Journal of Chromatography, 626 (1992) 109–116
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 215

Chromatographic determination of the position and configuration of isomers of methyl oleate hydroperoxides

Renzo Bortolomeazzi and Lorena Pizzale

Istituto di Tecnologie Alimentari, Università di Udine, Via Marangoni 97, 33100 Udine (Italy)

Giovanni Lercker

D.I.S.T.A.M., Università di Firenze, Via Donizetti 6, 50144 Firenze (Italy)

ABSTRACT

Oxygen can react with organic substrates (RH) to yield hydroperoxides, which in many instances are the first products of oxidation to be analytically isolated. A study of the structure of hydroperoxides could elucidate the reaction mechanisms activated during the first steps of oxidation processes. The simplest structural model used in the study of oxidation mechanisms of fats is methyl oleate. In this work the structures of methyl oleate hydroperoxides (MOHPs) were determined by gas chromatography–ion-trap detector mass spectrometry (GC–ITD-MS) of the corresponding hydroxystearates (MSHs). The hydroperoxides were reduced to methyl hydroxyoctadecenoates (MOHs), which were separated into the *cis* and *trans* fractions by argentation thin-layer chromatography. By hydrogenation of the double bond the *cis*- and *trans*-MOHs were reduced to MSHs for GC–ITD-MS analysis. Methods to isolate and determine the positional isomers of MOHPs were tested. The analytical techniques used were preparative high-performance liquid chromatography and GC–ITD-MS, solid-phase extraction–GC–ITD-MS and direct GC–ITD-MS.

INTRODUCTION

To understand the oxidation processes of unsaturated fractions of fatty substances, it is necessary to determine the relative composition and the concentration of the isomers of methyl oleate hydroperoxides (MOHPs). These isomers are defined with respect to double bond position and configuration, and peroxide group position.

The separation of MOHPs can be conveniently carried out only by liquid chromatography (LC) or high-performance liquid chromatography (HPLC), because they are thermally labile and chemically unstable. However, by using these techniques it is not possible to separate *cis* from *trans* isomers.

Grosh [1] described a method for the separation of MOHPs using HPLC, but the individual determination of *cis* and *trans* isomers for the MOHP system is limited to the use of gas chromatography (GC)–mass spectrometry (MS) and GC–nuclear magnetic resonance techniques [2,3]. The data obtained with these techniques are in agreement but, due to a lengthy sample preparation and to instrumental requirements, these techniques cannot be used for the analysis of a large number of samples.

This paper describes the HPLC separation of hydroxyoctadecenoates (MOHs) obtained from the reduction of MOHPs following the separation of isomers into *cis* and *trans* fractions by argentation thin-layer chromatography (TLC).

EXPERIMENTAL

Materials and reagents

Methyl oleate standard (>99%) was supplied by Nu-Chek-Prep (Elysian, MI, USA). Reagents and solvents (analytical or HPLC grade) were supplied by Carlo Erba (Milan, Italy).

Solid-phase extraction (SPE) columns (Bond

Correspondence to: Dr. G. Lercker, D.I.S.T.A.M., Università di Firenze, Via Donizetti 6, 50144 Firenze, Italy.

0021-9673/92/$05.00

Elut, Analytichem International, Varian, CA, USA) packed with 500 mg of silica were used.

HPLC

A Varian Model 5040 liquid chromatograph equipped with a Varian CDS 401 data system and a Varian UV 50 detector operating at 212 nm was used. The column (15 cm × 4.6 mm I.D.) was a Spherisorb CN (alkylnitrile) of 3 μm particle size (Phase Separations, Deeside, UK). Separations were performed under isocratic conditions at a flow-rate of 1.5 ml/min, using a solution of 0.3% anhydrous ethanol in *n*-hexane as the mobile phase.

GC and GC–ion-trap detector mass spectrometry

A Carlo Erba Model Mega 5160 high-resolution gas chromatograph equipped with a split–splitless injector and a flame ionization detector was used. The fused-silica column was an SP 2340 column (Supelco, Bellefonte, PA, USA), 30 m × 0.32 mm I.D., 0.2 μm film thickness, and the temperature ranged from 80 to 240°C with a gradient of 5°C/min. The carrier gas (helium) flow-rate was 1.6 ml/min and the split ratio 1:80 (v/v). The injector and detector temperatures were 230 and 240°C, respectively.

For GC–ion-trap detector mass spectrometric (ITD-MS) analysis a Varian 3400 capillary gas chromatograph, coupled to a Varian Saturn ion-trap detector, was used. The fused-silica column was a DB-5 column (5% phenylmethyl) (J&W, Folsom, CA, USA), 30 m × 0.255 mm I.D., 0.25 μm film thickness. The oven temperature was programmed from 220 to 300°C with a gradient of 5°C/min. The injection was in the split mode (split ratio 1:70) with helium as the carrier gas at a flow-rate of 1 ml/min. The injector, transfer line and manifold temperatures were 300, 300 and 320°C, respectively. The filament emission current was 10 mA. Samples were injected as trimethylsilyl (TMS) derivatives.

Preparation and isolation of MOHP

Before carrying out the oxidation process, the methyl oleate standard was purified by passing through an alumina (neutral) liquid chromatographic column (2 cm × 1 cm I.D.), using hexane as the elution solvent.

The oxidation step was carried out by transferring 1 g of methyl oleate in a PTFE-lined screw-cap tube and placing it in oven at 80°C for about 120–140 h. The sample was then dissolved in 1 ml of *n*-hexane and loaded onto a SPE silica column previously conditioned with 5 ml of *n*-hexane. The column was eluted with 10 ml of *n*-hexane (fraction 1) and with 5 ml of an *n*-hexane–diethyl ether (1:1, v/v) mixture (fraction 2).

Fraction 1 contained the main part of non-reacted methyl oleate, whereas fraction 2 contained MOHPs together with other polar products of the oxidation. Fraction 2 was loaded on a silica gel TLC plate, 20 × 20 cm, 0.25 mm thickness and eluted with an *n*-hexane–diethylether (65:35, v/v) mixture. The band corresponding to MOHPs, visualized under UV light (254 nm) after spraying with a 0.2% ethanolic solution of 2,7-dichlorofluorescein sodium salt, was scraped off, extracted with chloroform and evaporated under nitrogen flow.

Reduction of MOHPs

MOHPs were reduced to the corresponding MOHs in 1 ml of methanol by adding a spatula tip of solid $NaBH_4$ [4]. After the disappearance of bubbles (about 30 min), the solution was acidified with 2–3 drops of 6 *M* HCl and its volume reduced under a stream of nitrogen. After the addition of 0.5 ml of water, hydroxides were extracted twice with 2 ml of diethyl ether. The ether extract was dried with anhydrous Na_2SO_4 and evaporated to dryness under a flow of nitrogen.

Argentation thin-layer chromatography

MOHs were separated on a TLC plate, 15 × 20 cm, 0.25 mm thickness, impregnated with silver nitrate [5]. The two fractions were detected by viewing under UV light after spraying the plate with the 2,7-dichlorofluorescein solution. The two bands corresponding to *trans* and *cis* isomers were extracted with chloroform and evaporated to dryness under a nitrogen flow.

HPLC and GC–ITD-MS

MOHPs, the corresponding MOHs and the two *trans* and *cis* isomer fractions, were analysed by HPLC (Fig. 1). The *cis* and *trans* isomer mixtures of MOHs were fractionated into the single isomers by collecting the corresponding peaks at the outlet of the HPLC detector cell.

To confirm the structure of the eight positional

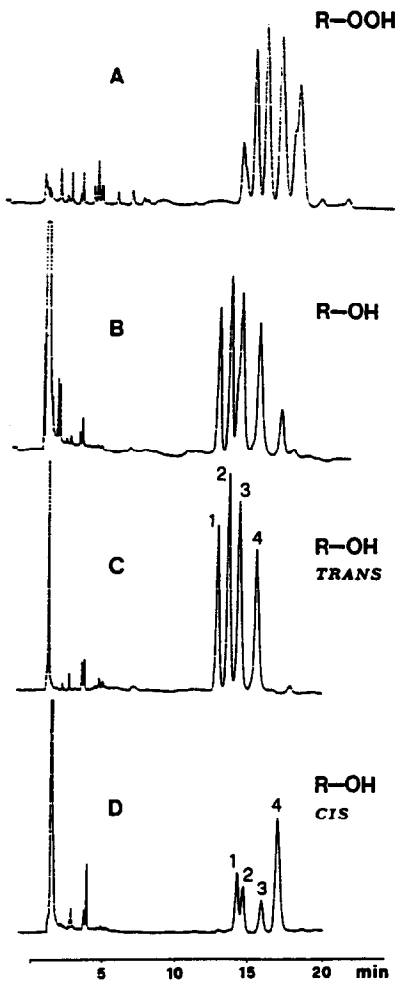

Fig. 1. HPLC chromatograms of (A) MOHPs; (B) MOHs; (C) *trans*-MOHs and (D) *cis*-MOHs.

and *cis*/*trans* isomers of MOHs, each single compound was analysed by GC–ITD-MS as the TMS ether. Using a similar procedure their corresponding methyl stearate hydroxides (MSHs), obtained by hydrogenation of each single MOH isomer using platinum as catalyst [2], were analysed.

For quantitative purposes the *trans* and *cis* isomer mixtures were analysed by GC–ITDMS with the same procedure before and after hydrogenation.

Preparation of the TMS ethers

The samples were silylated with about 0.1 ml of a pyridine–hexamethyldisilazane–trimethylchlorosilane (5:2:1, v/v) mixture, by storing them for 30 min at room temperature in a desiccator. After evaporation to dryness under nitrogen flow the samples were redissolved in 30–50 μl of benzene.

RESULTS AND DISCUSSION

For the collection of polar compounds from a methyl oleate peroxidation mixture we used an SPE column, which allows the elimination of the largest portion of non-reacted methyl oleate. This clean-up step made the improved isolation of the MOHP fraction from low oxidized systems possible.

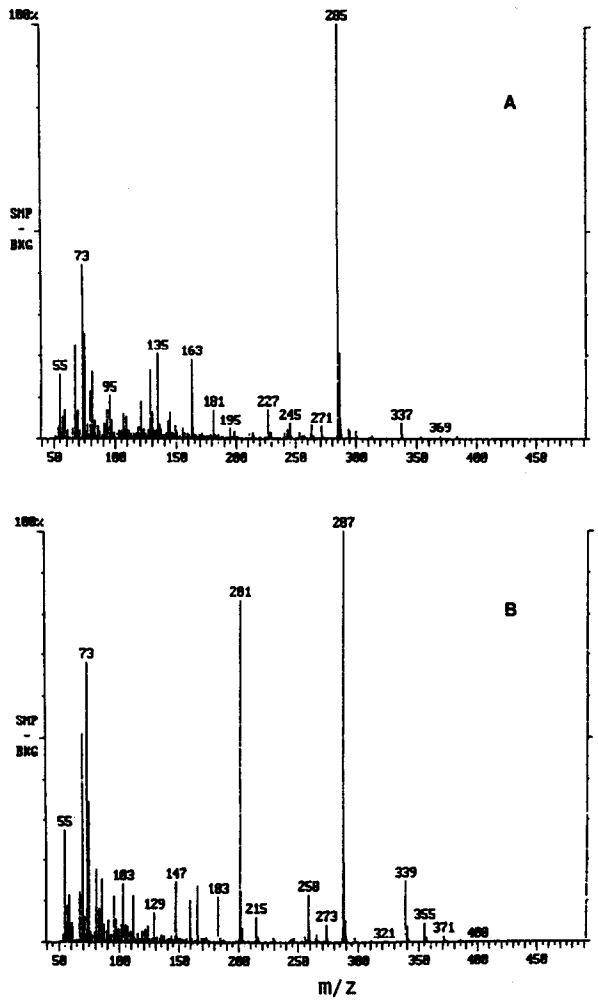

Fig. 2. ITD mass spectra of (A) peak 1 of Fig. 1C and (B) the same compound after hydrogenation.

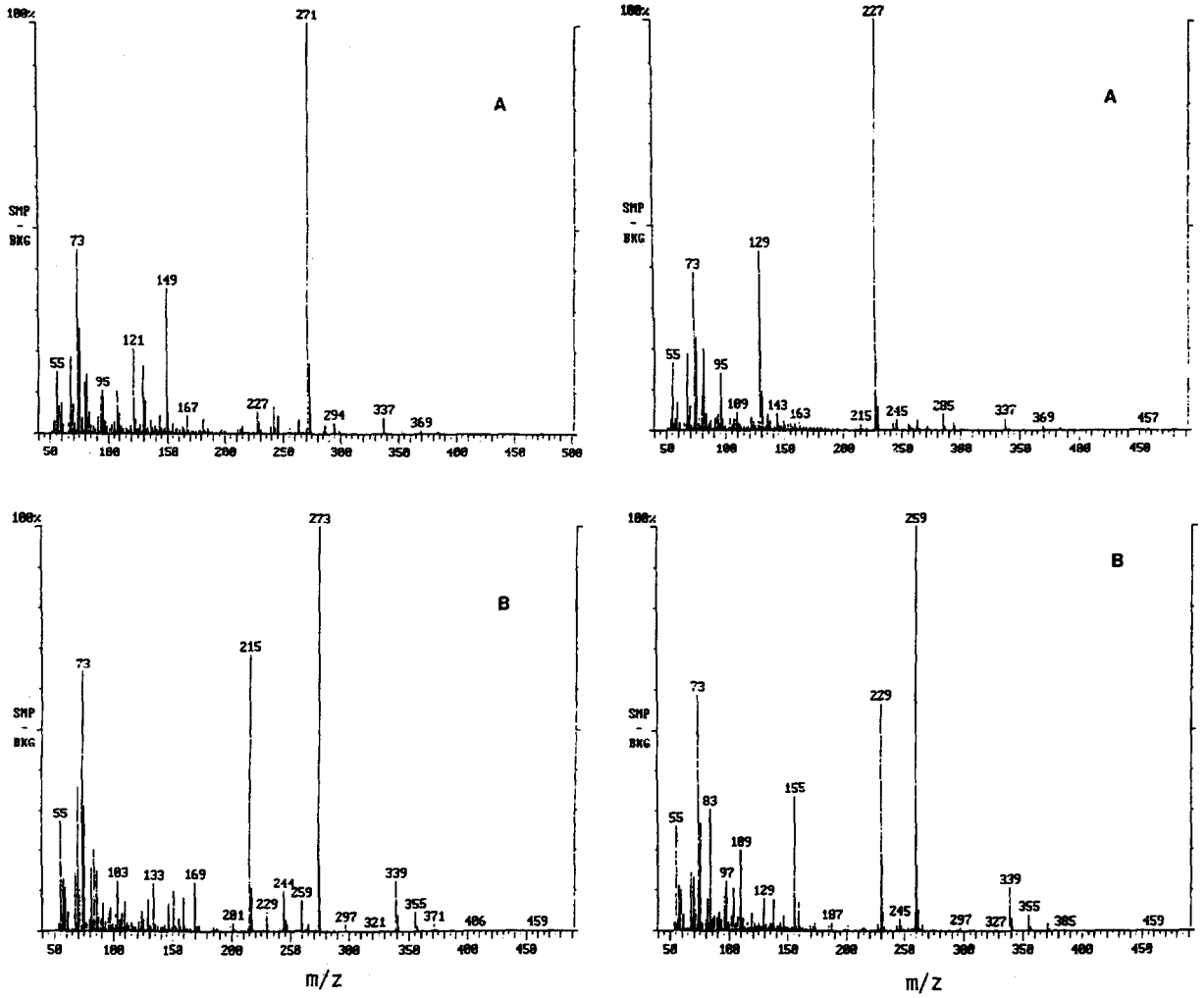

Fig. 3. ITD mass spectra of (A) peak 2 of Fig. 1C and (B) the same compound after hydrogenation.

Fig. 4. ITD mass spectra of (A) peak 3 of Fig. 1C and (B) the same compound after hydrogenation.

The HPLC chromatograms showing the separation of the MOHPs, the corresponding MOH and the *trans* and *cis* isomers mixture are presented in Fig. 1.

Similarly to what was observed for silica HPLC columns [6], with the alkylnitrile HPLC column it was not possible to separate either the eight MOHP isomers or the corresponding MOHs. In contrast, the *trans* isomers, and to a lesser degree the *cis* isomers, were fairly well resolved. This separation allowed the collection of each single isomer as it eluted from HPLC instrument.

In Figs. 2–5, the ITD mass spectra of peaks 1–4 in the HPLC chromatogram (Fig. 1C) of the *trans*-MOHs are shown. The corresponding spectra of peaks 1–4 in the HPLC chromatogram (Fig. 1D) of the *cis*-MOHPs are shown in Figs. 6–9.

The mass spectra of the four *trans* isomers of MOHs showed a very intense peak (base peak) at *m/z* 285, 271, 227 and 241, respectively. These ions are characteristic of MOH positional isomers, and have the –OH group in the 11, 10, 9 and 8 position, respectively. They originate from the homolytic cleavage of the C–C bond next to the –O–TMS

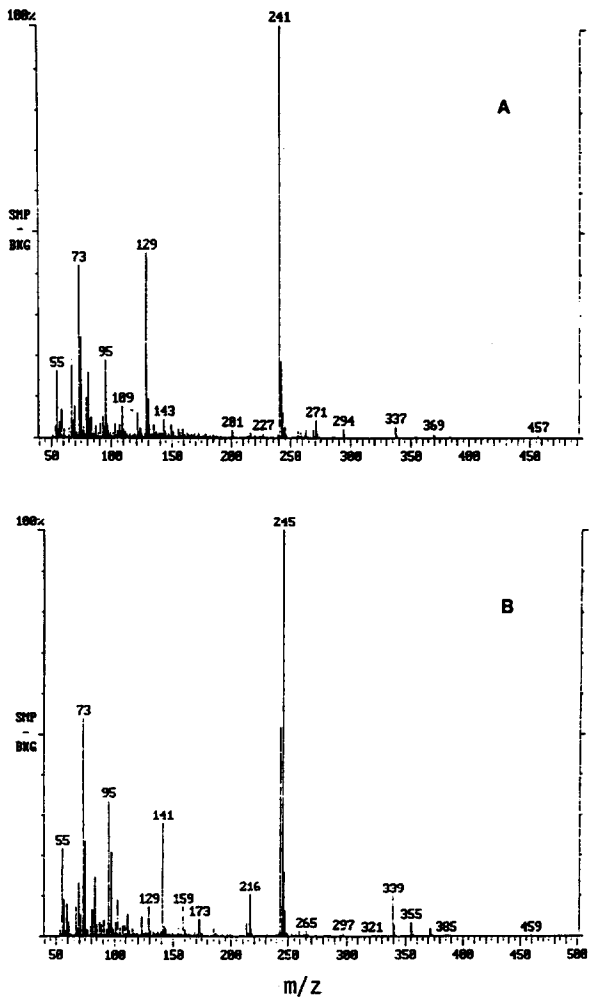

Fig. 5. ITD mass spectra of (A) peak 4 of Fig. 1C and (B) the same compound after hydrogenation.

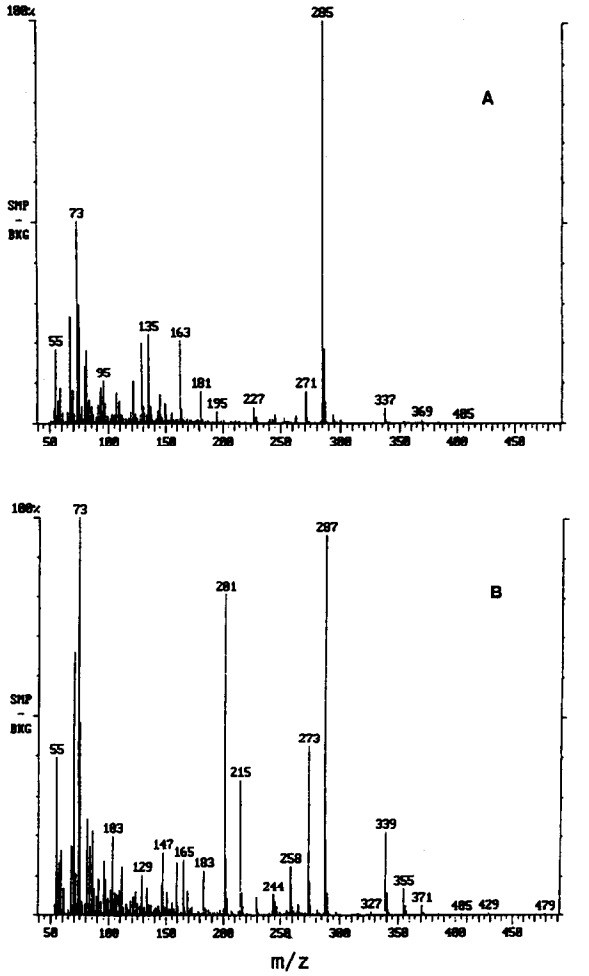

Fig. 6. ITD mass spectra of (A) peak 1 of Fig. 1D and (B) the same compound after hydrogenation.

group (α bond) on the opposite side of the double bond [7]. The structures of these ions are represented in Fig. 10.

The mass spectra of the corresponding MSHs show four pair of peaks at *m/z* 201/287, 215/273, 229/259 and 243/245, respectively. These pairs of ions originate from the homolytic cleavage of the C–C bonds next to the –O–TMS group and confirm the position that was previously assigned to the –OH group [7].

To the peaks in the HPLC chromatogram of *trans* MOHs (Fig. 1C) of the following positional isomers were assigned: 11-OH Δ^9 (peak 1); 10-OH Δ^8 (peak 2); 9-OH Δ^{10} (peak 3); 8-OH Δ^9 (peak 4).

A homologous sequence of positional isomers was obtained for the peaks in the HPLC chromatogram of *cis*-MOHs (Fig. 1D): 11-OH Δ^9 (peak 1); 10-OH Δ^8 (peak 2); 9-OH Δ^{10} (peak 3); 8-OH Δ^9 (peak 4).

The order of MOH elution obtained with this type of HPLC column is similar to that achieved in the separation of the corresponding MSH isomers

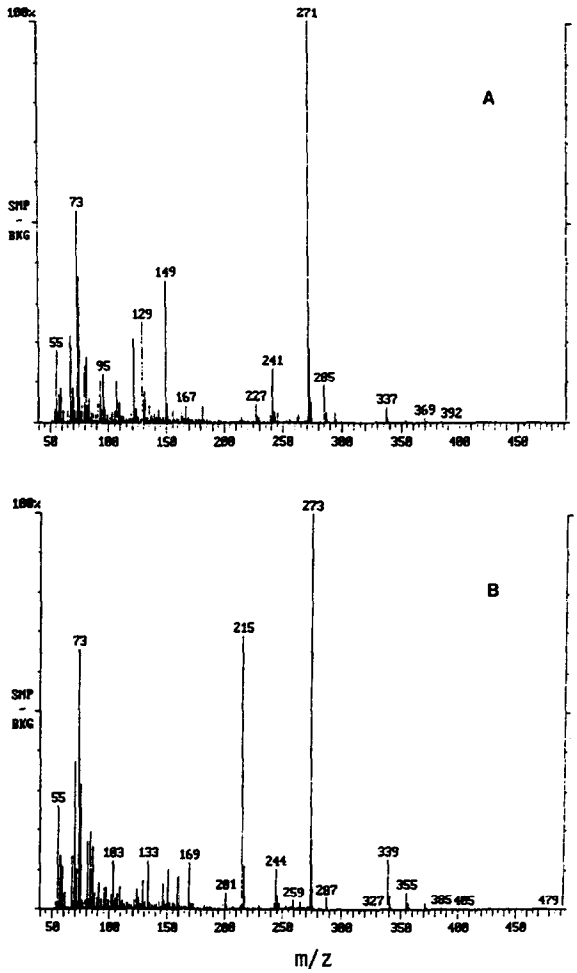

Fig. 7. ITD mass spectra of (A) peak 2 of Fig. 1D and (B) the same compound after hydrogenation.

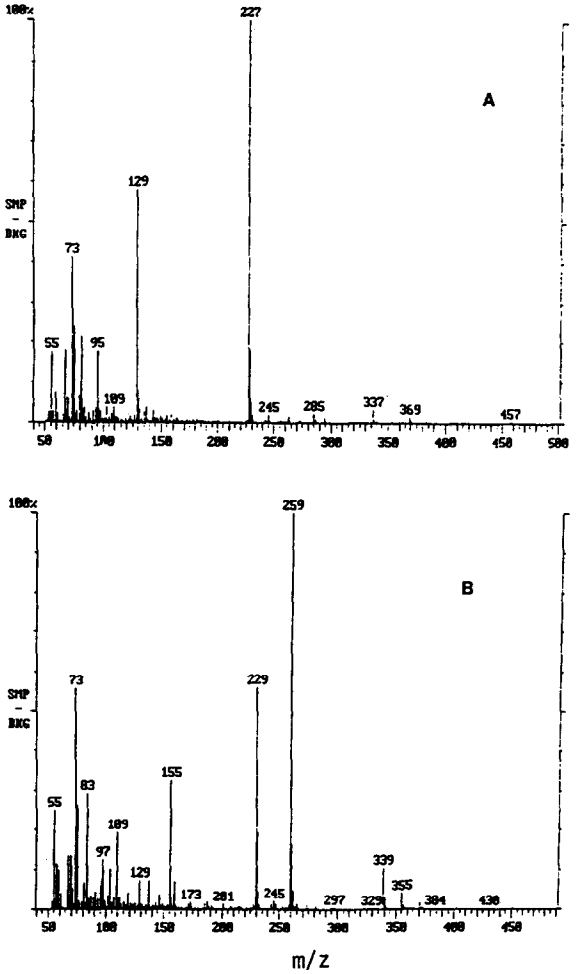

Fig. 8. ITD mass spectra of (A) peak 3 of Fig. 1D and (B) the same compound after hydrogenation.

using a silica column [6]. Thus we suggest that the position of the –OH group determines the order of elution, with 11-OH being the less retained isomer. With respect to the geometric isomerism, the *trans* isomers are slightly less retained than the corresponding *cis* isomers.

HPLC peak areas were used to calculate ratio compositions of each fraction of *trans* and *cis* positional isomers of MOHPs. The corresponding ratio compositions of each fraction of the *trans* and *cis* positional isomers of MSHs were also calculated using the relative intensities of the pairs of GC–MS ions, 201/287, 215/273, 229/259 and 243/245 for the 11-OH, 10-OH, 9-OH and 8-OH isomers, respectively. The mass spectra were obtained by computer processing, by averaging all scans containing the singlet ion monitoring (SIM) peaks. In fact, the SIM analysis showed that with our conditions only a partial separation of the MSH isomers can be achieved, as already noticed by other workers [3,8]. Compositions of *trans* and *cis* positional isomers, as calculated with the HPLC and GC–ITD-MS methods, are listed in Table I. The results obtained by comparing these two independent methods are in good agreement.

Isomers 9 and 10 were present in *trans* fractions

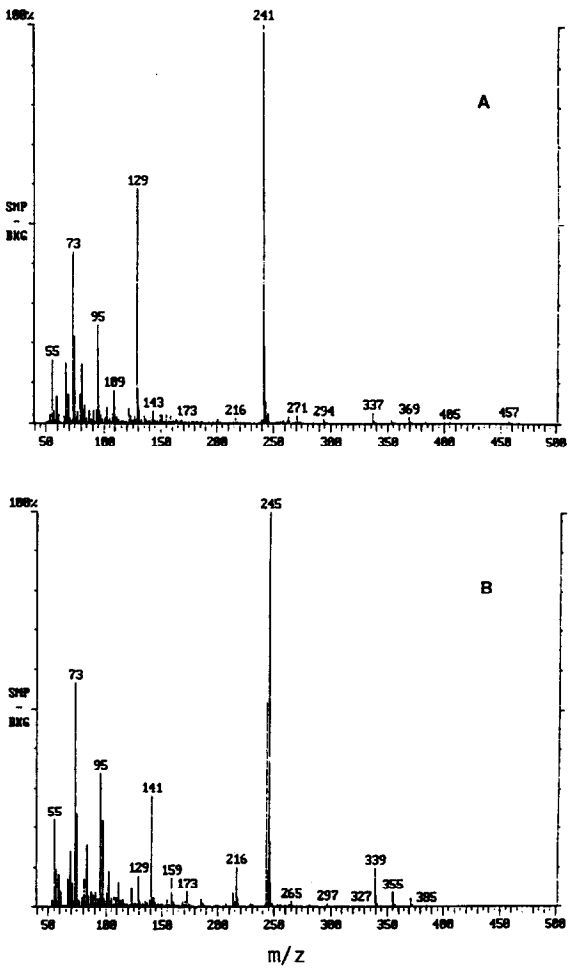

Fig. 9. ITD mass spectra of (A) peak 4 of Fig. 1D and (B) the same compound after hydrogenation.

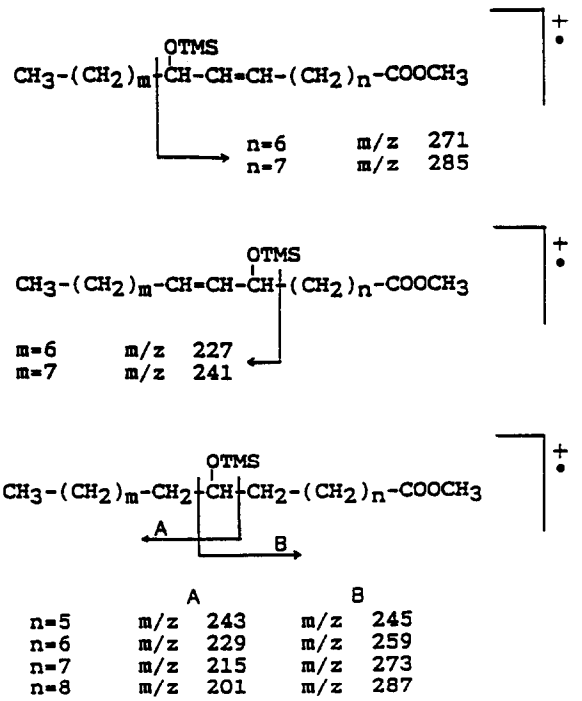

Fig. 10. Structures of ions present in the mass spectra of the four *trans*-MOH (or *cis*-MOH) isomers.

in larger amounts than isomers 8 and 11. In contrast isomers 8 and 11 predominated in the *cis* fractions; in particular, the isomer 8 alone accounted for about 50% of this fraction.

Table I also indicates the ratio compositions of the total *trans*- and *cis*-MOH isomers, as calculated using chromatographic areas obtained with the capillary column SP 2340, which resolved the *cis* from the *trans* isomers as TMS derivatives.

CONCLUSIONS

The use of an SPE column in the purification step of MOHPs allowed the elimination of the largest portion of non-reacted methyl oleate. This step made possible the isolation of MOHPs on a single TLC plate. With this procedure it is possible to collect very small amounts of MOHPs and this is a key factor in the study of the first steps of the reaction mechanisms in the oxidation of lipids. The composition of positional and *cis/trans* MOH isomers obtained by reducing the corresponding MOHPs and separating them by argentation TLC can be determined by a simple HPLC method. The results are in good agreement with those obtained by GC–ITD-MS. The use of a polar capillary column allows the rapid and direct determination of the *cis* and *trans* isomers ratio. This shortened procedure facilitates the complex analysis needed for the study of oxidation of model systems.

TABLE I

COMPARISON BETWEEN THE PERCENTAGE COMPOSITIONS OF THE POSITIONAL AND *trans/cis* ISOMERS OF THE METHYL HYDROXYOCTADECENOATES AS DETERMINED BY HPLC AND GC–ITD-MS

Conditions	Position isomers	*Trans* (84.8%)		*Cis* (15.2%)	
		HPLC	GC–ITD-MS[a]	HPLC	GC–ITD-MS[a]
80°C; 140 h	11-OH Δ^9	22.3	21.1	23.0	20.9
	10-OH Δ^8	27.1	28.0	17.3	20.1
	9-OH Δ^{10}	26.8	26.6	15.7	16.2
	8-OH Δ^9	23.8	24.3	44.0	42.8
		Trans (84.7%)[c]		*Cis* (15.3%)[c]	
		HPLC	GC–ITD-MS[a]	HPLC	GC–ITD-MS[a]
80°C; 120 h[b]	11-OH Δ^9	22.4	22.3	17.4	19.8
	10-OH Δ^8	29.0	28.2	16.3	17.8
	9-OH Δ^{10}	28.3	27.5	14.1	12.2
	8-OH Δ^9	20.3	22.0	52.2	50.2
80°C; 120 h[b]	11-OH Δ^9	20.1	24.1	19.6	21.2
	10-OH Δ^8	27.1	27.4	15.8	17.0
	9-OH Δ^{10}	28.4	25.3	12.6	11.4
	8-OH Δ^9	24.4	23.2	52.0	50.4

[a] Determined as methyl hydroxystearates TMS.
[b] Two sets of analyses shown for comparison.
[c] Mean of two determinations.

ACKNOWLEDGEMENTS

This research was supported by the National Research Council of Italy, Special Project RAISA, Sub-project No. 4, Paper No. 319.

REFERENCES

1 W. Grosch, *Lebensmittelchem. Gerichtl. Chem.*, 38 (1984) 81.
2 M. Piretti, B. Capella and U. Pallotta, *Riv. Ital. Sost. Grasse*, 46 (1969) 652.
3 E. N. Frankel, W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood and B. C. L. Weedon, *Lipids*, 12 (1977) 901.
4 G. Lercker, P. Capella, L. S. Conte and U. Pallotta, *Rev. Fr. Corps Gras*, 5 (1978) 227.
5 M. S. J. Dallas and F. B. Padley, *Lebensm.-Wiss. Technol.*, 10 (1977) 328.
6 H. W. S. Chan and G. Levett, *Chem. Ind.*, (1977) 692.
7 U. Pallotta, M. V. Piretti and P. Capella, *Riv. Ital. Sost. Grasse*, 47 (1970) 472.
8 R. F. Garwood, B. P. S. Khambay, B. C. L. Weedon and E. N. Frankel, *J. Chem. Soc. Chem. Commun.*, (1977) 364.