Confirmatory Mass-Spectral Data for Cyclic Fatty Acid Monomers

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ABSTRACT: Cyclic fatty acid monomers (CFAM) are degradation products found in heat-abused edible oils. This study confirms previously published data and reports the structural elucidation of hydrogenated and deuterated monocyclic and bicyclic CFAM prepared from the corresponding unsaturated species that were previously isolated from heated flaxseed (linseed) oil. CFAM structures were determined as 2-alkyl-4,4-dimethyloxazoline derivatives by using gas chromatography–electron ionization mass spectrometry. The observed retention times for the deuterated CFAM were about 0.1 min shorter than those of the corresponding hydrogenated species. For two minor six-membered ring CFAM components, an increase in the mass of the unsaturated ring by eight mass units upon deuteration indicated the addition of four deuterium atoms to two double bonds in that ring. These data unequivocally confirmed the identity of cyclohexadienyl CFAM species in the original CFAM mixture that was isolated from heated linseed oil. The observed electron ionization mass spectrometic data for minor hydrogenated and deuterated CFAM species, which correspond to the last two eluting monounsaturated species, were consistent with CFAM having bicyclic (fused 5- and 6-membered rings) structures. The location of the ring along the fatty acid chain was also confirmed for all saturated CFAM mixture components. The presence of a pair of deuterium atoms on two adjacent carbon atoms further confirmed the previously determined double-bond position along the fatty acid chain of the corresponding parent (unsaturated) compound.
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KEY WORDS: 2-Alkyl-4,4-dimethyloxazoline, cyclic fatty acid monomers, gas chromatography, electron ionization mass spectrometry.

The structural elucidation of potentially toxic (1-3) diunsaturated cyclic fatty acid monomers (CFAM) (4), formed in heat-abused flaxseed (linseed) oil, was recently reported (5–8). A complex mixture of diunsaturated CFAM methyl esters was successfully converted to 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivatives (5) and separated by gas chromatography (GC) into components that were found to inhibit double-bond migration during electron ionization (EI) in the mass spectrometer (9,10). The resulting CFAM DMOX derivatives exhibited definitive EI mass spectra with distinctive fragmentations, which indicated the molecular weight, and the locations of the ring and the double bond along the hydrocarbon chain of CFAM mixture components (5). GC–matrix isolation–Fourier transform infrared spectroscopy (11,12) was used to determine the double bond configuration (5) for these unsaturated CFAM.

Questions remained about the identity of four late-eluting minor unsaturated CFAM (GC peaks 12–15 in Fig. 1, top graph) (5,7,8). For each of the minor species that gave rise to GC peaks 12 and 13 (Fig. 1, top graph), the EI mass spectrometry (MS) data indicated the presence of a mixture of CFAM with cyclohexene and cyclohexadiene ring structures, and the infrared data suggested that both double bonds are in the six-membered ring. On the other hand, Dobson *et al.* (6) reported that the structure of each of these two components has a cyclohexene ring and a *cis* double bond on the chain (at C_{16} of the original fatty acid chain). Moreover, the EIMS data (7) observed for the other two minor CFAM components that eluted last (GC peaks 14 and 15 in Fig. 1, top graph) were the least informative, and were tentatively attributed to bicyclic monounsaturated CFAM structures. To verify these different assignments for the four late-eluting minor components (GC peaks 12–15) of the unsaturated CFAM mixture, different portions of the CFAM methyl ester mixture were hydrogenated, deuterated, and subsequently analyzed as DMOX derivatives by GC–EIMS. The present study reports the analysis of mass spectral data observed for both of these monocyclic and bicyclic, hydrogenated, and deuterated CFAM DMOX mixture components.

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); all solvents were reagent grade from Fisher

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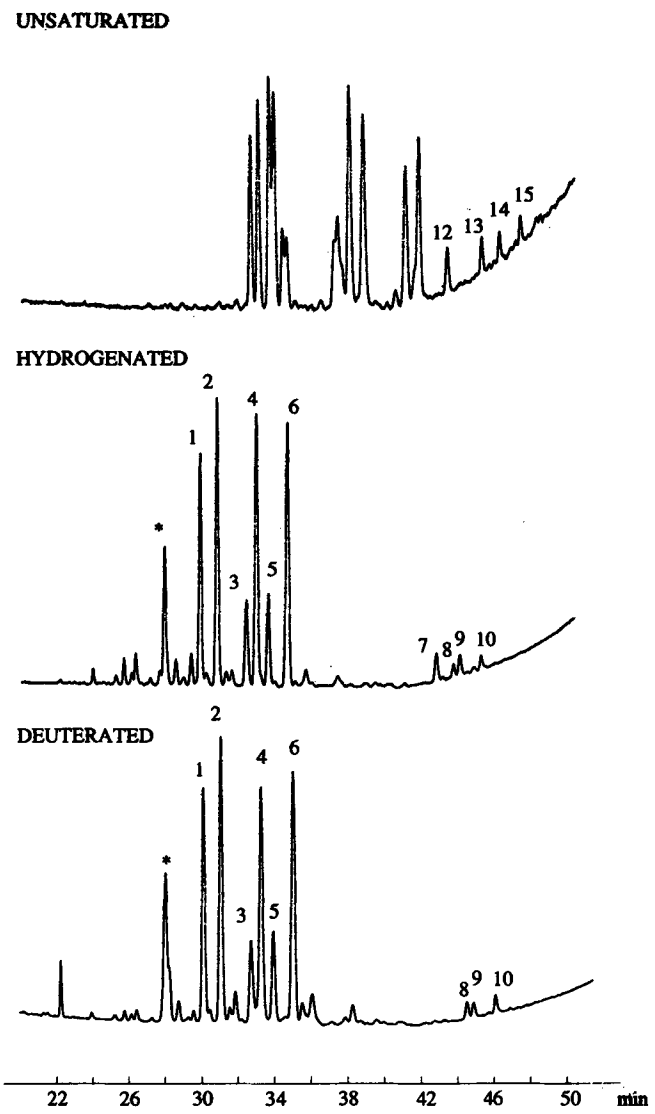

FIG. 1. Observed flame-ionization detection gas chromatograms for the mixture of unsaturated (top) C_{18} cyclic fatty acid monomer (CFAM) oxazolines, isolated from heated flaxseed (linseed) oil, and the corresponding hydrogenated (middle graph), and deuterated (bottom graph) analogues. The asterisks denote saturated noncyclic C_{18} species. Gas chromatographic peak 7H (middle graph) is due to a minor hydrogenated monocyclic CFAM (molecular weight of 2-alkenyl-4,4-dimethyloxazoline derivative was 335).

(Pittsburgh, PA); Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$] from Strem Chemical Co. (Newburyport, MA); and deuterium from Alpha Products (Ward Hill, MA).

A test portion of oil was heated at 275°C under nitrogen for 12 h as previously described (13). Previously described detailed procedures (4,13) were followed for the saponification of oil, esterification of fatty acids, separation of fatty acid methyl esters (FAME) from polar compounds by silicic acid column chromatography, and isolation of CFAM methyl esters 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. A portion of the isolated mixture of CFAM methyl esters was catalytically hydrogenated over platinum oxide with a microhydrogenator (Supelco, Inc., Bellefonte, PA) as described earlier (14). Unsaturated FAME were deuterated by the method of Rakoff and Emken (15).

The method of Fay and Richly (10) for derivatizing FAME to oxazolines was modified as follows: About 150 µL 2-amino-2-methyl-1-propanol was added to 24 mg neat methyl esters in a 2-mL reaction vial. The vial was suspended in a wax bath and held at 175°C for 6 h. The contents of the vial were cooled and transferred with 5 mL methylene chloride to a 250-mL separatory funnel that contained 40 mL petroleum ether. The funnel contents were shaken, the petroleum ether layer was washed with 40 mL deionized water, and then dried with sodium sulfate. The solution was evaporated under a stream of argon, and the residue was dissolved in isooctane.

Low-resolution GC–EIMS analyses were obtained with a Hewlett-Packard (Avondale, PA) 5890 series II gas chromatograph, coupled to a Fisons VG (Wytheshawe, United Kingdom) Autospec Q mass spectrometer and an OPUS 4000 data system (Fisons VG, Wytheshawe, United Kingdom). The GC/MS system was loaded with version 2.1BX software. The capillary GC column used was CP-Sil-88 (Chrompack, Inc., Bridgewater, NJ), 50 m × 0.22 mm (i.d.), with 0.19-µm stationary phase film. Adjusting the capillary GC column head pressure to 10 psi gave chromatographic profiles that were comparable to those found 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 daltons at 1 s/decade. Filament emission was 200 µA at 70 eV. Ion source temperature was 250°C.

RESULTS AND DISCUSSION

The flame ionization detector traces for the unsaturated, hydrogenated and deuterated CFAM DMOX mixtures are shown in Figure 1. In general, the chromatograms obtained for the hydrogenated and deuterated species were qualitatively similar, and the components of the latter mixture eluted about 0.1 min sooner than those of the former one under our experimental conditions.

In Figure 1, the major components (labeled 1–6) are saturation products of major and/or minor diunsaturated (parent) 5- or 6-membered ring CFAM mixture components. The different saturated structures are summarized in Figure 2. The CFAM that exhibited each of the pairs of components that gave rise to GC peaks 1 and 3, 2 and 5, and 4 and 6, are probably cyclic stereoisomers (Fig. 2). Typical mass-spectral evidence is presented in Figures 3–5 for several hydrogenated and deuterated CFAM. The application of EIMS to CFAM DMOX derivatives was detailed earlier (5,7). Consecutive fragments, separated by two adjacent intervals of 15 u each

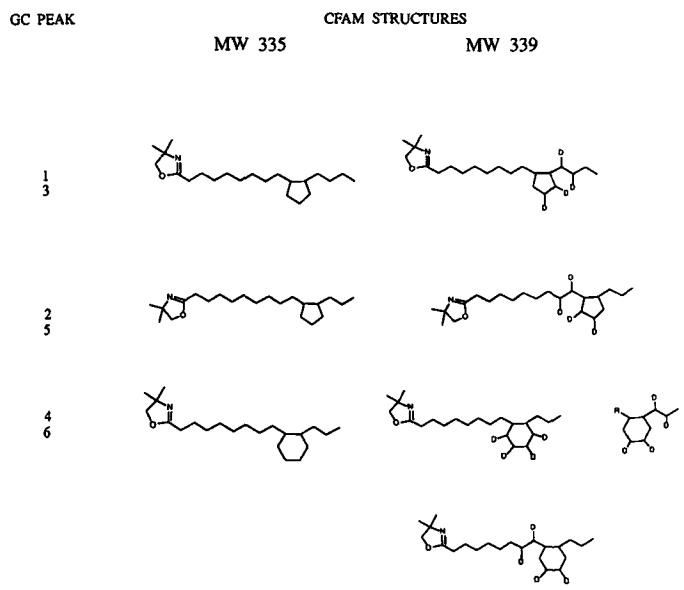

FIG. 2. CFAM structures for the hydrogenated and deuterated CFAM [molecular weights (MW) 335 and 339, respectively] that gave rise to the major gas chromatographic (GC) peaks 1–6 shown in Figure 1 (middle and bottom graphs). The CFAM that exhibited each of the pairs of components that gave rise to GC peaks 1 and 3, 2 and 5, and 4 and 6 are probably cyclic stereoisomers. These compounds may have originated from major and/or minor unsaturation (parent) monocyclic CFAM. The deuterium atoms in the five-membered rings may be at the 3,4- or 4,5-carbon positions. Three coeluting deuterated components were found for each of the GC peaks 4 and 6. See Figure 1 for abbreviations.

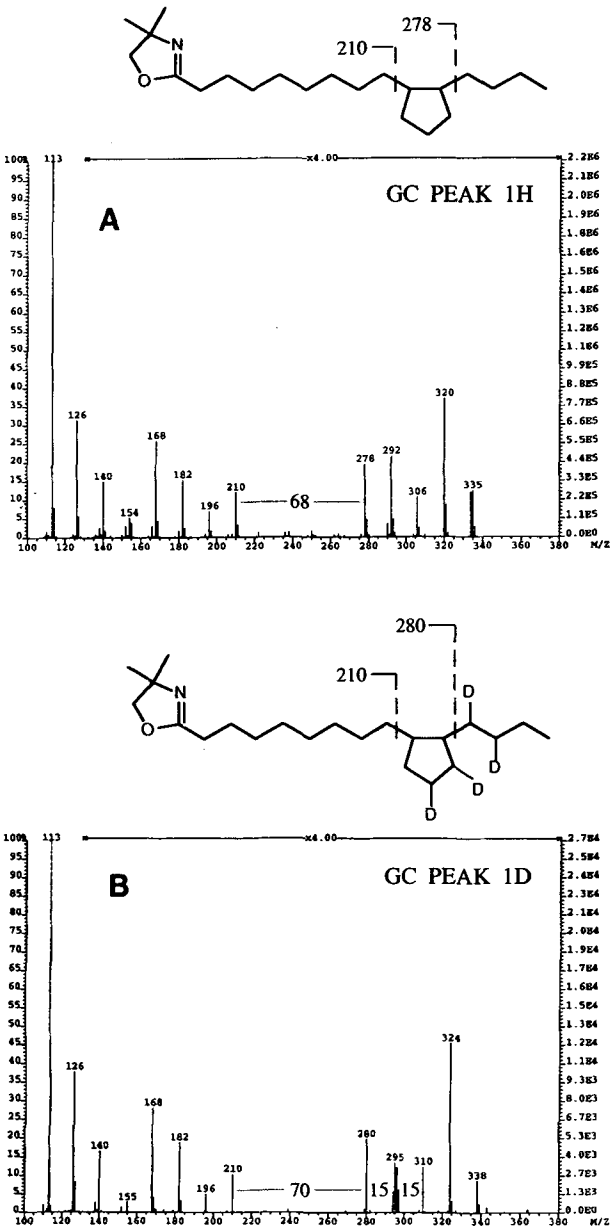

FIG. 3. Electron ionization mass spectra and structures for (A) the hydrogenated analyte that exhibited GC peak 1 in Figure 1 (middle graph), and (B) deuterated analyte that exhibited GC peak 1 in Figure 1 (bottom graph). The ring deuterium atoms may be at the 3,4- or 4,5-ring carbon positions. See Figures 1 and 2 for abbreviations.

(or a total of 30 u) in the mass spectra of deuterated CFAM, indicated the presence of a -CHD-CHD- moiety along the hydrocarbon chain (see for example Fig. 3B). Determination of the sites of deuterium atoms in the chains (Figs. 3 and 4) further confirmed the previously assigned (5,7) locations of double bonds along the carbon backbone of the corresponding parent (unsaturated) CFAM. The mass spectra shown in Figure 4B are attributed to three deuterated CFAM with an identical skeletal carbon structure (a cyclohexyl ring with a propyl substituent); although three CFAM coeluted by GC, the observed ions at *m/z* 294 and 296 in Figure 4B discriminated between two of them. The ion at *m/z* 296 is consistent with saturated species that have four deuterium atoms in a six-membered ring, and further supports infrared evidence (5,7,8) for the presence of a hexadienyl ring structure in the original unsaturated mixture (GC peaks 12 and 13 in Fig. 1, top graph). A minor deuterated GC component (r_t 38.5 min) gave rise to a mass spectrum (*m/z* 182, 196, 210, 296, 310, 324, and 339) that is consistent only with a cyclohexadienyl CFAM structure. Further inspection of Figure 4B indicates that several ions are also due to a third coeluting saturated CFAM structure (shown in Fig. 2) with two deuterium atoms in a cyclohexyl ring (gap of 84 u between *m/z* 212 and 296), and two deuterium atoms (two adjacent gaps of 15 u between *m/z* 182 and 197, as well as *m/z* 197 and 212) along the hydrocarbon chain: the ring is between carbons C_{10} and C_{15}, and the two deuteriums are on carbons C_8 and C_9 of the parent fatty acid; the corresponding diunsaturated CFAM structure was previously identified (5,7,8).

Inspection of the mass spectra in Figure 5, observed for the saturated components that correspond to GC peak 8 (Fig. 1, middle and bottom graphs), indicates the presence of large gaps of 122 and 124 mass units, respectively, due to the loss of a bicyclic fragment (fused five- and six-membered rings). These gaps found for the hydrogenated and deuterated components are 2 and 4 u higher, respectively, than the 120-u gap obtained for the corresponding unsaturated bicyclic CFAM component (GC peak 14 in Fig. 1, top graph) (7), and are con-

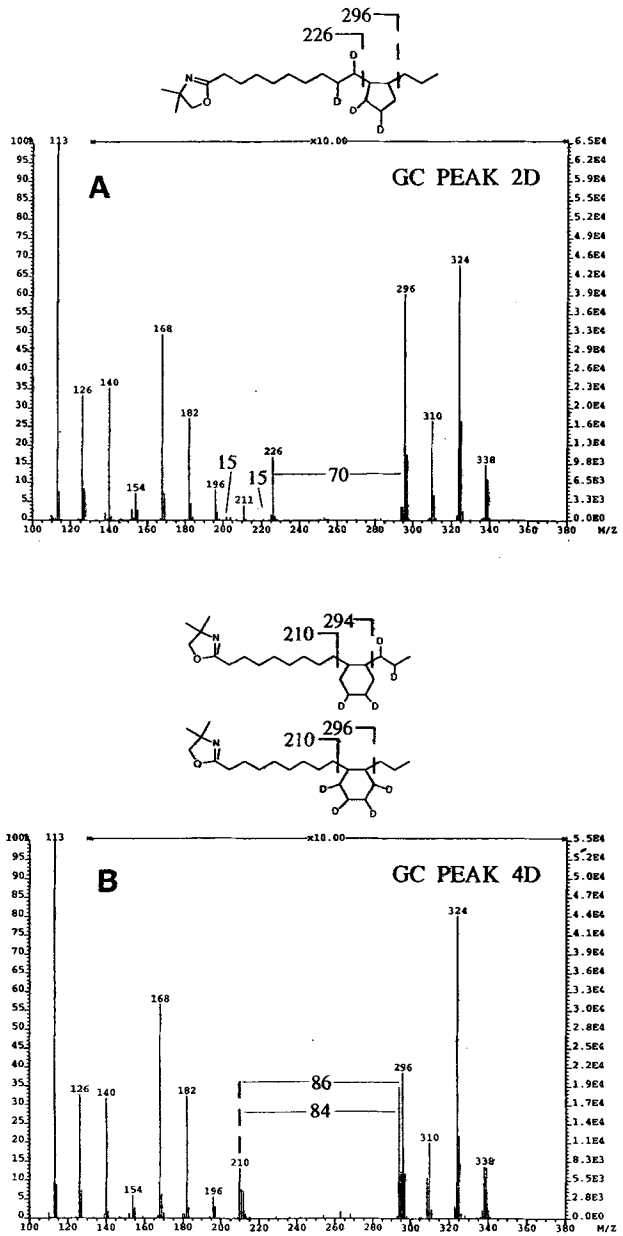

FIG. 4. Electron ionization mass spectra and structures (A) for the deuterated analyte that exhibited GC peak 2 in Figure 1 (bottom graph). The ring deuterium atoms may be at the 3,4- or 4,5-ring carbon positions, and (B) for the three coeluting deuterated analytes that gave rise to GC peak 4 in Figure 1 (bottom graph). A similar result was found for GC peak 6 in Figure 1 (bottom graph). The gap of 86 mass units does not indicate the location of the four deuterium atoms in the six-membered ring. The given structure is consistent with the assignment of the infrared data observed for the corresponding unsaturated (parent) species (Refs. 7,8). See Figure 2 for abbreviation.

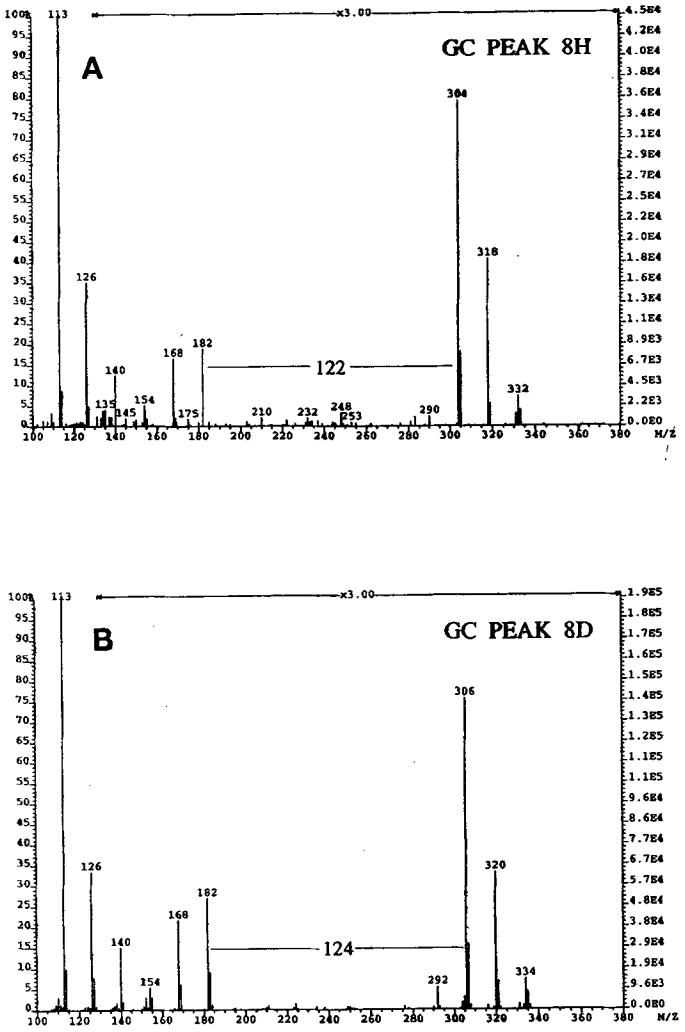

FIG. 5. Electron ionization mass spectra (A) for the hydrogenated analyte that exhibited GC peak 8 in Figure 1 (middle graph), and (B) for the deuterated analyte that gave rise to GC peak 8 in Figure 1 (bottom graph). These spectra are consistent with a bicyclic (fused five- and six-membered rings) structure with an ethyl substituent. See Figure 2 for abbreviation

sistent with the saturation of a single double bond. Mass spectra with similar gaps, between *m/z* 196 and 318 (hydrogenated), and *m/z* 196 and 320 (deuterated), were also obtained for the saturated components that gave rise to GC peaks 9 and 10. A methyl substituent on the bicyclic ring structure was indicated by a mass interval of 15 u at the high-mass end, between *m/z* 318 and 333 and between *m/z* 320 and 335, for the hydrogenated and deuterated compounds, respectively. The =C-H stretching vibration band, observed near 3025 cm^{-1} for the corresponding monounsaturated bicyclic CFAM components (GC peaks 14 and 15 in Fig. 1, top graph), is more likely to be due to a double bond in a six-membered ring (3030 cm^{-1}) than one in a five-membered ring (3060 cm^{-1}) (7,8). Similar bicyclic structures are formed during tall oil distillation (16).

Further mass-spectral evidence was observed for structural assignments that were previously reported (5,7,8) for the two minor diunsaturated monocyclic CFAM components with two double bonds in a six-membered ring (GC peaks 12 and 13). Mass-spectral support for the two minor monounsaturated bicyclic (fused five- and six-membered rings) CFAM structures (GC peaks 14 and 15) was also found.

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