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Journal of Chromatography A, 985 (2003) 333–342
JOURNAL OF CHROMATOGRAPHY A
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Analysis of the oxidation products of *cis*- and *trans*-octadecenoate methyl esters by capillary gas chromatography–ion-trap mass spectrometry
I. Epoxide and dimeric compounds

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Abstract

The oxidative behavior of methyl oleate (MeOl) and methyl elaidate (MeEl) was compared by hyphenated chromatographic techniques. MeOl and MeEl were separately oxidized (200 °C/30 min) and subjected to solid-phase extraction, in order to isolate the low polarity compounds. The two isomeric 9,10-epoxystearic methyl esters formed in both MeOl and MeEl at different *threo*/*erythro* ratios (2.3 and 6.2%, respectively). The dimeric products produced in the thermoxidized MeOl and MeEl (1.4 and 1.6%, respectively), showed similar gas chromatographic characteristics and mass spectra, suggesting similar molecular structures and formation mechanisms. A positional and probably configurational mixture of symmetric and asymmetric dehydrodimers was detected, whereas the occurrence of MeEl or MeOl dimeric ethers is to be confirmed.

Keywords: Methyl elaidate; Methyl oleate; Fatty acid methyl esters; Epoxides; Dimeric products

1. Introduction

Lipid peroxidation has been studied in depth in model and biological systems because of its in vivo effects, among which cellular damage and consequent aging are the most important. Hydroperoxide decomposition leads to the formation of free radicals, which react through a chain reaction mechanism. Although the oxidation of unsaturated fatty acids (especially methyl oleate) has been the subject of numerous investigations [1–4], some uncertainties still remain in relation to the mechanisms of formation and decomposition of hydroperoxides. The initial stage of olefins autoxidation is a key point for elucidation of the mechanisms of hydroperoxides decomposition.

In general, hydroperoxides are susceptible to homolysis of the O–O bond, which yields an alcohoxy radical and a hydroxyl radical [3]. A relatively small amount of energy (about 44 kcal mol^{-1}; 1 cal = 4.184 J) is required to cleave the O–O bond, which can be provided by heat [3]. These radicals can react between them, rearrange and give rise to a mixture of ether-linked [5] and carbon–carbon linked

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01466-8

dimers (dehydrodimers) and oligomers [6–8]. Dimers can be constituted by identical or different monomeric units and, thus, they can be symmetric or asymmetric, respectively. Dehydrodimers of unsaturated fatty acid esters can be obtained by subjecting them to heating alone (65–210 °C) [9], heating plus oxygen [10,11] and γ irradiation [12].

Thermal degradation of the hydroperoxy isomers of monounsaturated fatty acids generate many products, most of which have already been identified [13–20]. Epoxy esters with the oxirane group at the double bond site, are some of the major thermoxidation products of monounsaturated fatty acids, as reported by several researchers [1,2,11,16,17,21]. A bimolecular mechanism that involves a direct attack of hydroperoxides or peroxy radicals on the carbon–carbon double bond, is responsible for their formation [1,11,21]. Isolation of both *cis*- and *trans*-9,10-epoxyoctadecanoates was additional evidence that peroxide attack of the double bond readily occurred during autoxidation of methyl oleate [1,21].

Although it is known that octadecenoate methyl esters generate epoxy esters and dimers under thermoxidation conditions, a comparative study on the oxidative behavior of *cis*- and *trans*-octadecenoate methyl esters by hyphenated chromatographic techniques has not been performed yet. The aim of this study was to compare the thermoxidative behavior of methyl oleate (MeOl) and methyl elaidate (MeEl); in particular, epoxides and dimers generated from MeOl and MeEl, were determined by capillary gas chromatography (cGC) and gas chromatography–ion trap mass spectrometry (GC–ITDMS).

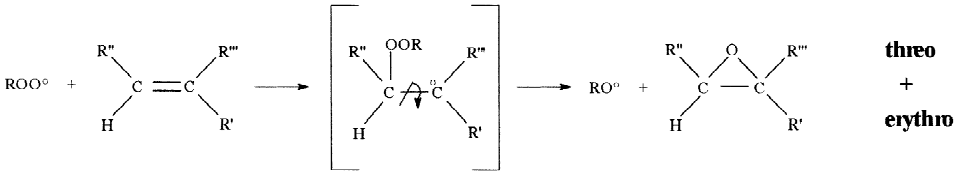
2. Experimental

2.1. Reagents and samples

Reagents and solvents were analytical or HPLC grade, supplied by Carlo Erba (Milan, Italy). Methyl oleate (MeOl) and methyl elaidate (MeEl) standards were purchased from Nu-Chek-Prep (Elysian, MI, USA); they were purified by passing them through an alumina (neutral) liquid chromatographic column (2×1 cm I.D.), using *n*-hexane as the elution solvent, and analyzed by cGC as suggested by Bortolomeazzi et al. [19].

2.2. Preparation and isolation of low polarity compounds

Samples of 0.5 g of MeOl and MeEl were separately oven-heated at 200 °C for 30 min in a 2-ml sealed screw cap container. The low polarity oxidation products (dimeric products and epoxides) were then separated from the thermal oxidation mixtures by solid-phase extraction (SPE) [19].



	R'	R"	R'''	C=C
Me Ol	H	$CH_3-(CH_2)_7$	$(CH_2)_7-COOCH_3$	*cis*
Me El	$(CH_2)_7-COOCH_3$	$CH_3-(CH_2)_7$	H	*trans*

ROO° hydroperoxy radical (mixture of eight different isomers)

RO° = alcohoxy radical (mixture of eight different isomers)

Fig. 1. Scheme of chemical reaction proposed for the formation mechanism of epoxide isomers originated from monounsaturated methyl ester oxidation, following a bimolecular radical reaction (by intermediate I formation) [1,16].

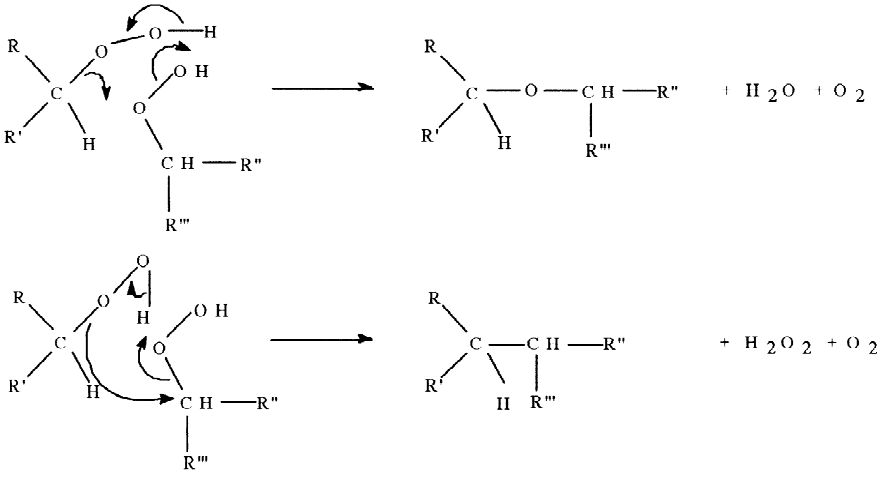
Table 1
Main thermal oxidation parameters of MeOl and MeEl

Fatty acid methyl ester	Oxidation extent (%)[a,b]	Epoxystearates (%)[a,b]	*threo*/*erythro* ratio[a]	Dimeric products (%)[a,b]
Methyl oleate (MeOl)	5.41±0.42	1.46±0.07	2.27±0.03	1.43±0.07
Methyl elaidate (MeEl)	5.51±0.75	1.75±0.34	6.22±0.08	1.64±0.14

[a] Values are the mean of three replicates and their standard deviation. All values were calculated by using the cGC peak areas.
[b] These parameters are calculated as internal percentages of the total cGC peak areas of the thermoxidized MeOl and MeEl.

2.3. Solid-phase extraction of low polarity compounds [19]

Each thermal oxidation mixture (about 100 mg) was dissolved in 220 μl of *n*-hexane and loaded onto a SPE silica column (500 mg) (Strata, Phenomenex, Torrence, CA, USA), which was preconditioned with *n*-hexane (3 ml). The cartridge was then eluted with 3 ml of *n*-hexane, 3 ml of *n*-hexane–diethyl ether (4:1, v/v), 4 ml of *n*-hexane–diethyl ether (1:1, v/v)



Substituents[a]		X[a]	Y[a]
R	$CH_3-(CH_2)_X$ or $CH_3-(CH_2)_X-CH=CH$	6 or 7	
R'	$CH=CH-(CH_2)_Y-COOCH_3$ or $(CH_2)_Y-COOCH_3$		6 or 7
R''	$CH_3-(CH_2)_X$ or $CH_3-(CH_2)_X-CH=CH$	6 or 7	
R'''	$CH=CH-(CH_2)_Y-COOCH_3$ or $(CH_2)_Y-COOCH_3$		6 or 7

[a] **X** + **Y** = 13

Fig. 2. Scheme of chemical reactions proposed for formation mechanisms of dimeric compounds, generated by monounsaturated methyl ester oxidation (MeOl and MeEl) [30].

and 3 ml of methanol. The first two SPE fractions that contained the low polarity products, were joined, taken to dryness and redissolved in 150 μl of *n*-hexane prior to cGC injection.

2.4. Analysis of MeOl and MeEl epoxides (epoxystearates)

Methyl (*R*)-(9),(*R*)-(10)-epoxy-octadecanoate (*erythro*) and methyl (*S*)-(9),(*R*)-(10)-epoxy-octadecanoate (*threo*) were determined by cGC analysis using a polar column (25 m×0.32 mm I.D.), coated with 0.2 μm of cyanopropylpolysiloxane stationary phase (Rtx 2330) (Restek, Bellefonte, PA, USA). Column, detector (flame ionization) and injector temperatures were 190, 220, and 220 °C, respectively. Helium was used as carrier gas at a pressure of 68.4 kPa. Injection was performed in the split injection mode at 1:50 split ratio.

2.5. Analysis of thermal oxidation products of methyl oleate and methyl elaidate

Analysis of the thermal oxidation products of MeOl and MeEl was performed in a Varian 3400 capillary GC, equipped with a low bleed ZB-5 fused-silica capillary column (30 m×0.25 mm I.D.; 0.25 μm film thickness) (Phenomenex) and coupled to a Finningan MAT ITS40 (San Jose, CA, USA) ion trap detector. The oven temperature was programmed from 50 to 300 °C with a rate of 5 °C/min. Injector, transfer line and manifold temperatures were 300 °C. The analyses were performed in the electron impact mode. The filament emission current was 10 μA and the electron impact energy was 70 eV.

3. Results and discussion

Positive evidence for the occurrence or non-occur-

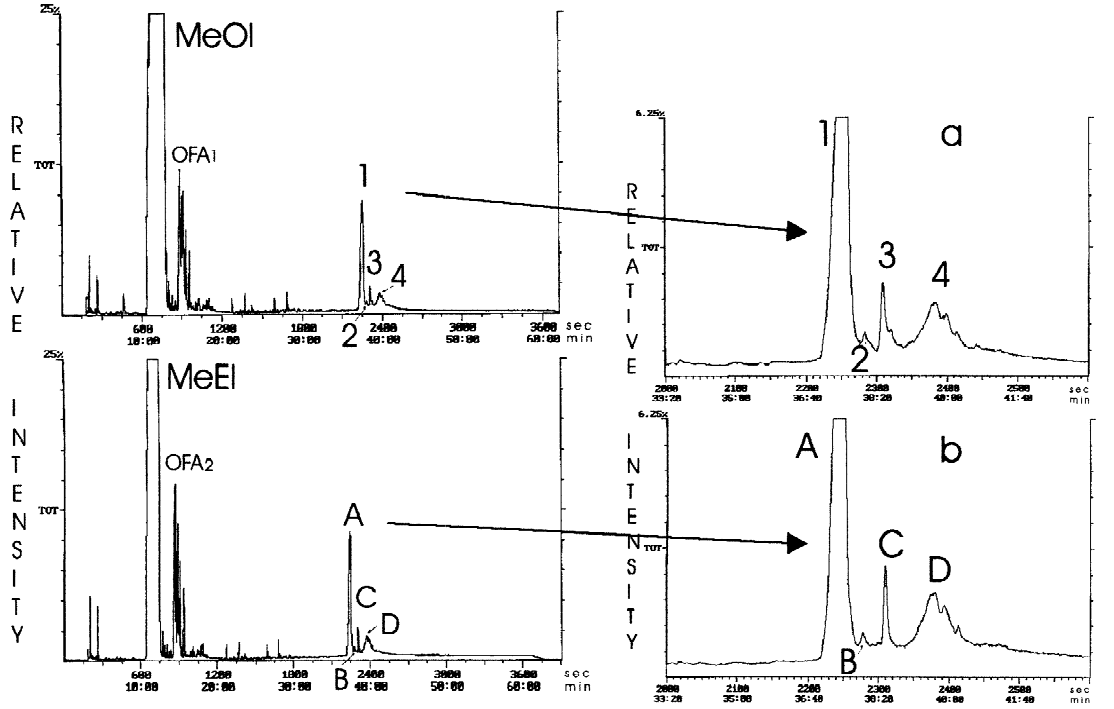


Fig. 3. Reconstructed GC–ITDMS traces of thermal oxidation mixture of MeOl (upper traces; right trace is an enlargement of the dimeric product peaks) and MeEl (lower traces; right trace is an enlargement of the dimeric product peaks). Abbreviations: OFA, oxidized fatty acids (mono- and di-oxygenated).

rence of *cis–trans* and positional isomerization is important from the theoretical standpoint, since it can be useful for supporting the assumption of a radical formation mechanism for the initiation step of lipid oxidation. Trace amounts of *trans* and *cis* isomers in the thermoxidized MeOl and MeEl were detected (0.03–0.07%), respectively; however, these values are extremely low to be able to confirm the occurrence of *cis–trans* isomerization in these experiments by a radical mechanism. Since previous tests demonstrated the formation of the isomers of opposite geometrical configuration from thermoxidized MeOl and MeEl (unpublished data), further investigations are required to verify the occurrence of *cis–trans* isomerization.

Table 1 reports the main thermal oxidation parameters of MeOl and MeEl (oxidation extent, epoxystearates, dimeric products and *threo*/*erythro* ratio); each parameter was calculated as internal percentage of the total cGC areas of the thermoxidized MeOl and MeEl. The oxidation extent of both isomers is analogous (about 5%), which confirms that oxidation of monounsaturated fatty acid methyl esters occurs with similar intensity under these thermal oxidation conditions, regardless of the double bond configuration.

Epoxy esters with the oxirane group at the double bond site were major products of the thermoxidation of MeOl and MeEl, as observed for thermoxidized mono-unsaturated short chain fatty acids [11]. A bimolecular mechanism is responsible for their formation (Fig. 1) [1,11]. The two isomeric 9,10-epoxystearic methyl esters are formed in both MeOl and MeEl oxidations at different ratios. The *threo* isomer in both cases is predominant over the *erythro* one, but the ratio *threo*/*erythro* is higher for MeEl than for MeOl (Table 1). This is probably due to the higher stability of the *threo* isomer as compared to the *erythro* one, which can be attributed to the low hindrance between the allylic groups in the *threo* structure; this behavior is analogous to what have been observed for the *cis* and *trans* double bond isomers.

Thermal decomposition of fatty ester hydroperoxides produces a variety of scission products and high-molecular mass material. The latter are mainly products with about twice as much the molecular mass of the original substrate [dehydrodimers and dimeric oxygenated products (DPs)] [7,22–29] and are formed by a bimolecular reaction mechanism (Fig. 2) [30]. DPs originated from thermoxidized MeOl and MeEl (Fig. 2) and are quite similar with respect to their cGC retention times, cGC relative areas (Fig. 3) and GC–ITDMS analysis (Figs. 4, 6–8). In fact, the percent DPs ranged between 1.4 and 1.6% in the thermoxidized MeOl and MeEl, respectively (Table 1). In order to describe the elution characteristics of DPs independently of experimental conditions, equivalent hydrocarbon lengths (EHLs) were calculated; the DPs peaks eluted in a 40–45 EHL range. Equivalent carbon length were not used, because DPs are all formed by 36 carbon atoms but are chemically different due to the functional groups attached to the carbon skeleton.

Fig. 3 displays the reconstructed GC–ITDMS traces of the thermal oxidation mixtures of MeOl and MeEl. It is evident that peaks 1–4, as well as peaks A–D, are constituted by various compounds (most

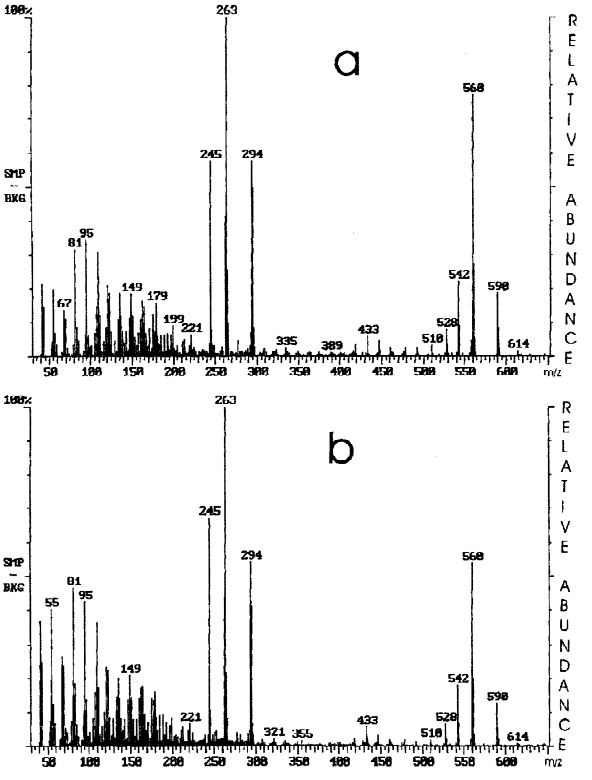


Fig. 4. Mass spectra of the GC–ITDMS peaks 1 (spectrum a) and A (spectrum b) of Fig. 3.

probably a mixture of positional and configurational isomers), which show similar GC behavior and are therefore partially overlapped. Although the identification of the molecular structures of the various isomer mixtures had already been performed in previous studies [3,23,25], it was important to verify if specific groups of oxidation products had similar composition in both thermoxidized MeOl and MeEl. To corroborate this hypothesis, the overall mass spectra of such groups of peaks were compared (Figs. 4, 6–8). Since these peaks are composed by different compounds, the quantitative distribution of the mass fragments of each set of spectra (reported in each figure), could be slightly different and some mass fragments could be due to the presence of compounds that do not exhibit identical molecular structure. Nevertheless, a preliminary evaluation shows a good correspondence between the spectra of MeOl and those of MeEl, having similar distribution pattern. This behavior evinces that the thermoxidation products of MeOl and MeEl are most probably generated by the same chemical formation mechanisms.

The mass spectra of Fig. 4 show some intense m/z fragments that are characteristic of a symmetric dimeric structure [23]. If m/z 590 is the molecular ion, m/z 560 is the loss of 30 u (CH_2O) and m/z 542 corresponds to the loss of another 18 u (H_2O). In addition, m/z 528 is the loss of 62 u (30 + 18 + 14 or more probably 30 + 32, where 32 should be CH_3OH), whereas m/z 510 corresponds to the loss of 80 u (30 + 18 + 32). The m/z 447 and 433 result from the loss of $-(CH_2)_n-COOCH_3$, with n equal to 6 and 7, respectively; these prominent signals indicate that these groups could be attached to a tertiary carbon and could be allylic as well. The m/z 295 is half of the m/z 590, which could correspond to cleavage of the branched bond that joins two oleate or elaidate segments; this bond would be prone to cleavage, since it links two tertiary carbons and is allylic to two double bonds. The m/z 294 is practically identical to m/z 295, but without a H atom in

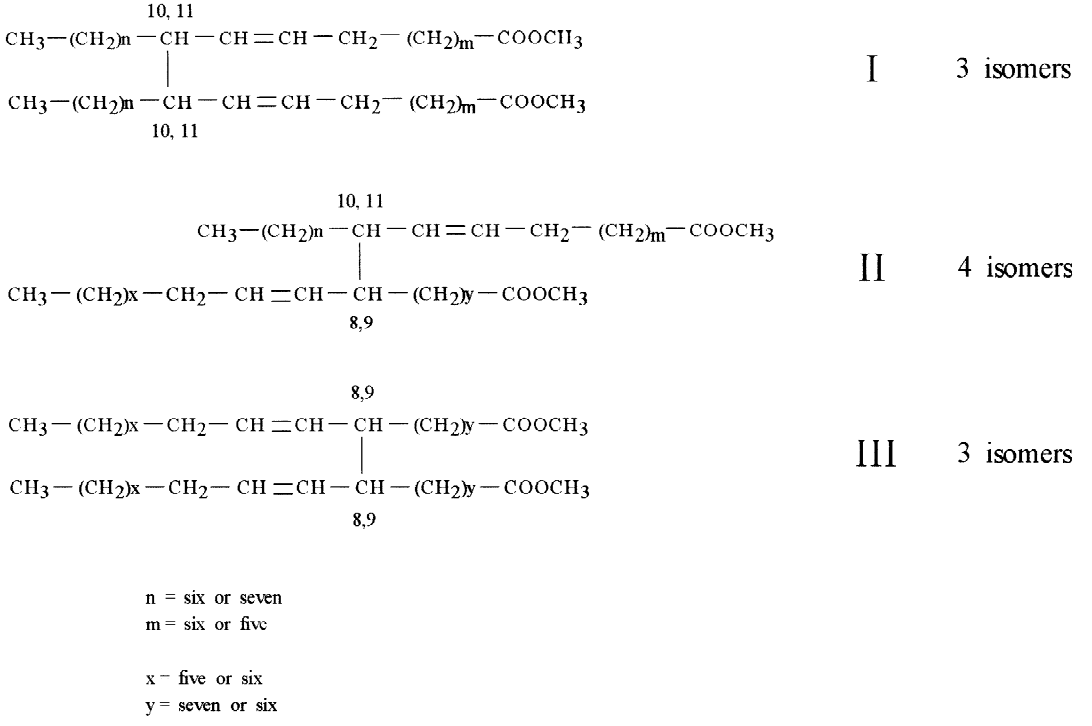


Fig. 5. Scheme of molecular structures of dehydrodimers originated from monounsaturated methyl ester oxidation (MeOl and MeEl).

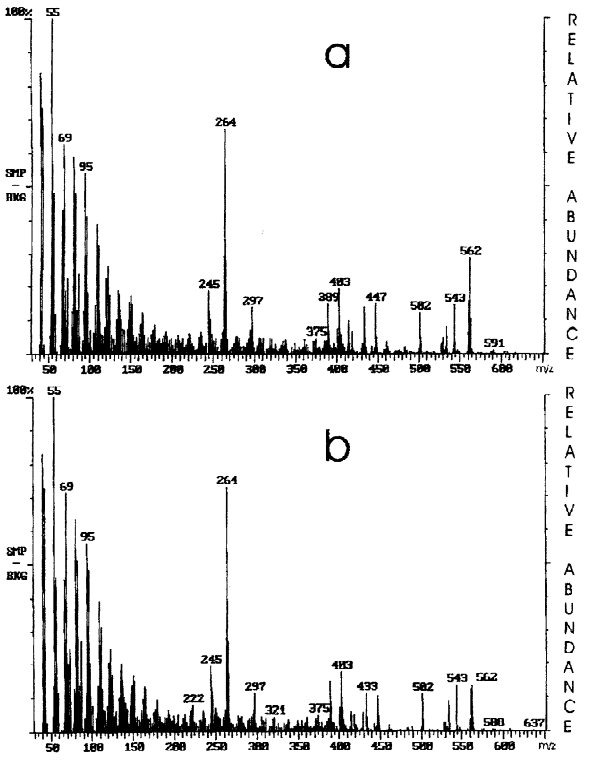


Fig. 6. Mass spectra of the GC–ITDMS peaks 2 (spectrum a) and B (spectrum b) of Fig. 3.

its structure. The *m*/*z* 263 originates from the loss of either 32 u from the *m*/*z* 295, or 31 u from the *m*/*z* 294. The *m*/*z* 245 is generated from an additional loss of 18 u. The presence of all these fragments leads to the conclusion that peaks 1 and A of Fig. 4, that show almost identical mass spectra, are positional and probably configurational isomer mixtures of dehydrodimers (structures I–III of Fig. 5) of MeOl and MeEl, respectively.

The mass spectra of Fig. 6 are almost identical and they have some *m*/*z* fragments in common with Fig. 4, such as *m*/*z* 447 and 433, which represent the loss of $-(CH_2)_n-COOCH_3$ with *n* equal to 6 and 7, respectively. In addition, the presence of *m*/*z* fragments 445 and 431, that have 2 u less than the previous *m*/*z* series, could justify the presence of a hydroxyl group in one of the dimeric units; this hydroxyl group could be eliminated as H_2O, leading to the formation of an additional unsaturation with respect to the dimers of Fig. 4. If *m*/*z* 606 is the molecular ion, *m*/*z* 562 results from the loss of CH_3CH_2 and CH_3. The *m*/*z* 562 looses 60 u ($HCOOCH_3$ or 30+30) and gives a strong signal at *m*/*z* 502. The *m*/*z* 264 results from the loss of 33 u (H_2O+CH_3) from *m*/*z* 297. The *m*/*z* 543 is produced from the loss of 63 u (CH_3O+CH_3OH) from M^+, whereas *m*/*z* 403 results from the loss of 30 u (CH_2O) from *m*/*z* 433. An additional loss of 30 u from *m*/*z* 419 is observed, giving rise to *m*/*z* 389. The strong signal at *m*/*z* 297 may result from the loss of 309 u ($(M^+-15)-294$); the latter indicates that half molecule contains an oxygen atom as an hydroxyl group. The uncertainty of the exact *m*/*z* fragment of the molecular ion and the absence of a *m*/*z* 295, together with the presence of *m*/*z* 297, confirm the existence of an OH group in the dimeric structure.

The mass spectra of Fig. 7 show a series of *m*/*z* fragments that are also present in the mass spectra already examined, such as *m*/*z* 447 and 433. The

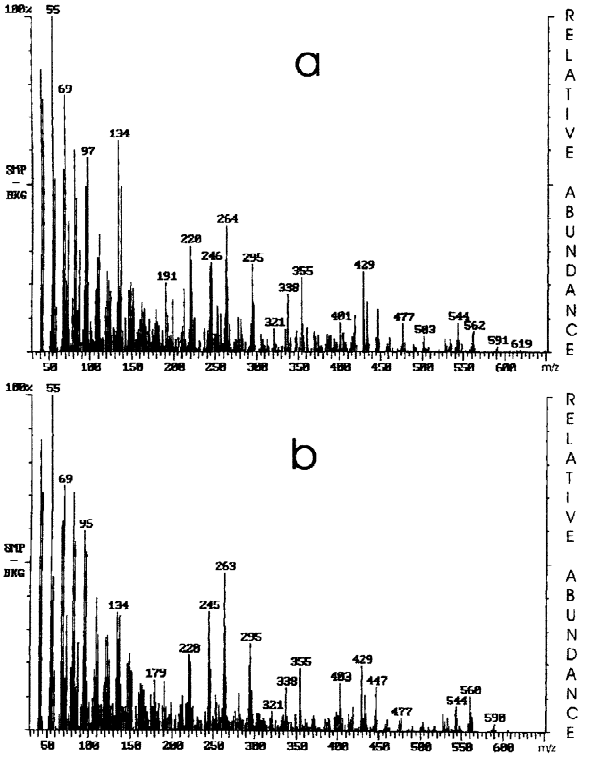


Fig. 7. Mass spectra of the GC–ITDMS peaks 3 (spectrum a) and C (spectrum b) of Fig. 3.

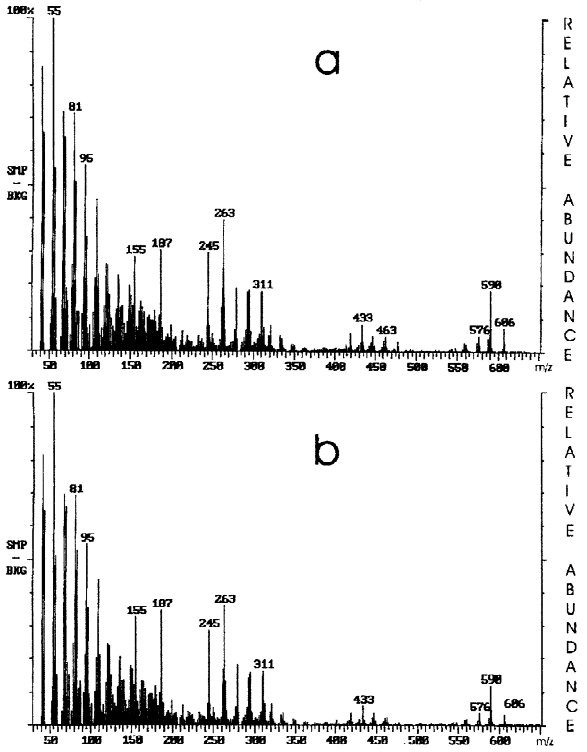


Fig. 8. Mass spectra of the GC–ITDMS peaks 4 (spectrum a) and D (spectrum b) of Fig. 3.

m/z 590 probably derives from the molecular ion. An intense m/z 295 justifies the presence of a half structure similar to that of the dimers described in Fig. 4. The other half of the molecular structure is not clear enough; particular m/z fragments are observed in this figure (such as 134, 136, 220 and 221), but they cannot be attributed to ion structures that could be helpful elucidating the molecular structure of these compound mixture. The gas chromatographic behavior apparently indicates that the peaks are an isomer mixture of dimers with an additional oxygen atom with respect to the dimers of Fig. 4. Since there are no m/z fragments that reveal the presence of an oxygen of a hydroxyl group, it could be hypothesized the occurrence of an oxygen atom that does not modify the dimension of the main fragments, such an oxygen atom of a carbonylic group.

The mass spectra of Fig. 8 show a series of m/z fragments that are also present in the mass spectra of Figs. 4, 6 and 7, such as m/z 447, 433, 294, 295, 263 and 245. The molecular ion seems to be m/z 606. Other characteristic m/z fragments are 311 (m/z 295 plus 16 u), 590 (loss of 16 u from the molecular ion), 279 (loss of CH_3OH from m/z 311). In addition, m/z 187 and 155 are typical fragments that derive

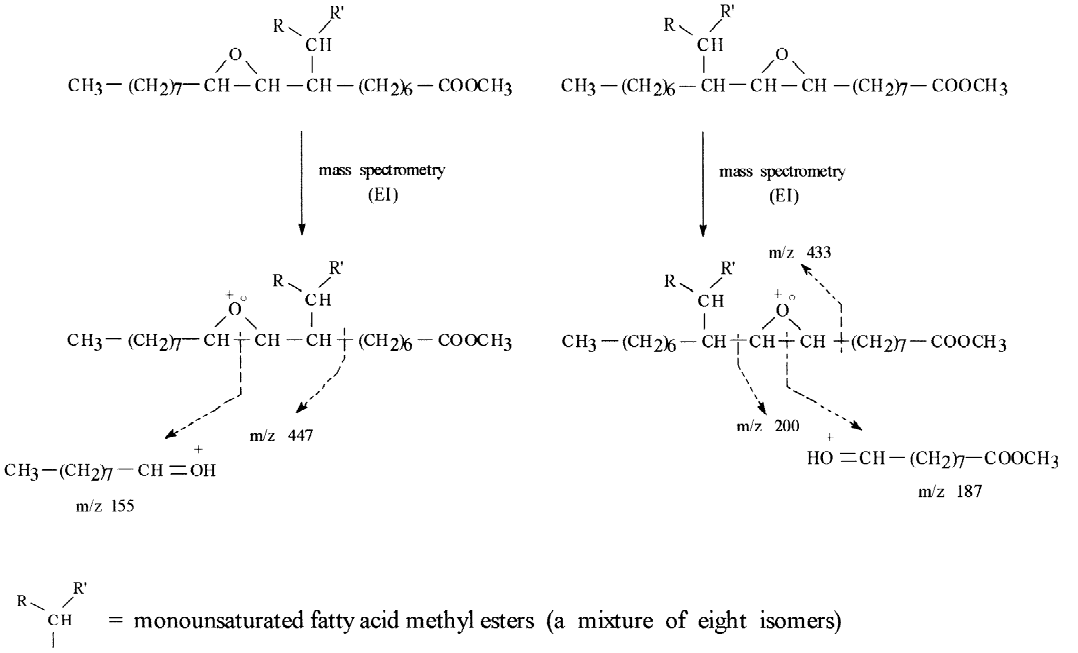


Fig. 9. Scheme of proposed mechanisms for the formation of the characteristic fragments of the epoxy-dimeric compounds found in the thermoxidized monounsaturated methyl esters (MeOl and MeEl). See Fig. 2 for the description of the substituents R and R'.

from an epoxy group in position 9,10 of the octadecanoate methyl ester molecule (Fig. 9). These fragments indicate that the dimers are the combination of a methyl oleate (or methyl elaidate) and a unit with the same number of carbon atoms that contains an epoxy group (an extra oxygen atom). Considering these facts, it can be stated that peaks 4 and D of Fig. 3 correspond mainly to a positional isomer mixture of methyl octadecenoate groups bonded to a 9,10-epoxy-octadecanoate derivative. However, it should be pointed out that it cannot be excluded the fact that MeEl or MeOl dimeric ethers can also be present in this mixture.

On the other hand, it must be noticed that small dimension differences between some *m*/*z* fragments of the thermoxidized MeOl and those of the thermoxidized MeEl (for example, 263 and 264 for Fig. 7a,b, respectively), were observed. Although these minor variations could be observed when injecting the same compound at different times, the differences in this case could be due to different ratios between the isomers present in the mixtures.

The mass spectra here presented confirm the fact that these isomer mixtures from thermoxidized MeEl and MeOl show similar distribution pattern and structures. Since the cGC–ITDMS is not able to provide information about the configuration and position of the double bonds present in these compounds, further studies with other analytical and derivatization techniques would be required to elucidate the complete structure of such groups of isomers.

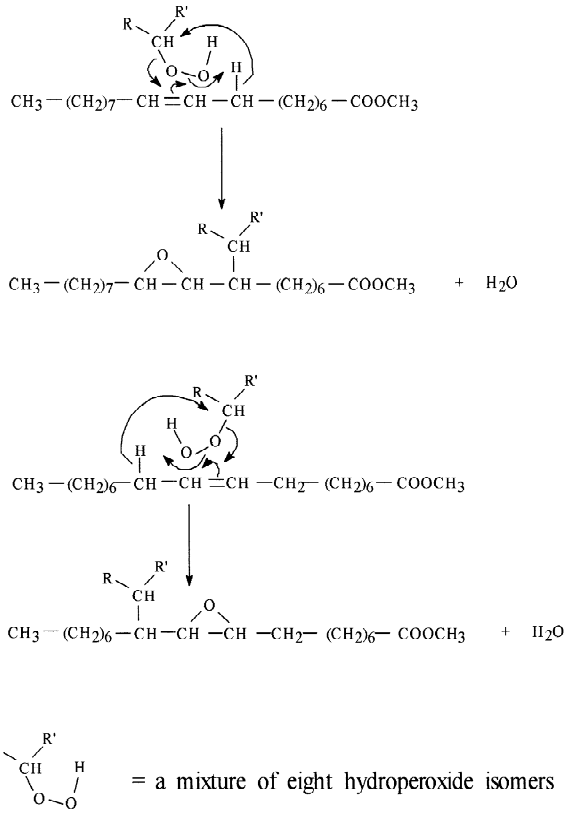


Fig. 10. Scheme of chemical reactions proposed for formation mechanisms of epoxy-dimeric compounds, generated by thermoxidation of monounsaturated methyl esters (MeOl and MeEl). See Fig. 2 for the description of the substituents *R* and *R′*.

4. Conclusions

The thermoxidative behavior of MeOl and MeEl was compared by using hyphenated chromatographic techniques. MeOl and MeEl were separately oxidized to generate the corresponding oxidation products. No evidence for the occurrence of *cis–trans* isomerization was found under these experimental conditions. The two isomeric 9,10-epoxystearic methyl esters formed in both MeOl and MeEl oxidations, but at different *threo*/*erythro* ratios; the *threo* isomer in both cases was predominant over the *erythro* one. Dimeric products originated by thermoxidation of MeOl and MeEl, had similar cGC characteristics and mass spectra, which suggests that the molecular structures and the formation mechanisms are similar (Fig. 10).

References

[1] G. Lercker, P. Capella, L.S. Conte, U. Pallotta, Rev. Fr. Corps Gras 25 (1978) 227.
[2] P. Capella, M.F. Caboni, G. Bonaga, G. Lercker, Riv. Ital. Sostanze Grasse 65 (1988) 629.
[3] H.V. Gardner, in: H.W.-S. Chan (Ed.), Autoxidation of Unsaturated Lipids, Academic Press, London, Orlando, San Diego, 1987, p. 51.
[4] R.F. Garwood, B.P.S. Kambay, B.C.L. Weedon, E.N. Frankel, J. Chem. Soc., Chem. Commun. (1977) 364.
[5] D. Swern, H.B. Knoght, J.T. Scalan, W.C. Ault, J. Am. Chem. Soc. 67 (1945) 1132.
[6] L. Williamson, J. Appl. Chem. 3 (1953) 301.
[7] E.N. Frankel, C.D. Evans, J.C. Cowan, J. Am. Oil Chem. Soc. 37 (1960) 418.

[8] M.M. Paulose, S.S. Chang, J. Am. Oil Chem. Soc. 55 (1978) 375.
[9] A.K. Sen Gupta, Fette Seifen Anstrichm. 69 (1967) 907.
[10] O.S. Privett, J. Am. Oil. Chem. Soc. 36 (1959) 507.
[11] C.B. Whitlock, W.W. Nawar, J. Am. Oil Chem. Soc. 53 (1976) 586.
[12] D.R.F. Howton, Radiat. Res. 20 (1963) 161.
[13] W. Grosch, in: H.W.-S. Chan (Ed.), Autoxidation of Unsaturated Lipids, Academic Press, London, Orlando, San Diego, 1987, p. 95.
[14] N.A. Khan, Oléagineux 19 (1964) 397.
[15] E.N. Frankel, W.E. Neef, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood, B.C.L. Weedon, Lipids 12 (1977) 901.
[16] G. Lercker, P. Capella, L.S. Conte, Riv. Ital. Sostanze Grasse 61 (1984) 337.
[17] G. Lercker, P. Capella, L.S. Conte, Riv. Ital. Sostanze Grasse 61 (1984) 623.
[18] E.N. Frankel, J. Am. Oil Chem. Soc. 61 (1984) 1908.
[19] R. Bortolomeazzi, L. Pizzale, G. Lercker, J. Chromatogr. 626 (1992) 109.
[20] G. Lercker, R. Bortolomeazzi, L. Pizzale, J. Am. Oil Chem. Soc. 75 (1998) 1115.
[21] W.E. Neff, E.N. Frankel, C.R. Scholfield, D. Weisleder, Lipids 13 (1978) 415.
[22] E.N. Frankel, C.D. Evans, H.A. Moser, D.G. McConnel, J.C. Cowan, J. Am. Oil Chem. Soc. 38 (1961) 130.
[23] R.F. Paschke, L.E. Peterson, S.A. Harrison, D.H. Wheeler, J. Am. Oil Chem. Soc. 41 (1964) 56.
[24] D.H. Wheeler, J. White, J. Am. Oil Chem. Soc. 44 (1967) 298.
[25] G. Lercker, J. Chromatogr. 279 (1983) 543.
[26] R.L. Veazey, J. Am. Oil Chem. Soc. 63 (1986) 1043.
[27] N. Jensen, J. Moller, Fette Seifen Anstrichm. 88 (1986) 352.
[28] C.N. Christopoulou, G. Perkins, J. Am. Oil Chem. Soc. 66 (1989) 1353.
[29] A. Bendini, T. Gallina Toschi, G. Lercker, Riv. Ital. Sostanze Grasse 76 (1999) 221.
[30] G. Lercker, T. Gallina Toschi, Riv. Sci. Alimentazione 27 (1998) 159.