Characterization of Fatty Acid Isomers in Dehydrated Castor Oil by Gas Chromatography and Gas Chromatography–Mass Spectrometry Techniques

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ABSTRACT: Fatty acid isomers present in dehydrated castor oil were analyzed by gas chromatography and gas chromatography–mass spectrometry (GC–MS) of their dimethyl oxazoline derivatives. Conjugated linoleic acid 9,11 and 10,12 isomers were identified by GC based on equivalent chainlengths. Segmental peak analysis of GC–MS total ion chromatogram mass fragmentation pattern revealed the presence of 7,9 and 8,10 conjugated linoleic acids along with 9,11 and 10,12 conjugated linoleic acids. Interestingly, a reverse elution of *trans,trans*-conjugated linoleic acids was observed, *viz.* 10,12 was followed by 9,11, which was in turn followed by the 8,10 and 7,9 isomers. The observed reverse order of elution was in contrast to the conventional elution pattern of both nonconjugated and conjugated *cis,cis*-, *cis,trans*- and *trans,cis*-isomers.
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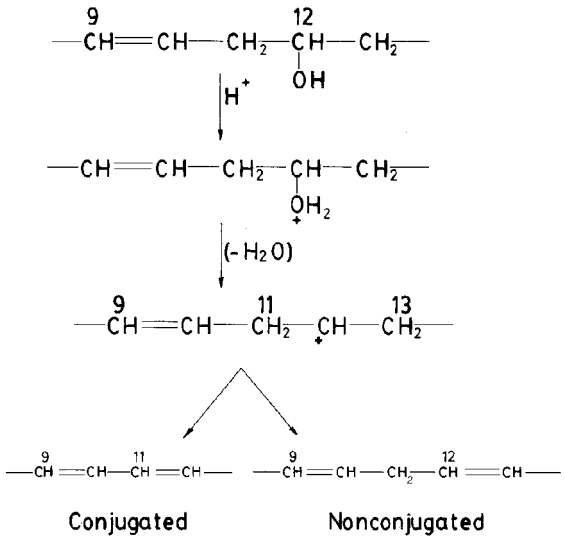
KEY WORDS: Castor oil, conjugated dienes, conjugated linoleic acid, dehydrated castor oil, dehydration, 4,4-dimethyloxazoline derivative, GC–MS, segmental peak analysis, SPA.



SCHEME 1

Dehydrated castor oil (DCO) is extensively used in the surface coating industry and is prepared by catalytic dehydration of castor oil (1,2). During the dehydration process, the hydroxyl group of ricinoleic acid ($C_{18:1}$; 12-hydroxy-9-*cis*-octadecenoic acid) is replaced by a double bond. The extent of dehydration of castor oil attains a plateau when around 95% of the hydroxyl functionality is replaced. The acid-catalyzed mechanism proceeds through the initial protonation of the hydroxyl group, resulting in a hydroxonium ion that subsequently loses water to form a carbonium ion (Scheme 1; adapted from Ref. 2). Owing to the availability of a proton from one or another of the neighboring carbon atoms, different products are formed, i.e., the nonconjugated linoleic acids (NCLA) and the conjugated linoleic acids (CLA), based on the position of the newly formed double bond in relation to the existing double bond (2). Because of the operating conditions (high temperature, long time), different positional and geometric fatty acid isomers are formed. CLA isomers have attracted much attention recently because of their proposed anticarcinogenic and anticholesterolemic activities (3–5). They are present naturally in milk, dairy products, and meat from ruminants and are formed during high-temperature refining of vegetable oils (6).

Identification of the various fatty acid isomers using chemical methods is rather laborious and often inconclusive (7). Therefore, instrumental techniques such as gas chromatography (GC) employing equivalent chainlength (ECL) determination are widely used in identifying the fatty acid isomers (8–10). CLA have been identified based on their ECL in GC by comparison with those of available standards, *viz.* commercially available geometric mixtures of 9,11 and 10,12 CLA (11). The position of the double bond in a fatty acid chain can be identified by GC–mass spectrometry (MS) of derivatized fatty acids (12). Recently, the use of DMOX (4,4-dimethyloxazoline) derivatives has been shown clearly to identify the double bond position in both conjugated and nonconjugated systems (13–16).

Though the foregoing techniques are well known for the identification of fatty acid isomers, an attempt has been made in this paper to extend GC–MS analyses of fatty acid DMOX derivatives toward the detailed characterization of conjugated fatty acids in dehydrated castor oil with the aid of segmental peak analysis (SPA).

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MATERIALS AND METHODS

Bleached castor oil was obtained from Hathibhai Bhulakidas (Mumbai, India). Standard CLA, a mixture of geometric isomers of 9,11- and 10,12-linoleic acid, and methyl esters were obtained from Nu-Chek-Prep Inc. (Elysian, MN). A normal (straight chain, no conjugation or branching) fatty acid methyl ester (FAME) standard was supplied along with the column used for GC. All chemicals and solvents used were of analytical grade.

GC. Gas chromatographic analyses of FAME were performed on a Shimadzu GC 17A (Shimadzu Corporation Inc., Kyoto, Japan) gas chromatograph equipped with a flame-ionization detector (FID). The injector and detector temperatures were maintained at 250°C. Helium was the carrier gas at 20 cm/s with a split ratio of 1:100. The analyses were carried out on a CPSIL-88 (Chrompack, Middelburg, The Netherlands; 50 m × 0.25 mm i.d., 0.20 μm film thickness) column under isothermal conditions (175°C). Prior to each GC run, the column was conditioned at 220°C for 2 h to ensure noninterference by extraneous peaks. This step was necessary because of the elution of methyl ricinoleate after 100 min under the above conditions.

To estimate methyl ricinoleate, FAME analysis was carried out using 2% OV-17 on 80/100 Chromosorb WHP packed column (Chromatopack, Mumbai, India; 1′ × 1/8″ i.d.). Nitrogen was the carrier gas at 10 mL/min. The injector and FID detector were kept at 230 and 250°C, respectively. The GC oven was temperature programmed as follows: 150°C for 2 min, then raised to 230°C at the rate of 8°C/min. and held at 230°C for 3 min. Under these conditions, methyl ricinoleate eluted after 9.5 min.

GC–MS. A Finnigan Mat GCQ GC–MS (Finnigan Mat Inc., Austin, Texas) was used for mass spectral analyses. The GC separation of fatty acid DMOX derivatives was performed on CPSIL-88 column as described above, with oven temperature of 200°C. Helium was the carrier gas at 40 cm/s. MS was used in the electron impact mode at 70 eV with a source temperature of 250°C. Splitless injections were employed with the injector port at 250°C.

Mass spectra of the DMOX derivatives of standard samples of oleate ($C_{18:1}$ *cis*), elaidate ($C_{18:1}$ *trans*), linoleate ($C_{18:2}$ *cis*,*cis*), linoelaidate ($C_{18:2}$ *trans*,*trans*), and CLA (mixture of geometric 9,11 and 10,12 isomers) obtained on the Finnigan Mat GCQ GC–MS (ion-trap mass detector) were compared with mass spectral data from the Shimadzu QP 5000 (quadrupole mass detector) to ensure identical fragmentation patterns.

Dehydration of castor oil. Bleached castor oil [400 g; 89% ricinoleic acid by GC, iodine value (IV) (Wijs) 85.5, acid value (AV) 0.1, hydroxyl value 160.0 (17)] was heated to 240°C under vacuum of 5 mm Hg in the presence of 1.0% sodium bisulfate in a Parr autoclave with constant stirring for 3 h. The filtered DCO product had an IV (Wijs) 132.0, AV 3.0, and hydroxyl value 9.0 (17).

FAME preparation. DCO (250 mg) was mixed with 50 mL of methanol and 1.5 mL of 13% methanolic potassium hydroxide at 60°C for 30 min; 3 mL of boron trifluoride/methanol was added and boiled under reflux for 30 min. The FAME were extracted with hexane (5 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. Standard fatty acids were also subjected to the above treatment. Standard solutions (10%, wt/vol) were prepared in chloroform; 0.5 μL of the FAME solution was injected into the gas chromatograph.

Fatty acid DMOX derivative preparation. FAME (100 mg) were taken along with 2-amino-2-methyl propanol (0.25 mL) in a tube, flushed with nitrogen, and capped. The capped tube was heated at 180°C in a constant-temperature oil bath for 6 h (12). The reaction mixture was cooled and mixed with dichloromethane (2 mL) and water (2 mL). The organic layer was transferred to another glass tube, and dichloromethane was evaporated under nitrogen atmosphere. Hexane (2 mL) was added to the residue and dried over anhydrous sodium sulfate. Hexane was further removed under nitrogen, and the fatty DMOX derivatives were then finally dissolved in chloroform for GC and GC–MS analysis.

RESULTS AND DISCUSSION

The fatty acid composition of castor oil and DCO is presented in Table 1. After dehydration for 3 h, about 95% of the ricinoleic acid was converted, resulting in the formation of both NCLA and CLA. A typical gas chromatographic trace of DCO FAME is presented in Figure 1C along with a trace of the standard FAME containing both regular (Fig. 1B) and CLA (Fig. 1A). The CLA elute at a greater elapsed time than the NCLA isomers and do not overlap with the fatty acids that are normally present in castor oil.

GC peaks of DCO FAME were tentatively identified based on the ECL of known FAME on a 50-m CPSIL capillary column (presented as fatty acid, NCLA, or CLA, followed by ECL): t_9-18:1, 18.46; c_9-18:1, 18.66; c_{11}-18:1, 18.73; t_9,t_{12}-18:2, 19.20; c_9,t_{12}-18:2, 19.40; t_9,c_{12}-18:2, 19.49; c_9,c_{12}-18:2, 19.61; c_9,t_{11}-18:2 and t_9,c_{11}-18:2, 20.84; c_{10},t_{12}-18:2, 20.94; t_{10},c_{12}-18:2, 20.99; c_9,c_{11}-18.2, 21.07–21.10; c_{10},c_{12}-18:2, 21.13; t_9,t_{11}-18:2 and t_{10},t_{12}-18:2, 21.32. Various geometrical isomers (*trans*,*trans*; *cis,trans*; *trans*,*cis*; and *cis*,*cis,* in that

TABLE 1
Relative Fatty Acid Composition (%) of Castor Oil and DCO

Fatty acid	Castor oil[a]	DCO[b]
16:0	1.0	1.2
18:0	1.2	1.5
18:1	3.8	4.0
18:2 NCLA	4.5	48.0
18:2 CLA	—	37.0
18:1, OH	88.0	5.0
Others	1.5	3.3

[a]Analyzed on OV-17 packed column (Chromatopack, Mumbai, India).
[b]Analyzed on CPSIL-88 capillary column (Chrompack, Middelburg, The Netherlands). Abbreviations: DCO, dehydrated castor oil; NCLA, nonconjugated linoleic acids; CLA, conjugated linoleic acids; 18:1, OH, ricinoleic acid; —, not detected or below detection limit.

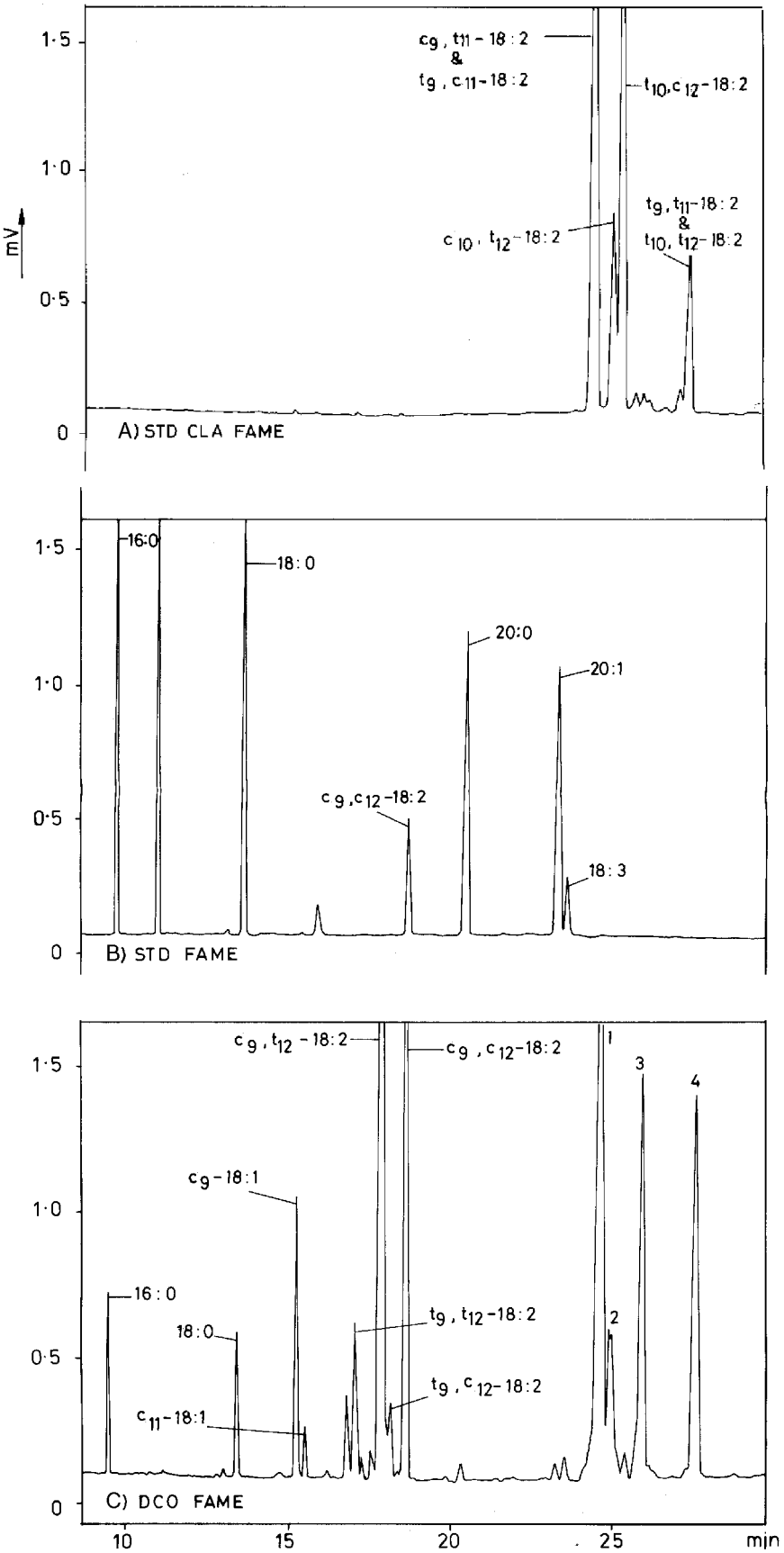

FIG. 1. Gas chromatography trace of (A) standard conjugated linoleic acid (CLA) fatty acid methyl esters (FAME) (B) standard FAME, and (C) dehydrated castor oil (DCO) FAME, on CP SIL-88 column (Chrompack, Middelburg, The Netherlands).

order) of NCLA were observed. Such isomers have previously been identified during the analysis of partially hydrogenated oils and fats, where they are present in substantial amounts (18). Further confirmation of their structure was carried out in this study by GC–MS analyses. Of the four isomers of NCLA, the *cis*,*trans* and *cis*,*cis* isomers were the major components and comprised *ca.* 61 and 30%, respectively. About 70% of the NCLA contained at least one *trans*-double bond. Further discussion in this paper will relate to the identification of CLA isomers.

Four major peaks in the CLA portion of the trace were labeled 1, 2, 3, and 4 in order of increasing elution time (Fig. 1C). Based on ECL, peak 1 was tentatively projected as a mixture of 9,11-*cis*,*trans* and *trans*,*cis* isomers; peak 2 as unidentified components; peak 3 as 9,11-*cis*,*cis* and peak 4 as a mixture of 9,11- and 10,12-*trans*,*trans* isomers. Further confirmation was attempted by GC–MS of DMOX derivatives.

Mass spectra of standard fatty acid DMOX derivatives ($C_{18:1}$ *cis*, $C_{18:1}$ *trans*, $C_{18:2}$ 9,12-*cis*,*cis* and $C_{18:2}$ 9,12-*trans*,*trans*) were analyzed. The spectra were distinct, with intense molecular ions and characteristic diagnostic ions indicating the positions of the double bond. $C_{18:1}$ *cis* and $C_{18:1}$ *trans* showed intense molecular ions (*m/z* 335) and characteristic diagnostic fragments at *m/z* 196 and 208 (*i.e.,* 12 mass units between C8 and C9, indicating the location of the double bond between C9 and C10, in contrast to the normal fragmentation of 14 units for methylene groups in the fatty acyl chain) (13). The geometric *cis* and *trans* isomers could not be distinguished from their mass spectra. $C_{18:2}$ 9,12-*cis*,*cis* and $C_{18:2}$ 9,12-*trans*,*trans* isomers showed intense molecular ions (*m/z* 333) characteristic of linoleic acid and diagnostic fragments at *m/z* 196, 208 (for double bond at C9), 236, and 248 (for double bond at C12) (15).

SPA of the total ion chromatograms for oleic and linoleic acid DMOX derivatives (both *cis* and *trans*) was carried out. SPA involves obtaining and analyzing the mass spectrum of the ion chromatogram peak at progressive retention times within the peak area. When two components coelute and are observed as a single peak, SPA can be used to detect the two components by studying the changing intensities of the characteristic fragmentation ions. The spectra obtained for oleic and linoleic DMOX derivatives were clearly identical at all retention times within the peak area.

The total ion chromatogram of the standard CLA mixture is shown in Figure 2. The fatty acid DMOX derivatives were generally well resolved. Four major peaks were observed and labeled 5, 6, 7, and 8. Peak 7 was clearly seen as a combination of two peaks that were not base-resolved.

Table 2 lists the characteristic mass fragmentation intensities by SPA of the various peaks of Figure 2. Peaks 5 and 6 were pure peaks containing only single components of 9,11 and 10,12 CLA isomers, respectively. Peak 5 gave a fragmentation pattern characteristic of 9,11 CLA (Fig. 3); *i.e.*, ions with *m/z* 12 difference between 196 and 208 (for the double bond at C9) and another *m/z* 12 difference between *m/z* 222 and 234 (for the double bond at C11). Similarly, peak 6 gave fragmentation ions with *m/z* 12 difference between *m/z* 210, and 222 (for the double bond at C10) and another *m/z* 12 difference between *m/z* 236 and 248 (for the double bond at C12). Mass spectra of 9,11 and 10,12 CLA DMOX derivatives have been reported earlier, and our results are in agreement with earlier reports (14,16). SPA of Peak 7 revealed the coelution of 9,11 and 10,12 isomers (*cis*,*cis,* based on their ECL). In this peak, the 9,11 isomer appears to elute before the 10,12 isomer, as revealed by the characteristic fragmentation ion intensities (Table 2). SPA of Peak 8 gave interesting results. Here again the peak was a mixture of 9,11 and 10,12 CLA isomers (*trans*,*trans* based on their ECL). SPA of the

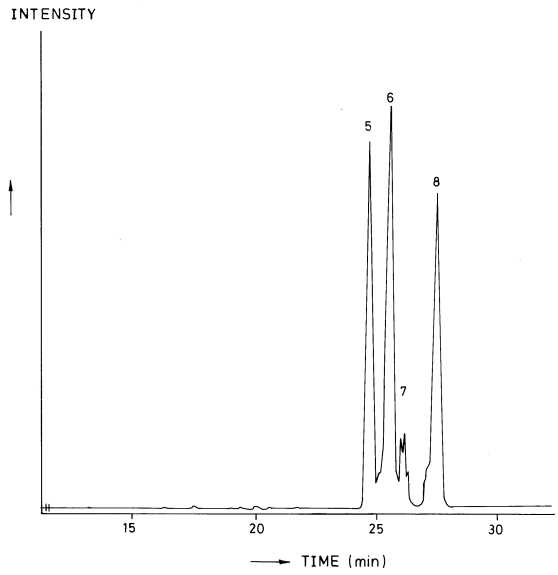

FIG. 2. Total ion chromatogram of standard CLA dimethyloxazoline (DMOX) derivative. For other abbreviation see Figure 1.

TABLE 2
Diagnositic Fragmentation Ions and Intensities of Standard CLA DMOX Derivatives by SPA

Peak no.	Identification[a]	Diagnostic ions *m/z* (% intensity)			
5	9,11	196 (7.6)	208 (7.4)	222 (17.5)	234 (15.4)
6	10,12	210 (5.1)	222 (14.4)	236 (10.2)	248 (2.9)
7	9,11 (up)	196 (7.1)	208 (5.6)	222 (13.7)	234 (10.5)
	9,11 (apex)	196 (9.9)	208 (5.3)	222 (18.8)	234 (8.1)
	10,12 (apex)	210 (3.6)	222 (18.8)	236 (8.4)	248 (7.1)
	10,12 (down)	210 (5.2)	222 (13.2)	236 (8.3)	248 (6.3)
8	10,12 (up)	210 (7.3)	222 (9.1)	236 (10.7)	248 (3.7)
	10,12 (apex)	210 (2.9)	222 (16.6)	236 (7.6)	248 (9.2)
	9,11 (apex)	196 (8.1)	208 (6.2)	222 (16.6)	234 (9.8)
	9,11 (down)	196 (8.6)	208 (6.1)	222 (16.9)	234 (10.3)

[a]Molecular ion (*m/z*): 333; base ion (*m/z*): 126. DMOX, 4,4-dimethyloxazoline; SPA, segmental peak analysis; for other abbreviation see Table 1.

upslope yielded fragmentation ions characteristic of the 10,12 CLA isomer, whereas the downslope yielded ions characteristic of the 9,11 CLA isomer. The elution of 10,12 CLA *trans*,*trans* isomer before the 9,11 CLA *trans*,*trans* isomer, which is contrary to the elution order for *cis*,*cis* or *cis*,*trans* and *trans*,*cis* CLA isomers, is now reported for the first time.

The total ion chromatogram of DCO fatty acid DMOX derivatives is shown in Figure 4. Four major peaks were observed and labeled as 9, 10, 11, and 12. Table 3 lists the characteristic mass fragmentation intensities by SPA of the various peaks of Figure 4. Peak 9 gave a fragmentation pattern that indicated the coelution of 7,9/8,10 and 9,11 CLA isomers. SPA showed the presence of 7,9/8,10 and 9,11 isomers (in order of increasing retention time) in the upslope of the peak; only the 9,11 isomer appeared at the apex; and 8,10 isomer in the downslope of the peak. The diagnostic fragmentation ions for 7,9 and 8,10 isomers (presented in Table 3) are consistent with the formation of *m/z* 12 units that indicate the location of the double bonds. It appears that within Peak 9, two 8,10 geometric isomers are separated by a 9,11 isomer. Peak 10 was found to be a mixture of 9,11 and 10,12 isomers, with the 9,11 isomer eluting before the 10,12 isomer. Peak 11 was purely 9,11 isomer (*cis*,*cis* by ECL), as shown by the presence of fragmentation ions characteristic of that isomer. The unusual pattern of elution of *trans*,*trans* CLA standard isomers was also seen from SPA of peak 12, which was a mixture of all four *trans*,*trans* CLA isomers (10, 12/9, 11/8, 10/7, 9) in the increasing order of elution. We offer no clear explanation for this peculiar observation, though the stereogeometry of the molecules seems to play an important role. Further

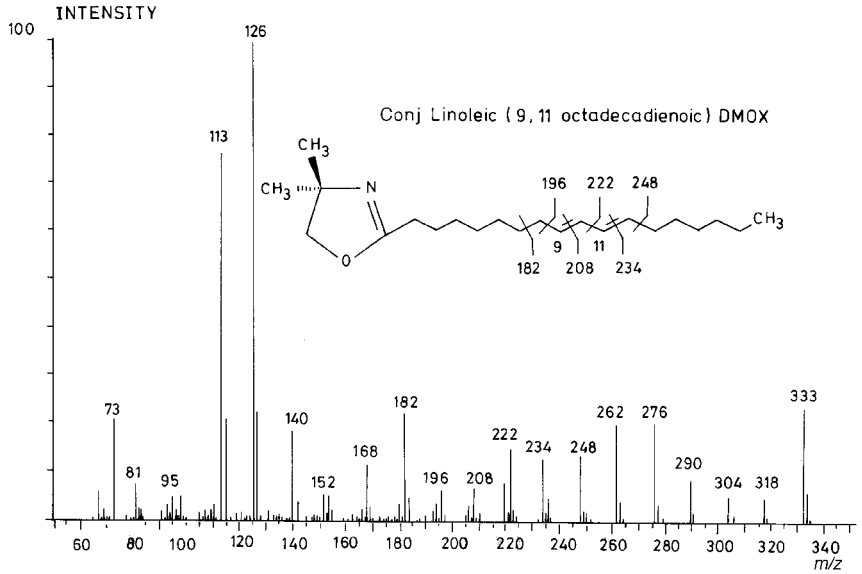

FIG. 3. Electron impact mass spectrum of 9,11 CLA DMOX derivative. For abbreviations see Figures 1 and 2.

TABLE 3
Diagnostic Fragmentation Ions and Intensities of DCO Fatty Acid DMOX Derivatives by SPA

Peak no.	Identification[a]	Diagnostic ions *m/z* (% intensity)			
9	7,9 (up)	168 (12.4)	180 (6.4)	194 (6.1)	206 (6.3)
	8,10 (up)	182 (23.6)	194 (6.1)	208 (7.7)	220 (32.8)
	9,11 (up)	196 (6.6)	208 (7.7)	222 (15.8)	234 (40.8)
	9,11 (apex)	196 (6.7)	208 (7.7)	222 (15.6)	234 (21.2)
	8,10 (down)	182 (21.2)	194 (7.3)	208 (7.8)	220 (20.2)
10	9,11 (up)	196 (7.1)	208 (5.6)	222 (13.7)	234 (10.5)
	9,11 (apex)	196 (9.9)	208 (5.3)	222 (18.8)	234 (8.1)
	10,12 (apex)	210 (3.6)	222 (18.8)	236 (8.4)	248 (7.1)
	10,12 (down)	210 (5.2)	222 (13.2)	236 (8.3)	248 (6.3)
11	9,11	196 (7.8)	208 (8.4)	222 (16.8)	234 (12.8)
12	10,12 (up)	210 (6.4)	222 (12.1)	236 (10.8)	248 (7.3)
	9,11 (apex L)	196 (7.0)	208 (7.5)	222 (14.3)	234 (20.0)
	8,10 (apex L)	182 (21.7)	194 (5.9)	208 (7.5)	220 (14.8)
	8,10 (apex R)	182 (23.0)	194 (9.1)	208 (8.7)	220 (34.0)
	7,9 (apex R)	168 (13.7)	180 (22.5)	194 (9.1)	206 (28.6)
	7,9 (down)	168 (13.8)	180 (23.4)	194 (8.5)	206 (27.6)

[a]Molecular ion (*m/z*): 333; base ion (*m/z*): 126. For abbreviations see Tables 1 and 2.

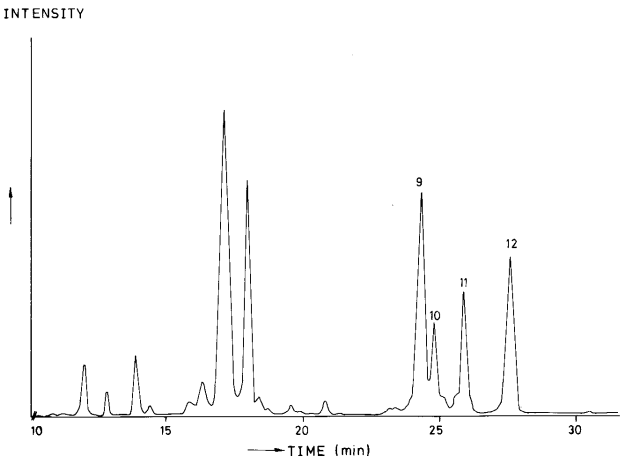

FIG. 4. Total ion chromatogram of DCO fatty acid DMOX derivative. For abbreviations see Figures 1 and 2.

study of the individual isomers in pure form will be undertaken to investigate this behavior.

The detailed relative fatty acid composition of DCO after SPA is as follows (fatty acid, %): C16:0, 1.2; C18:0, 1.5; C18:1, 4.0; NCLA, 48 (total percentage); t_9,t_{12}-18:2, 2.2; c_9,t_{12}-18:2, 30.6; t_9,c_{12}-18:2, 1.1; c_9,c_{12}-18:2, 14.1; CLA, 37 (total percentage); Peak 9 (7, 9/8,10/9, 11), 16.3; Peak 10 (9, 11/10, 12), 4.0; Peak 11 (9, 11), 8.3; Peak 12 (10, 12/9, 11/8, 10/7,9), 8.4; *c*9, OH-18:1, 5.0; unidentified, 3.3. About 21% of the total CLA isomers (37%) is comprised of the 9,11-*cis*,*cis* isomer (peak 11 as a single component). The large amount of 9,11-*cis*,*cis* isomers seems to indicate that the majority of the CLA in DCO is made up of the 9,11 isomers (*cis*,*cis*, *cis*,*trans* and *trans*,*cis* and *trans*,*trans*). The total *cis*,*trans* and *trans*,*cis* isomers constitute about 44% of the total CLA isomers present, whereas the *trans*,*trans* isomers are formed to the same extent as the 9,11-*cis*,*cis* isomer.

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