Analysis of Cyclic Fatty Acid Monomer 2-Alkenyl-4,4-dimethyloxazoline Derivatives by Gas Chromatography−Matrix Isolation−Fourier Transform Infrared Spectroscopy

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Recently, cyclic fatty acid monomers (CFAMs) were isolated from heated flaxseed (linseed) oil and analyzed as methyl ester (ME) derivatives by capillary gas chromatography−matrix isolation−Fourier transform infrared spectroscopy (GC−MI−FTIR) and as 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives by GC−electron ionization mass spectrometry (EIMS). The latter produced fragmentation patterns that were used to determine ring and double-bond positions along the hydrocarbon chain. However, for four CFAM ME derivatives, the FTIR spectra were consistent with CFAM structures having either a cyclohexenyl or a cyclohexadienyl ring, whereas those found by EIMS for the corresponding CFAM DMOX derivatives were consistent with both ring structures. In the present study, the FTIR spectra observed for the DMOX derivatives of this CFAM mixture were consistent with the earlier FTIR results obtained for the corresponding ME derivatives. Mass spectral data observed for deuterated analogues are also reported.

Keywords: *Cyclic fatty acid monomers; infrared*

INTRODUCTION

Complex patterns of oxidative and thermolytic reactions take place in fats and oils during heating and deep-fat frying, including polymerization, hydrolysis, isomerization, and cyclization (White, 1991). The chemical products of these reactions are of great interest to both the consumer and the food industry because the nature of these materials may affect the nutritive value and the sensory quality of the oil and food fried in it. Many studies have linked the ingestion of heated fats and oils to harmful biological effects (Sebedio and Grandgirard, 1989). The most detrimental chemicals formed during thermal abuse are cyclized monomers (Sebedio and Grandgirard, 1989; Crampton *et al.*, 1956; Firestone *et al.*, 1991). These reportedly toxic (Crampton *et al.*, 1956; Saito and Kaneda, 1976; Combe *et al.*, 1978; Iwaoka and Perkins, 1978) cyclic fatty acid monomers (CFAMs) are readily absorbed by the digestive system (Saito and Kaneda, 1976; Combe *et al.*, 1978).

Since the formation of unsaturated CFAMs in frying oils was first suspected 4 decades ago, several studies to elucidate their structures by various analytical techniques have been reported (Sebedio and Grandgirard, 1989; Dobson *et al.*, 1995). Recently, we described a method (Mossoba *et al.*, 1994, 1995a,b) for determining the double-bond configuration and the location of unsaturation sites for a complex mixture of diunsaturated CFAMs isolated from heated flaxseed (linseed) oil. Mixtures of CFAMs were analyzed by gas chromatography−matrix isolation−Fourier transform infrared spectroscopy (GC−MI−FTIR) (Mossoba *et al.*, 1994, 1995a,b) as methyl ester (ME) derivatives. Because double-bond migration obscures the location of double bonds in the electron ionization (EI) mass spectra of ME derivatives of CFAMs (Sebedio and Grandgirard, 1989), the mixture of CFAM ME derivatives was first converted to the 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives and subsequently analyzed by GC−EI mass spectrometry (MS) (Mossoba *et al.*, 1994, 1995b).

Complementary GC−MI−FTIR and GC−EIMS techniques (Mossoba *et al.*, 1994, 1995a,b) were used to establish the location of double bonds and rings along the hydrocarbon chain in CFAMs, the double-bond configuration, the molecular weight, the ring size, and the degree of unsaturation. However, for 4 of the 15 components of this mixture, a discrepancy was found between the GC−MI−FTIR data for the CFAM ME derivatives and the GC−EIMS data for the CFAM DMOX derivatives (Mossoba *et al.*, 1995b). To examine this inconsistency, in the present study the same mixture of CFAMs was further analyzed by GC−MI−FTIR as DMOX derivatives, and the infrared data observed for both ME and DMOX derivatives were compared.

EXPERIMENTAL PROCEDURES

Materials and Methods. Materials were obtained as follows: refined linseed oil from Cargill (Riverside, ND), silica gel from Mallinckrodt (St. Louis, MO), urea from International Biotechnologies, Inc. (New Haven, CT), Wilkinson's catalyst from Strem Chemical Co. (Newburyport, MA), and deuterium from Alpha Products (Ward Hill, MA). All solvents were Fisher (Pittsburgh, PA) reagent grade. A test portion of oil was heated at 275 °C under nitrogen for 12 h as previously described (Sebedio *et al.*, 1987).

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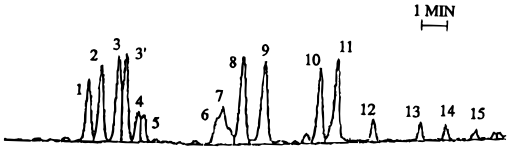

Figure 1. Gas chromatogram for the mixture of diunsaturated C18 CFAM oxazoline derivatives isolated from heated flaxseed (linseed) oil. Peak 5 is due to the DMOX derivative of linoleic acid. Peaks 14 and 15 are due to monounsaturated bicyclic CFAM structures (Mossoba *et al.*, 1995b).

Previously described procedures (Sebedio and Grandgirard, 1989; Sebedio *et al.*, 1987) were followed for the saponification of oil, esterification of fatty acids, separation of fatty acid methyl esters (FAMEs) from polar compounds by silicic acid column chromatography, and isolation of CFAM ME derivatives by urea fractionation of the nonpolar FAME fraction. The urea fractionation step was carried out twice, and the optimum ratio of urea to FAME was 3:1. Unsaturated FAMEs were deuterated by the method of Rakoff and Emken (1978). The method of Fay and Richly (1991) for the oxazoline derivatization of CFAM MEs was modified as described earlier (Mossoba *et al.*, 1995b).

Instrumentation. Gas chromatographic separations were performed on a Hewlett-Packard Model 5890 instrument (Avondale, PA) equipped with a flame ionization detector and a Hewlett-Packard Model 3392A integrator. A 50 m × 0.22 mm (i.d.) CP-Sil-88 capillary column (Chrompack, Inc., Bridgewater, NJ) with a 0.19-μm stationary phase film was used. A Sirius Model 100 FT-IR spectrometer (Mattson Instruments, Inc., Madison, WI) equipped with an MI Cryolect interface operating at 12 K under vacuum was used. This system has been described in detail (Bourne *et al.*, 1984; Reedy *et al.*, 1985). The experimental conditions were the same as those reported earlier for the CFAM ME derivatives (Mossoba *et al.*, 1995a,b).

Low-resolution GC−EIMS analyses were performed with a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Fisons VG (Wythenshawe, U.K.) Autospec Q mass spectrometer and OPUS 2000 data system. The GC−MS system used version 1.6C software. The capillary GC column was CP-Sil-88 (Chrompack, Inc.), 50 m × 0.22 mm (i.d.), with a 0.19-μm stationary phase film. Adjusting the capillary GC column head pressure to 10 psi gave chromatographic profiles comparable to those obtained with a flame-ionization detector. The GC−MS conditions were as follows: splitless injection with helium sweep restored 1 min after injection; injector and transfer lines held at 230 °C; oven temperature program, 75 °C for 2 min after injection, 20 °C/min to 185 °C, hold for 15 min, 4 °C/min to 225 °C, hold for 5 min. The mass spectrometer was tuned to a resolution of 1000 (5% valley) by observing *m*/*z* 305 in the EI mass spectrum of perfluorokerosene (PFK). The mass scale was calibrated with PFK for magnet scans from 440 to 44 Da at 1 s/decade. The filament emission was 200 μA at 70 eV. The ion source temperature was 250 °C.

RESULTS AND DISCUSSION

The gas chromatogram (Figure 1) for the DMOX derivatives of the unsaturated CFAM mixture isolated from heated flaxseed (linseed) oil was qualitatively similar to that of the ME derivatives of the same mixture (Mossoba *et al.*, 1995b). The position of the ring and double bond in the chain of these CFAM DMOX derivatives as determined by GC−EIMS was reported recently (Mossoba *et al.*, 1994, 1995b). Specifically, in the cases of GC peaks 10−13, the observed mass spectra were similar (Figure 2); some ions in this mass spectrum (Figure 2) are consistent with a cyclohexadienyl ring (a gap of 78 u between *m*/*z* 210 and 288), while others are consistent with a cyclohexenyl ring (a gap of 80 u between *m*/*z* 210 and 290) and a double bond at C16 in the chain (a gap of 12 u between *m*/*z* 290 and 302). The ion at *m*/*z* 277, which may arise *via* a retro-Diels−Alder

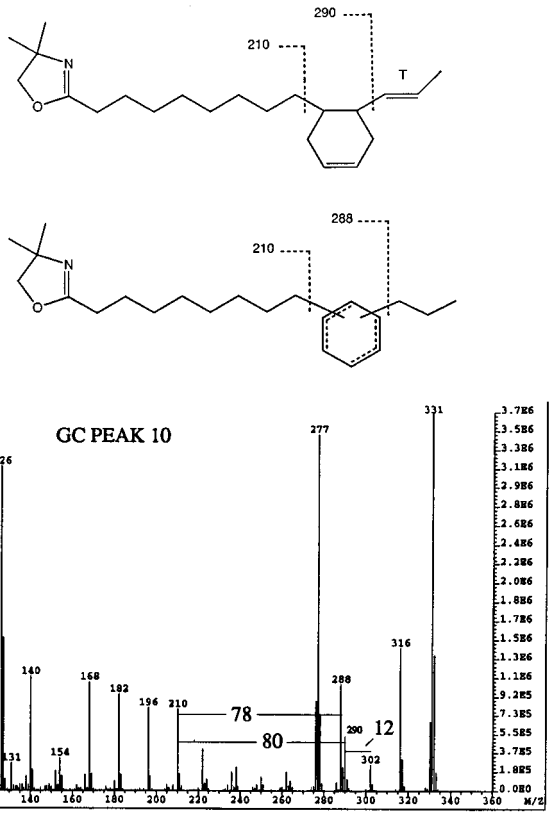

Figure 2. EI mass spectrum obtained for the CFAM DMOX derivative that gave rise to GC peak 10 (Mossoba *et al.*, 1995b). Similar spectra were found for GC peaks 11−13.

reaction, further supports the cyclohexenyl structure. Therefore, this spectrum does not unequivocally confirm the identity of any of the species that gave rise to these four GC peaks. Similar DMOX mass spectral data for these four GC components were also observed by Dobson *et al.* (1995), who reported that the ion at *m*/*z* 288 is presumably due to the radical-induced formation of an additional double bond in the ring after fragmentation. On the other hand, our infrared data (Table 1) observed for CFAM ME derivatives (Mossoba *et al.*, 1995a,b) were consistent with either the cyclohexene structure (GC peaks 10 and 11) or the cyclohexadiene structure (GC peaks 12 and 13). In order to confirm the identity of the CFAM structures that gave rise to GC peaks 10−13, their DMOX derivatives were further analyzed by GC−MI−FTIR.

Representative CFAM DMOX FTIR spectra are shown in Figures 3 and 4. As expected, the bands due to the oxazoline ring are common to all the spectra. These include the ring C=N stretching vibration at 1678 cm^{-1}, the C−O cyclic ether band, which is split with a maximum at 1002 cm^{-1}, and three weaker components near 1018, 979, and 954 cm^{-1}, as well as several weak features in the fingerprint region that are probably due to the oxazoline ring skeletal vibrations. The =C−H deformation vibration near 974 cm^{-1} for a double bond with *trans* configuration (Figures 3A and 4A) is relatively more intense than the adjacent oxazoline ring C−O bands.

The CFAM DMOX FTIR spectral data characteristic of unsaturation sites in a hydrocarbon chain or ring are summarized in Table 1. Those for the corresponding ME derivatives (Mossoba *et al.*, 1995a,b) are listed for comparison. Inspection of these data shows that the FTIR band positions for double-bond stretching or deformation vibrations are not significantly affected by the nature of the CFAM derivative. Thus, the double-

Table 1. FTIR Bands (cm^{-1}) Attributed to Unsaturation Sites in CFAM DMOX Derivatives

GC peak	ring 5-mem	chain *trans*	ring 6-mem	chain *cis*	chain *trans*	chain *trans*	ring 6-mem	ring 5-mem	ring 6-mem
1	3058	3035[a]			3003[a]	973		720	
	3060[a]					970[a]		719[a]	
2	3058	3035[a]			3003[a]	973		720	
	3061[a]					970[a]		719[a]	
3+3′	3060			3009				716	
	3061[a]			3005[a]				716[a]	
4	3060	3032[a]			3005[a]	976		716	
	3063[a]					979[a]		716[a]	
6	3058			3009				711	
	3063[a]			3006[a]				711[a]	
7	3058			3009				711	
	3063[a]			3006[a]				711[a]	
8			3030		3000[a]	974			662
			3032[a]			976[a]			663[a]
9			3030		3005[a]	974			662
			3032[a]			972[a]			664[a]
10			3031		3005[a]	974			662
			3032[a]			972[a]			664[a]
11			3030		3004[a]	975			662
			3032[a]			975[a]			663[a]
12			3030				722		663
			3031[a]				723[a]		664[a]
13			3030				722		661
			3031[a]				725[a]		662[a]
14			3025						
			3025[a]						
15			3022						
			3021[a]						

[a] FTIR data for CFAM ME derivatives (Mossoba *et al.*, 1995a,b) are included for comparison; the weak features near 3035 and 3003 cm^{-1} were not observed in the spectra for the DMOX derivatives.

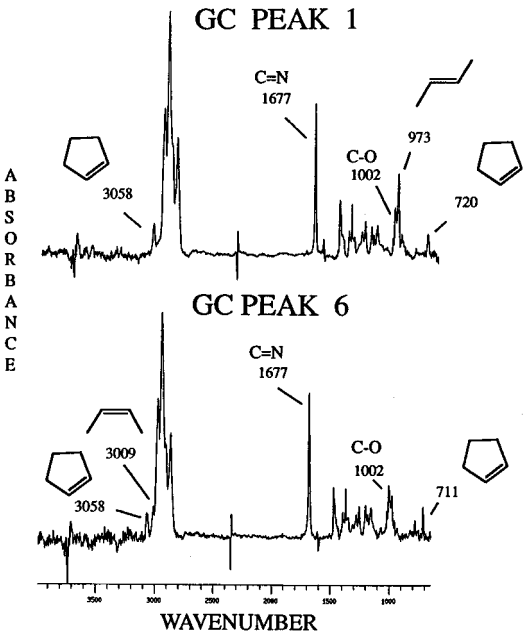

Figure 3. MI−FTIR spectra observed at 4 cm^{-1} resolution for the analytes that gave rise to (top) GC peak 1 and (bottom) GC peak 6.

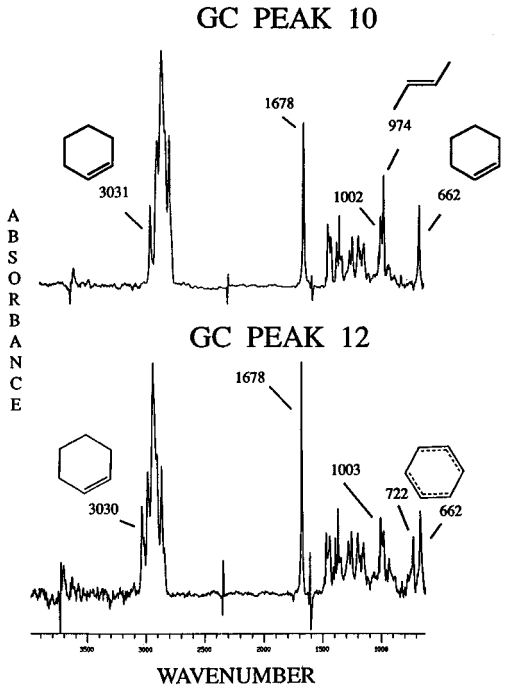

Figure 4. MI−FTIR spectra observed at 4 cm^{-1} resolution for the analytes that gave rise to (top) GC peak 10 (similar result obtained for GC peak 11) and (bottom) GC peak 12 (similar result obtained for GC peak 13). The locations of the two double bonds in the ring were not confirmed.

bond configurations for the CFAM DMOX derivatives are the same as those for the previously assigned ME derivatives (Mossoba *et al.*, 1995a,b). The double-bond configuration for all the analytes that gave rise to GC peaks 1−15 (Figure 1) is also specified in Table 1. In particular, the analytes that gave rise to GC peaks 10 and 11 were confirmed to have a *cis* double bond in a 6-membered ring and a *trans* double bond along the hydrocarbon chain of a ring substituent (Figure 4, top). This conclusion was also reached by Dobson *et al.* (1995).

A different spectrum (Figure 4, bottom) was observed for the other two minor diunsaturated CFAMs (GC peaks 12 and 13), each exhibiting an additional sharp band at 722 cm^{-1} that is consistent with a 6-membered ring having a second double bond (Mossoba *et al.*, 1995a,b). The locations of the two double bonds in the ring were not confirmed. Dobson *et al.* (1995) stated for these two minor components (labeled l and m, respectively) that the structure of the first one (l) has a cyclohexene ring and a *cis* double bond in the hydrocarbon chain (at C16 of the original fatty acid chain). They also reported that it was not possible to determine by FTIR the double-bond configuration for the second

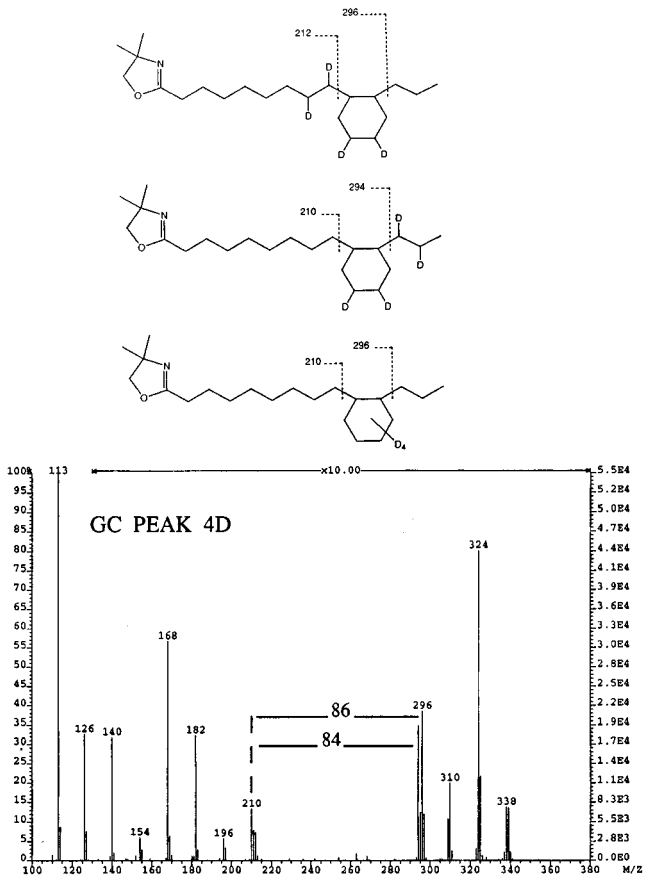

Figure 5. EI mass spectrum for three coeluting saturated DMOX components. The locations of the four deuterium atoms in the ring (bottom structure) were not confirmed. The structures shown from top to bottom are the deuterated products of the diunsaturated (parent) 6-membered ring CFAMs that gave rise to GC peaks 8 and 9, 10 and 11, and 12 and 13, respectively (see GC in Figure 1).

compound (m) due to its low abundance. The possibility of having a double bond in a hydrocarbon chain with a *cis* configuration may not be ruled out, since it usually does not give rise to any characteristic deformation band but only to a weak and broad feature near 730 cm^{-1} (Mossoba *et al.*, 1990). On the other hand, further support for CFAM structures with a cyclohexadiene ring was obtained by deuterating the mixture of unsaturated CFAM ME derivatives, converting its components to DMOX derivatives, and analyzing them by GC−EIMS. The ions at *m/z* 210 and 296 (Figure 5) clearly indicate the presence of saturated species having four deuterium atoms in a 6-membered ring (gap of 86 u). The positions of the four deuterium atoms on the ring were not confirmed. In addition, a minor deuterated GC component exhibited a mass spectrum (*m/z* 182, 196, 210, 296, 324, and 339) that is consistent only with the bottom structure in Figure 5.

CONCLUSION

By using the nondestructive infrared technique, vibrational spectra observed for the DMOX derivatives of the diunsaturated C18 CFAMs that exhibited GC peaks 10−13 were shown to be consistent with structures having a cyclohexenyl ring and a *trans* double bond on a hydrocarbon chain (GC peaks 10 and 11) or a cyclohexadienyl ring (GC peaks 12 and 13).

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