Heat Treatment of Vegetable Oils III. GC-MS Characterization of Cyclic Fatty Acid Monomers in Heated Sunflower and Linseed Oils After Total Hydrogenation[1]

J.L. Sebedio[a], J.L. LeQuere[b], O. Morin[c], J.M. Vatele[d], and A Grandgirard[a]

[a]INRA—Station de Recherches sur la Qualité des Aliments de l'Homme, 17 rue Sully, B.V. 1540, 21034 Dijon Cedex, France; [b]INRA—Laboratoire de Recherches sur les Arômes; [c]ITERG—Parc Industriel de Pessac, and [d]Université Claude Bernard, Laboratoire de Chimie Organique, UA 0467 CNRS

Fractions of cyclic fatty acid monomers (CFAM) were isolated from linseed oil heated at 275°C for 12 hr under nitrogen, at 240°C for 10 hr under nitrogen and at 240°C for 10 hr under air. Cyclic fatty acid monomers fractions were also isolated from a sunflower oil heated at 275°C for 12 hr under nitrogen and at 200°C for 48 hr in a commercial fryer. The CFAM fractions were hydrogenated and their composition studied by *gas liquid chromatography* coupled with mass spectrometry (GC-MS). The CFAM in the fraction isolated from heated linseed oil samples were a mixture (1:1) of *cis* and *trans* cyclopentyl and cyclohexyl isomers, while the CFAM in the fractions isolated from heated sunflower oils were mostly cyclopentyl isomers. The major cyclopentyl isomers were *trans* and *cis* methyl 7-(2'-hexylcyclopentyl)-heptanoate, methyl 9-(2'-butylcyclopentyl)-nonanoate and methyl 10-(2'-propylcyclopentyl)-decanoate. The major cyclohexyl isomers were the *trans* and *cis* methyl 9-(2'-propylcyclohexyl)-nonanoate which represented about 50% of the CFAM isomers isolated from heated linseed oil samples.

Recent studies (1, 2) have confirmed an earlier work (3) showing the formation of C_{18} α-disubstituted cyclopentane isomers after the heat treatment of vegetable oils along with C_{18} α-disubstituted cyclohexane monomers. Even though the structures of the cyclohexane isomers begin to be well established, especially by GC-MS and by comparison with some synthesized molecules (4-9), much work still needs to be carried out on the cyclopentane derivatives.

Recently we have shown that an oil rich in linolenic acid (linseed) gave mainly some Z, E diethylenic cyclic fatty acids while an oil rich in linoleic acid (sunflower) gave a mixture of Z and E monoethylenic CFAM isomers (1). In each case, both cyclohexyl and cyclopentyl structures were detected. However, the complete structure of these isomers could not be elucidated due to the complexity of the isolated CFAM mixtures. During that work, we also proposed a more complex MS fragmentation for the cyclopentane derivatives if compared to the well-known one for the cyclohexyl CFAM (3).

The purpose of this work was to establish the complete structure of these mono- and di-unsaturated CFAM esters, and it was necessary as a first step to hydrogenate the CFAM fractions. The effects of the hydrogenation are to eliminate the geometrical and positional isomers and thus to simplify the resulting CFAM mixtures. It was then possible to establish the carbon skeleton of the components from the different fractions (nature of the substituents, size of the ring). Furthermore, the MS spectra of the isolated CFAM esters were compared with those of some synthesized C_{18} α-disubstituted cyclopentane monomers (10).

The comparison of the MS spectra of the synthesized molecules and those obtained in fractions isolated from heated oils and further hydrogenated showed that the heat treatment of an oil rich in linolenic acid gives a mixture of C_{18}-cyclohexane and cyclopentane monomers, while that of an oil rich in linoleic acid gives mainly some C_{18}-cyclopentane isomers and minor amounts of C_{18}-cyclohexane monomers.

MATERIALS AND METHODS

Purification of solvents. All the solvents were redistilled before use.

Heating conditions and isolation procedure. The linseed oil was heated at 275°C for 12 hr under nitrogen, at 240°C for 10 hr under nitrogen and at 240°C for 10 hr under air. The sunflower oil was heated at 275°C for 12 hr under nitrogen and at 200°C for 48 hr as previously described (1, 11). CFAM fractions were isolated by a combination of column chromatography, urea adduct fractionation and high performance liquid chromatography as previously described (11).

Hydrogenation of cyclic fatty acid monomers. The hydrogenation was effected using platinum oxide as catalyst in 10 ml of a mixture of chloroform and methanol (2:1) as solvent and a hydrogen pressure of 2 bars. Each reaction was allowed to proceed 3 hr. The catalyst was removed by filtration and the saturated methyl esters were extracted with chloroform after the addition of water.

Gas liquid chromatography-mass spectrometry (GC-MS). The GC-MS analyses were effected on a Ribermag R 10-10C (Nermag) quadrupole mass spectrometer coupled to a Girdel 31 gas chromatograph fitted with a capillary column coated with Carbowax 20M (35 m × 0.32 mm i.d., film thickness 0.25μm). Helium was used as the carrier gas with a linear velocity of 35 cm/sec, and the oven temperature was maintained at 180°C. Mass spectra were generated at 70 eV with a source temperature of 150°C.

A Hewlett-Packard 5970 Mass Selective Detector coupled with a Hewlett-Packard gas chromatograph (model 5890) was also used for some GC-MS studies. The column used was a fused silica column (J & W Scientific Rancho Cordo, California) coated with DB Wax (30 m long and 0.25 mm i.d., film thickness 0.5 μm). The temperature was programmed from 50 to 200°C at 20°C/min, held at 200°C for 25 min, then programmed from 200°C to 220°C and held at 220°C until completion of the analyses. Splitless injection was used in all cases, and the injection port was maintained at 240°C.

[1]For part II in this series see Ref. 1.

RESULTS AND DISCUSSION

Heated linseed oils. The total ion chromatograms of the hydrogenated CFAM fraction isolated from a linseed oil heated under nitrogen and under air are shown in Figure 1. The peak identification code numbers and letters used in the present study are included in this figure. Mass spectra were obtained for all the numbered peaks with molecular ions at m/z 296, confirming that these peaks are hydrogenated CFAM. The expected fragmentations for saturated cyclic acid esters (1) were observed for each spectrum (Table 1). Ion fragments A to D and their rearrangement ions D-32, D-32-18 and B +1 derived from a well established fragmentation of the substituents in the α-position of the ring (12-14). The structures were unambiguously assigned to each peak (Table 2) on the basis of their individual mass spectrum. As already postulated (1) and verified on MS of synthesized disubstituted cyclopentane derivatives (10), fragmentations of the alkyl chain in the disubstituted cyclopentyl esters take place in α- and β-positions, leading to two series of ions D, D-32 and D-32-18 (Table 1).

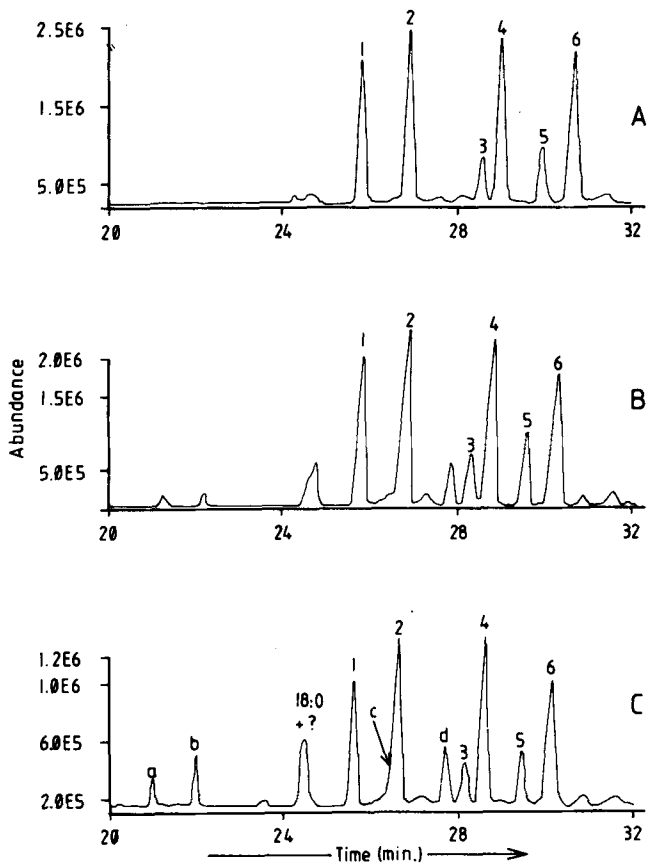

FIG. 1. Total ion chromatograms (DB Wax column, 30 m long × 0.25 mm i.d.) of the hydrogenated CFAM isolated from a linseed oil heated at A, 275°C/12 hr/N_2; B, 240°C/10 hr/N_2; and C, 240°C/10 hr/air.

The structures of the *trans*- and *cis*-isomers of methyl 9-(2′-butylcyclopentyl)-nonanoate (Fig. 2, MS of the *trans*-isomer) were definitely established by comparison of their respective retention times and MS with those of authentic samples (10).

The configurations reported in Table 2, the *trans*- one being assigned to the earlier eluting isomer, were also confirmed for the disubstituted cyclopentane isomers (10).

The only cyclohexyl esters isolated from linseed oil heated under nitrogen were the *trans*- and *cis*-isomers of methyl 9-(2′-propylcyclohexyl)-nonanoate. These cyclic fatty acid esters isomers were the major product characterized in previous studies on heated linseed oil (3, 14). This was also a confirmation of our previous hypothesis (1), i.e., the cyclohexenyl-alkenoates characterized in linseed oil heated under nitrogen differ only by the position of the E-ethylenic bond present on the carboxylate moiety.

For linseed oil heated under air (Fig. 1C), a higher level of methyl stearate appeared in the CFAM fraction, probably resulting from a less efficient nonurea adduct fractionation. Moreover, four extra major peaks were detected (identified with letters in Fig. 1). Peaks a and b with respective retention times of 21.06 and 22.02 min on a DB Wax Column under the conditions described in the experimental section have mass spectra very similar to that of methyl stearate with a molecular ion at m/z 298. These were identified as some methyl-branched chain isomers of methyl stearate.

Some branched fatty acids can be found naturally in small amounts in some vegetable oils (15), and these were

TABLE 1

GC-MS Fragmentation of Some Hydrogenated Cyclic Fatty Acid Monomers isolated From a Heated Linseed Oil (275C, 12 hr, N_2)

Compound (Fig. 1)	Ion fragment: m/z (% relative intensity)										
	M*	M-32	M-31	D[a]	D-32	D-32-18	B	B+1	C	A	Base
1	296(5.2)	264(4.9)	265(2.4)	239(2.7) 253(3.7)	207(3.9) 221(1.1)	189(4.6) 203(1.1)	171(1.3)	172(2.4)	125(17.5)	57(23.8)	55
2	296(12.2)	264(6.5)	265(3.1)	253(7.2) 267(0.8)	221(4.4) 235(0.8)	203(2.1) 217(0.4)	185(5.4)	186(3.3)	111(30.8)	43(43.2)	55
3	296(3.8)	264(5.0)	265(1.1)	239(1.6) 253(3.3)	207(3.8) 221(2.2)	189(2.7) 203(1.6)	171(1.1)	172(2.7)	125(18.8)	57(22.2)	55
4	296(6.7)	264(1.0)	265(1.6)	253(10.5)	221(8.0)	203(5.1)	171(1.2)	172(8.6)	125(42.3)	43(37.9)	55
5	296(10.0)	264(6.3)	265(2.9)	253(6.3) 267(0.3)	221(4.1) 235(0.7)	203(2.6) 217(0.7)	185(3.7)	186(2.9)	111(30.2)	43(45.8)	55
6	296(7.2)	264(1.2)	265(2.0)	253(13.5)	221(10.2)	203(5.6)	171(1.1)	172(10.2)	125(53.0)	43(39.8)	55

[a]Successive cleavage of the alkyl moiety for the cyclopentyl derivatives.

TABLE 2

Hydrogenated CFAM Identified by GC-MS in Heated Linseed Oil

Peak code[a]	ECL[b]		Component	Configuration
	Supelcowax[c]	Carbowax 20M, 180°C		
1	18.12	18.12	methyl 9-(2'-butylcyclopentyl)-nonanoate	*trans*-
2	18.27	18.28	methy 10-(2'-propylcyclopentyl)-decanoate	*trans*-
3	18.49	18.51	methyl 9-(2'-butylcyclopentyl)-nonanoate	*cis*-
4	18.54	18.54	methyl 9-(2'-propylcyclohexyl)-nonanoate	*trans*-
5	18.67	18.70	methyl 10-(2'-propylcyclopentyl)-decanoate	*cis*-
6	18.74	18.76	methyl 9-(2'-propylcyclohexyl)-nonanoate	*cis*-

[a]Peak code according to Fig. 1.
[b]Equivalent chain length.
[c]See Ref. 2.

also reported in oxidized soybean oil (16) and in partially hydrogenated soybean oil heated in simulated deep fat frying conditions (2). The iso- and anteiso-isomers are those most often found in nature, the iso- compound being eluted first (15,17,18). In fact, their respective mass spectra were very similar to those of the 16-methylheptadecanoate (iso-18) and 15-methylheptadecanoate (anteiso-18) published by Ryhage and Stenhagen (19).

Peak d (Fig. 1) was unambiguously identified using the mass spectrum (2) as methyl 8-(2'-butylcyclohexyl)-octanoate (*trans*). Peak c (Fig. 1), despite a poor chromatographic resolution for the GC-MS analysis (shoulder in Fig. 1) which did not allow a clear mass spectrum, was tentatively identified as methyl 7-(2'-pentylcyclohexyl)-heptanoate (*trans*). Their configurations were tentatively proposed from the observation that in all the heated oils presently studied *trans*-isomers are in higher proportion than their corresponding *cis*-counterparts. Moreover, their relative retention times agreed well with those of the *trans*-isomers characterized in partially hydrogenated soybean oil (2).

No major differences were observed in the two CFAM fractions isolated from linseed oil samples heated at 240°C with or without nitrogen. (Fig. 1B and C). However, at 275°C (Fig 1A), only traces of components a, b and d were observed. Scanning of peak 2 did not permit detection of component c, which was detected for the oils heated to 240°C with or without nitrogen. In fact, it is very difficult to make conclusions about the influence of the temperature on the formation of cyclic fatty acids after heat treatment of linseed oil. As mentioned earlier, the urea fractionation was not as good for samples B and C as for sample A. It is, therefore, possible that some of the branched fatty acids (a and b) could have been lost during the urea fractionation. However, this would not in theory explain the differences observed for components c and d which seem to be some cyclohexyl isomers.

Heated sunflower oils. For all the peaks identified by code numbers or letters in the total chromatograms of

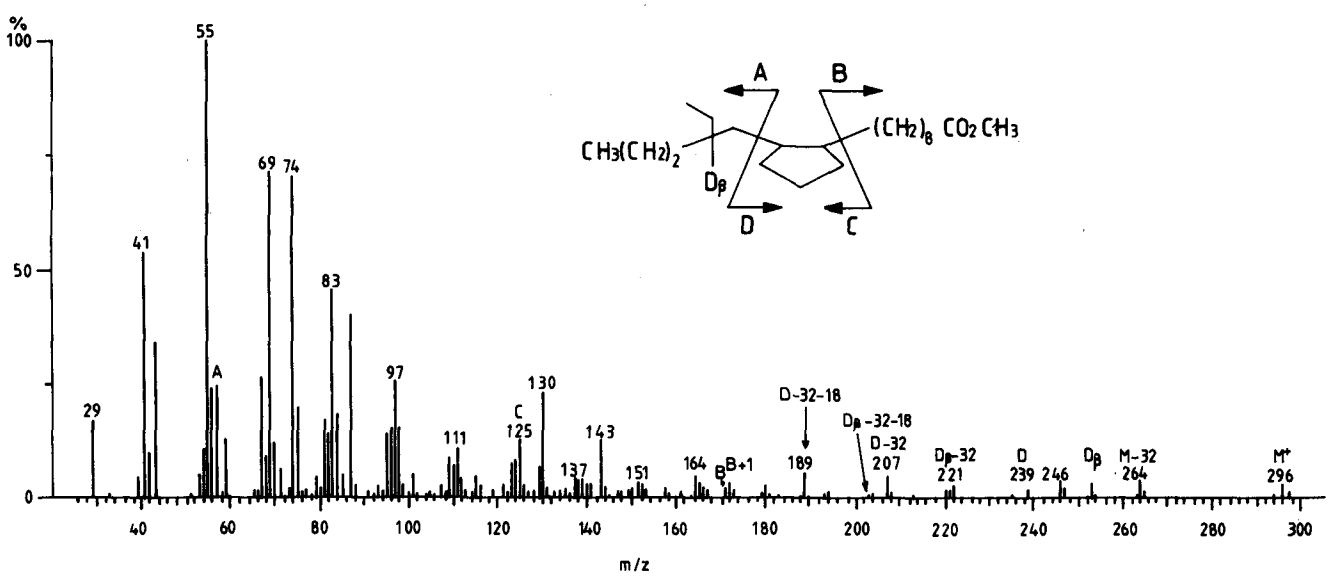

FIG. 2. Mass spectrum of peak 1 (Fig. 1) identified as *trans*-methyl 9-(2-butylcyclopentyl)-nonanoate.

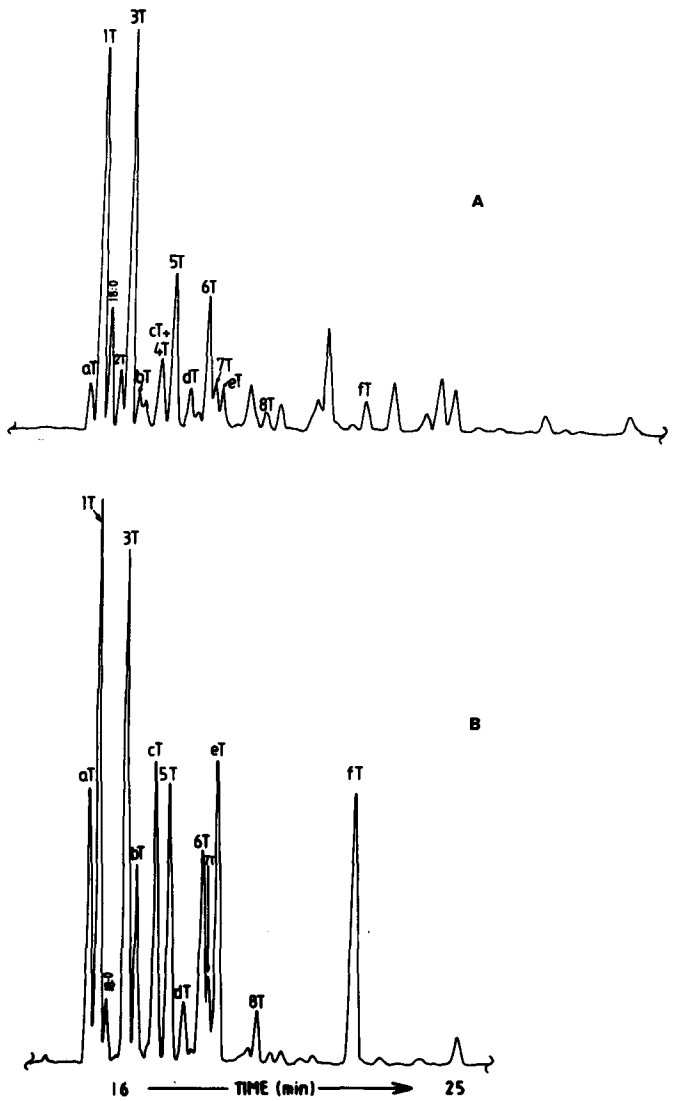

FIG.3. GLC analyses on a Carbowax 20 M, glass capillary column (35 m long × 0.32 mm i.d.) of the hydrogenated CFAM from a sunflower oil heated at 275°C for 12 hr under N_2 (A) and at 200°C for 24 × 2 hr under air (B).

the hydrogenated CFAM fractions isolated from a sunflower oil heated under nitrogen and under air (Fig. 3), the mass spectra were those of saturated cyclic esters, with molecular ions at m/z 296 or characteristic ions derived from them: M-18, M-32 and M-32-18 at m/z 278, 264 and 246, respectively. Structures of all the numbered peaks (1T-8T) were deduced from their respective mass spectra (Table 3) where the expected fragmentations for CFAM were always observed. Characteristic fragments for some of the CFAM, newly identified in this study, are presented in Table 4. As already stated, more than one fragmentation occurred in the alkyl moiety of the cyclopentane derivatives.

The structures of the *trans*- and *cis*-isomers of the methyl 7-(2′-hexylcyclopentyl)-heptanoate were definitely established by comparison of their respective retention times and MS with those of a synthetic sample (10). Mass spectra of peaks 2T and 4T, tentatively identified as the *trans*- and *cis*-methyl 4-(2′-octylcyclohexyl)-butanoates, were very similar with the published spectrum of an authentic methyl 4-(2′-octylcyclohexyl)-butanoate (20). The main feature of this spectrum is a very intense ion fragment at m/z 220. This M-76 fragment was attributed to a rearrangement ion with successive loss of CH_3OH and CH_2=CHOH from the molecular ion, and is characteristic of fatty acid esters branched on C-6 of the carboxylate moiety (21). Substitution in the 4- or 5-position affords only small peaks at the parent mass less 76, and on branching in other positions, no peak appears (21).

The mass spectrum of peak aT (Fig. 4) is obviously related to this mechanism of fragmentation, with an intense ion at m/z 220. Owing to its retention time (ECL =17.90), an attractive candidate was therefore methyl 5-(2′octylcyclopentyl)-pentanoate. However, the mass spectrum of an authentic sample of methyl 5-(2′-octylcyclopentyl)-pentanoate was found significantly different (10). Particularly, an unattributed ion fragment at m/z 148 appeared at m/z 134 in the mass spectrum of aT. After a careful examination of the spectrum, however, peak aT was tentatively identified as *trans*-methyl 4-(2′-nonylcyclopentyl)-butanoate, peak cT (ECL = 18.26) being identified as its *cis*-isomer. However, these compounds should be confirmed by synthesis. As previously outlined (10), the intensity of fragment C decreases when the length of the alkyl chain increases. Thus, fragment C is

TABLE 3

Hydrogenated CFAM Identified by GC-MS in Heated Sunflower Oil

Peak code[a]	ECL[b] Carbowax 20M, 180°C	Component	Configuration
1T	17.96	methyl 7-(2′-hexylcyclopentyl)-heptanoate	*trans*-
2T	18.07	methyl 4-(2′-octylcyclohexyl)-butanoate[c]	*trans*-
3T	18.12	methyl 9-(2′-butylcyclopentyl)-nonanoate	*trans*-
4T	18.28	methyl 4-(2′-octylcyclohexyl)-butanoate[c]	*cis*-
5T	18.34	methyl 7-(2′-hexylcyclopentyl)-heptanoate	*cis*-
6T	18.51	methyl 9-(2′-butylcyclopentyl)-nonanoate	*cis*-
7T	18.54	methyl 9-(2′-propylcyclohexyl)-nonanoate	*trans*-
8T	18.76	methyl 9-(2′-propylcyclohexyl)-nonanoate	*cis*-

[a]Peak code according to Fig. 3.
[b]Equivalent chain length.
[c]See Ref. 20.

TABLE 4

GC-MS Fragmentation of Some Hydrogenated Cyclic Fatty Acid Monomers Isolated From Heated Sunflower Oils (200°C, 24 × 2 hr under air and 275°C, 12 hr under N_2)

Compound	Ion fragment: m/z (% relative intensity)										
(Fig. 3)	M⁺	M-32	M-31	D[a]	D[a]-32	D[a]-32-18	B	B+1	C	A	base
IT	296(15.8)	264(7.1)	265(3.4)	211(3.7) 225 (0.6) 239(1.6) 253(1.9)	179(11.8) 193(1.2) 207(0.6) 221(0.7)	161(5.8) 175(2.3) 189(2.0) 203(0.2)	143(42.7)	144(7.1)	153(2.2)	85(7.5)	55
2T[b]	296(3.2)	264(9.7)	265(4.4)	183(4.3)	151(23.7)	133(20.8)	101(11.2)	102(10.1)	195(0.7)	113(2.3)	74
5T	296(11.3)	264(5.6)	265(2.8)	211(3.7 225(0.2) 239(2.9) 253(1.1)	179(9.1) 193(1.2) 207(2.8) 221(0.6)	161(4.9) 175(1.7) 189(3.2) 203(/)	143(37.4)	144(6.5)	153(1.7)	85(8.0)	55

[a]Successive cleavage of the alkyl moiety for the cyclopentane derivatives.
[b]Spectrum of the *cis*-isomer 4T was obtained in mixture with cT.

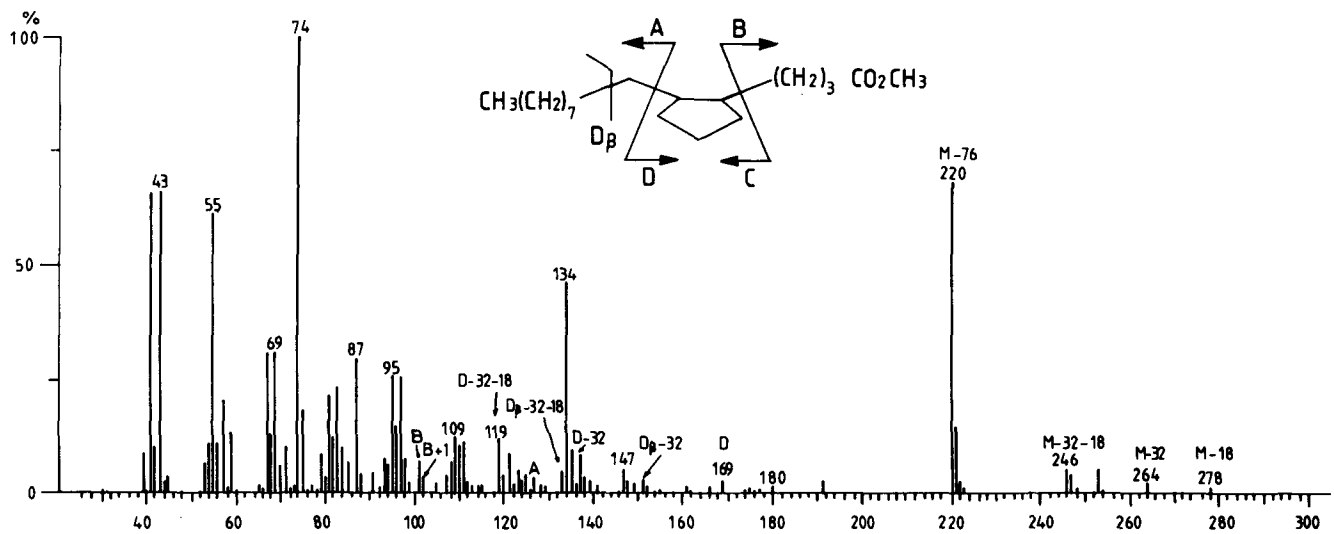

FIG. 4. Mass spectrum of peak aT (Fig. 3) tentatively identified as *trans*-methyl 4-(2-nonylcyclopentyl)-butanoate.

absent in the spectra of the octylcyclohexyl (2T and 4T) and nonylcyclopentyl (aT and cT) derivatives.

The MS fragmentations of the remaining peaks identified with letters indicate that the peaks bT (ECL = 18.17) and dT (ECL = 18.42) are ring isomers. This is also the case for the pair eT (ECL = 18.58) and fT (ECL = 19.17).

The isomers bT and dT were not identified, even though their ECL values were in good correspondence with the ECL values given for *trans*- and *cis*-methyl 7-(2′-pentylcyclohexyl)-heptanoate (2). Peaks eT and fT were tentatively identified as the *trans*- and *cis*-isomers of methyl 10-(2′-ethylcyclohexyl)-decanoate, but abundances of the diagnostic ions were rather low. Moreover, their ECL values did not correspond to those given for the methyl 10-(2′-ethylcyclohexyl)-decanoates characterized in partially hydrogenated soybean oil (2).

Other minor components in the chromatogram presented in Figure 3A were not identified. The mass spectra of some of them displayed a molecular ion at m/z 294. This could indicate that they are bicyclic components, as already signaled in another fraction of the same sunflower oil heated under air (1).

For oils heated under the same conditions there are about 10 times more cyclic fatty acid monomers in heated linseed oil which contains both linoleic and linolenic acid than in heated sunflower oil which only contains traces of linolenic acid (1,11). Furthermore, the structures of the CFAM formed from linolenic acid are different from those arising from linoleic acid. Linoleic acid gives mainly a mixture of *cis*- and *trans*-cyclopentyl CFAM while linolenic acid gave a mixture of *cis*- and *trans*-cyclopentyl and cyclohexyl components. In the latter case only two major cyclohexyl isomers were observed, the *cis*- and *trans*-methyl 9-(2′-propylcyclohexyl)-nonanoates.

From these data, it is difficult to make conclusions on the respective influence of the temperature and the oxygen. Under low or high temperatures the same important CFAM are formed. Differences were observed only in their relative proportions and their amounts in the oils. Most of the major cyclic fatty acids were identified by GC-MS. However, some structures still have to be confirmed by synthesis. These are methyl 4-(2′-nonylcyclopentyl)-butanoate and methyl 10-(2′-ethylcyclohexyl)-decanoate.

REFERENCES

1. Sébédio, J.L., J.L. Le Quéré, E. Sémon, O. Morin, J. Prévost and A. Grandgirard, *J. Am. Oil Chem. Soc. 64*:1324 (1987).
2. Rojo, J.A., and E.G. Perkins, *Ibid. 64*:414 (1987).
3. Potteau, B., P. Dubois and J. Rigaud, *Ann. Technol. Agric. 27*:655 (1978).
4. Hutchison, R.B. and J.C. Alexander, *J. Org. Chem. 28*:2522 (1963).
5. MacDonald, J.A., *J. Am. Oil Chem. Soc. 33*:394 (1956).
6. McInnes, A.G., F.P. Cooper and J.A. MacDonald, *Can. J. Chem. 39*:1906 (1961).
7. Graille, J., A. Bonfand, P. Perfetti and M. Naudet, *Chem. Phys. Lipids 27*:23 (1980).
8. Awl, R.A., and E.N. Frankel, *Lipids 17*:414 (1982).
9. Awl, R.A., E.N. Frankel and L.W. Tjarks, *Chem. Phys. Lipids 34*:25 (1983).
10. Vatèle, J.M., J.L. Sébédio and J.L. LeQuéré, *Chem. Phys. Lipids,48*:119 (1988).
11. Sébédio, J.L., J. Prévost and A. Grandgirard, *J. Am. Oil Chem. Soc. 64*:1026 (1987).
12. Michael, W.R., *Lipids 1*:359 (1966).
13. Michael, W.R., *Ibid 1*:365 (1966).
14. Friedrich, J.P., *J. Am. Oil Chem. Soc. 44*:244 (1967).
15. Christie, W.W., *Lipid Analysis. Isolation, Separation, Identification and Structure Analysis of Lipids*, 1st edn, Pergamon Press, Oxford, 1973.
16. Artman, N.R., and J.C. Alexander, *J. Am. Oil Chem. Soc. 45*:643 (1968).
17. Ackman, R.G., J.C. Sipos, and C.S. Tocher, *J. Fish. Res. Bd. Canada 24*:635 (1967).
18. Ackman, R.G., and C.A Eaton, *Fette, Seifen, Anstrichm. 80*:21 (1978).
19. Ryhage, R., and E. Stenhagen, *J. Lipid Res. 1*:361 (1960).
20. Zeman, A., and H. Scharmann, *Fette, Seifen, Anstrichm. 75*:32 (1973).
21. Meyerson, S., and L.C. Leitch, *J. Am. Chem. Soc. 88*:56 (1966).

[Received April 4, 1988; accepted September 18, 1988]