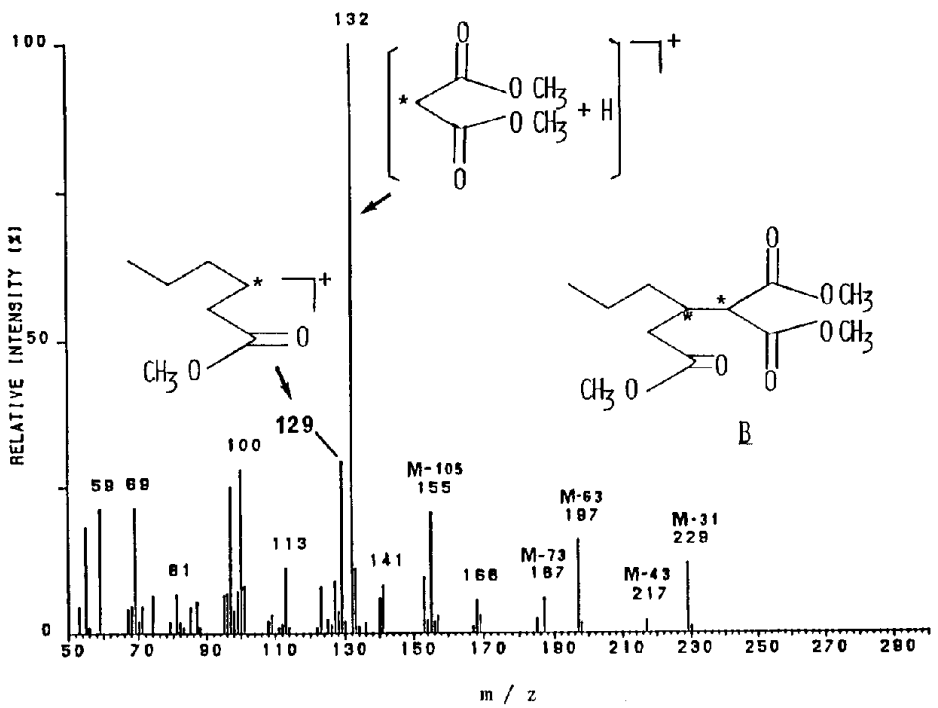

Fig. 9. Mass spectrum (70 eV) of the trimethyl ester (peak B in Fig. 8) identified as dimethyl 2-carbomethoxy-3-propylglutarate.

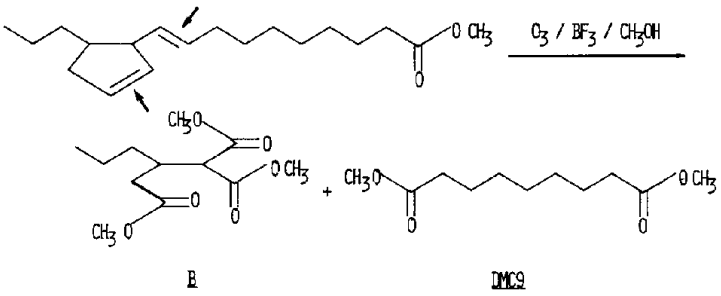

Fig. 10. The formation of DMC9 by oxidative ozonolysis.

An *n*-propyltrimethyl ester (2-carbomethoxy-3-propylglutarate) is a possible structure [13], and would account for the main fragment ions at *m/z* 132 and 129, as depicted in Fig. 9. This hypothesis has been confirmed recently by synthesis of the triester [30].

This structure is, therefore, the result of the oxidative ozonolysis of methyl 10-(5′-propyl-2′-cyclopentenyl)-9-decenoate (Fig. 10).

Peak A was unambiguously identified as methyl-3-propylglutarate by its mass spectrum and by synthesis [30]. It probably derived by *in situ* decarboxylation of B during oxidative ozonolysis.

The other structures have not been yet confirmed by synthesis, even though their mass spectra suggest they are di- or trimethyl esters. For instance, peak E has a molecular mass of 330 (determined by ammonia CI-MS) and fragment ions characteristic of a di- or triester. These data suggest it is a trimethyl ester of a branched C_{14} tricarboxylic acid. A structure for the cyclic acid, compatible with this hypothesis, could be methyl 9-(6′-propyl-3′-cyclohexenyl)-4-nonenoate (Fig. 11), a structure already suggested, but not retained, by McInnes *et al.* [31] in a study on oxidation of CFAM from linseed oil with periodate-permanganate.

Oxidative ozonolysis of such a compound would lead to a trimethyl ester of a C_{14} triacid (E) and to dimethyl succinate (DMC4). Absence of a detectable

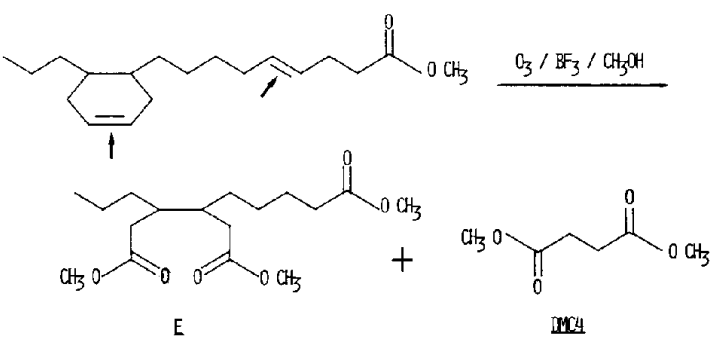

Fig. 11. The formation of DMC4 by oxidative ozonolysis.

amount of dimethyl succinate in the reaction mixture could be explained by its evaporation under the experimental conditions used.

However, other structures are also compatible with the spectrometric data, *i.e.* methyl 9-(5′-butyl-2′-cyclopentenyl)-4-nonenoate and methyl 9-(5′-butyl-3′-cyclopentenyl)-4-nonenoate. Therefore, synthesis is necessary in order to confirm all the hypotheses concerning the structures of the di- and triesters formed by oxidative ozonolysis. This very important drawback was much more obvious when considering the oxidative ozonolysis of the CFAMs isolated from sunflower oil. In the very complex reaction mixture, only the dimethyl esters octanedioate (DMC8), nonanedioate (DCM9) and decanedioate (DMC10) were unambiguously identified as products of the ozonolysis. These poor results did not permit any sensible structure for the CFAMs isolated from sunflower oil to be proposed. The complex nature of the initial CFAM mixture could certainly explain in part the complexity of the oxidative ozonolysis.

The difficulties encountered with oxidative ozonolysis, even though some interesting results were obtained, show the need to develop a new analytical approach for accurate assignment of the structures of the CFAMs and particularly the location of the double bonds.

MS–MS of unsaturated cyclopentyl acids

FAB-desorbed CAD-MIKE spectra of synthetic unsaturated cyclopentyl [14] and cyclopentenyl [30] acids were recorded in order to investigate the remote charge-site fragmentation process for locating rings and double bonds in a single run. All the acquired spectra displayed the same features (Fig. 12). The main characteristic fragment ion (a) corresponds to cleavage of the alkyl substituent α to the cyclopentane or cyclopentene moiety. The second important fragment (b) is formed by an allylic cleavage on the carboxylate side, and this behaviour corresponds to the allylic cleavage observed for mono-unsaturated fatty acids [20,21]. Fragmentation of the carboxylate moiety in the α-position of the ring occurs with very low intensity (fragments c in Fig. 12), probably because the corresponding bond is adjacent to the double bond.

Another important fragment (d in Fig. 12) corresponds to the ring cleavage reported above. This fragment, however, has a very low abundance in the CAD-MIKE spectrum of the 10-(5′-propyl-1′-cyclopentenyl)-9-decenoate (Fig. 12C). This could confirm that it involves a favoured cleavage of the cyclopentane ring, but not of the 1-cyclopentene ring; this fragment seems characteristic of cyclopentane-disubstituted acids.

In conclusion, remote charge-site fragmentation was observed for cyclopentyl- and cyclohexyl-disubstituted acids after collisional activation of their carboxylate anions. This fragmentation process allowed precise location of the rings in hydrogenated cyclic fatty acid monomers isolated from heated fats. The generation of carboxylate anions by electron-capture ionization of pentafluorobenzyl cyclic fatty acid esters allowed capillary GC to be performed prior to the MS–MS

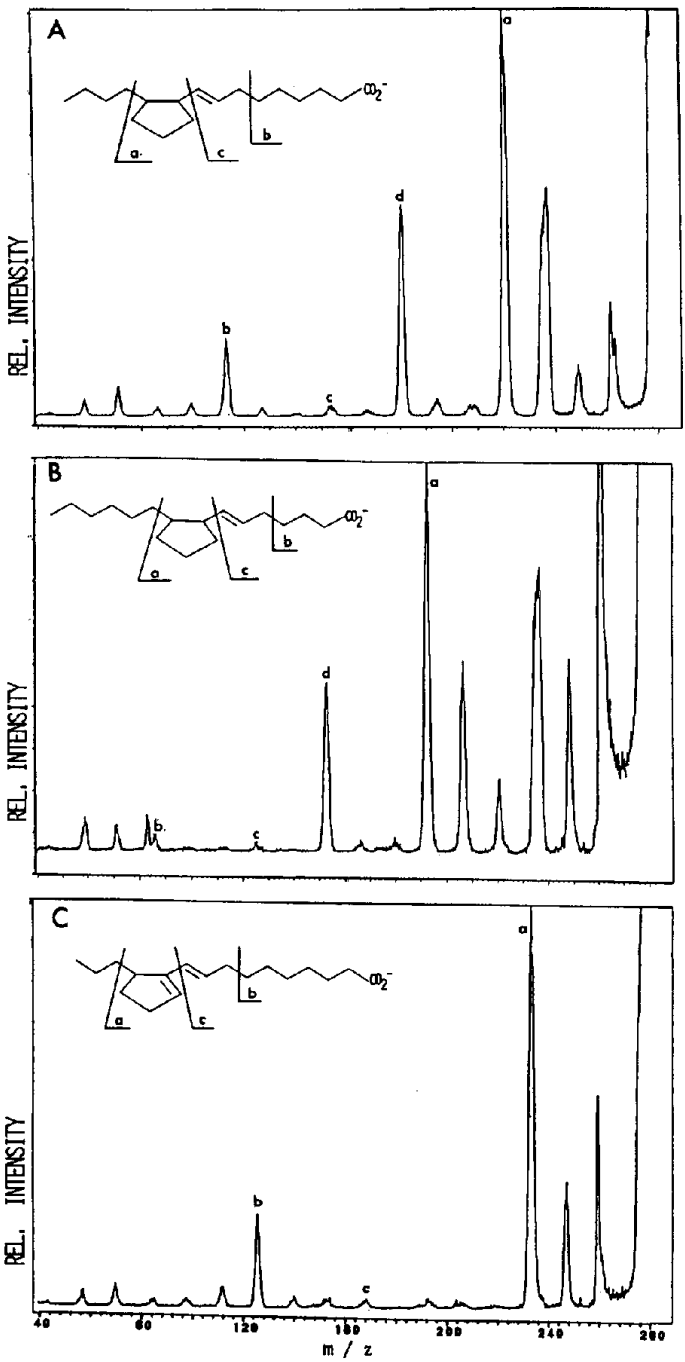

Fig. 12. CAD-MIKE spectra of carboxylate anions of unsaturated 1,2-disubstituted cyclopentyl and cyclopentenyl acids measured from negative FAB-generated parent ions (*m/z* 279 for A and B, *m/z* 277 for C).

analyses. Preliminary results obtained for synthetic unsaturated models make this approach an interesting alternative for a complete structural elucidation of CFAMs formed during the heating of fats and oils.

ACKNOWLEDGEMENTS

This work was partially supported by a grant of the Ministère de la Recherche et de la Technologie. J. Prévost and E. Ribot are thanked for technical support.

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