Elucidation of Cyclic Fatty Acid Monomer Structures. Cyclic and Bicyclic Ring Sizes and Double Bond Position and Configuration

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ABSTRACT: Gas chromatography (GC)–electron ionization mass spectrometry of 2-alkenyl-4,4-dimethyl-oxazoline derivatives was used to confirm the identities of a complex mixture of C_{18} diunsaturated cyclic fatty acid monomers (CFAMs) that were isolated from heated flaxseed (linseed) oil. The positions of double bonds and 1,2-disubstituted unsaturated 5- and 6-membered rings along the fatty acid hydrocarbon chains were established by this method. The oxazoline spectra exhibited a homologous ion series with a pattern of peaks that were 14 u (u = atomic mass unit) apart but interrupted when a double bond (12-u mass interval) or a ring was present along the fatty acid chain. The identity and location of a ring were indicated by a large interval of 68, 82, 66, 80, 78, or 120 u for a saturated 5- or 6-membered ring, monounsaturated 5- or 6-membered ring, diunsaturated 6-membered ring, or monounsaturated bicyclic ring system (fused 5- and 6-membered rings), respectively. The double bond configuration for the methyl ester derivatives of these CFAMs was established by GC–matrix isolation–Fourier transform infrared spectroscopy. The elucidated alkenyl structures at C_2 in diunsaturated 2-[alkenyl]-4,4-dimethyloxazolines were 8-(2-but-*trans*-1-enyl-cyclopentenyl)octyl, 9-(2-propyl-cyclopentenyl)non-*trans*-8-enyl, 9-(2-propyl-cyclopentenyl)non-*cis*-7-enyl, 8-(2-but-*cis*-1-enyl-cyclopentenyl)octyl, 9-(2-propylcyclopentenyl)non-*cis*-8-enyl, 8-(2-propyl-cyclohex-*cis*-4-enyl)oct-*trans*-7-enyl, 8-(prop-*trans*-1-enyl-cyclohex-*cis*-4-enyl)octyl, and 8-(2-propyl-cyclohexa-*cis,cis*-3,5-dienyl)octyl.

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KEY WORDS: Cyclic fatty acid monomers, double bond configuration, double bond position, infrared, mass spectrometry.

Intramolecular cyclization of polyunsaturated fatty acids occurs when fat and oil frying operations take place at a high temperature for a long period or under other abusive conditions. Cyclic fatty acid monomer (CFAM) products, which are easily absorbed by the digestive system, are of concern from a dietary–toxicity point of view (1). Hence, efforts to synthesize CFAMs (2–8) or isolate them from heated oils (2,3,8–15) and characterize their structures by chemical, chromatographic, and spectrometric methods have been pursued for several decades. Recently, important progress concerning the analysis of CFAMs was reported by Christie (16,17), Sebedio (18), and their co-workers. The nature of the cyclic monoenoic fatty acids formed from linoleic acid in sunflower oil heated to 275°C was determined by gas chromatography/mass spectrometry (GC/MS) of the picolinyl ester derivatives after simplification by silver-ion high-performance liquid chromatography (HPLC) (16). An HPLC method was developed as an enrichment step prior to GC/MS analysis to determine traces of CFAMs in oils and animal tissue (18).

To date, the classical problem of locating double bonds for a complex mixture of diunsaturated CFAM methyl esters isolated from heated oils has been only partially solved by MS (1,8,19). In the present work, the locations of the ring and the double bonds in the ring substituents in these C_{18} CFAM molecules were confirmed by applying capillary GC–electron ionization MS (GC–EIMS) to a derivative, 2-alkenyl-4,4-dimethyl-oxazoline (20), that can inhibit the migration of double bonds during EI in the mass spectrometer. This derivative was used in the present investigation because it appeared to be promising for the structural determination of fatty acids that contain double bonds (20), methyl branching (21), and/or terminal (monosubstituted) 5-membered rings (22).

In the present study, a mixture of diunsaturated CFAM methyl esters with 1,2-disubstituted 5- or 6-membered rings was isolated from heated flaxseed (linseed) oil. A portion of this mixture was analyzed by GC/matrix isolation–Fourier transform infrared (GC/MI–FTIR), and the remainder was converted to 2-alkenyl-4,4-dimethyl-oxazoline derivatives and analyzed by GC–EIMS. These techniques established the molecular weight, the double bond location on the ring substituents (hydrocarbon chain or oxazoline moiety), and the position of the unsaturated ring, as well as the double bond configuration in the CFAM molecules. The preliminary results were recently reported (23,24).

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MATERIALS AND METHODS

Materials. Refined linseed oil was purchased from Cargill (Riverside, ND), silica gel from Mallinckrodt (St. Louis, MO), and urea from International Biotechnologies, Inc. (New Haven, CT). All solvents were from Fisher (Pittsburgh, PA) and were reagent-grade.

Instrumentation. Low-resolution GC–EIMS analyses were obtained with a Hewlett-Packard (Avondale, PA) 5890 series II gas chromatograph coupled to a Fisons VG (Wythenshawe, United Kingdom) 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., Bridgewater, NJ), 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 daltons at 1 s per decade. The filament emission was 200 μA at 70 eV. The ion source temperature was 250°C.

With respect to the GC/MI–FTIR analyses, GC separations were performed on a Hewlett-Packard Model 5890 instrument, 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.) with a 0.19-μm stationary phase film was used. Helium containing 1.5% argon (Matheson Gas Products, Secaucus, NJ) at approximately 27 cm/s linear velocity was used as the carrier gas, and helium (99.995%) at 30 mL/min was used as the makeup gas to the detector. The injector and detector temperatures were 250 and 300°C, respectively. The carrier gas mixture was purified by a Hydro-Purge II filter (Alltech Associates, Deerfield, IL) and a heated gas purifier filter (Supelco, Bellefonte, PA). The injection mode was splitless, and a 10-μL Hamilton 701N syringe was used. Injections of about 1 μL were made 6 s after the start of a run, and the total time the syringe needle was in the injector was 15 s. The injector was purged 1 min after the start of the run. The initial column oven temperature was 75°C with a 2-min hold, followed by a 20°C/min increase to 220°C; the oven was held at this temperature for about 15 min until the analysis was complete.

A column effluent split ratio of 4:1, infrared (IR)/GC, was calculated from the flame-ionization detection (FID) response factors (area counts per nanogram injected) obtained with the column directly inserted into the flame-ionization detector, and again with the column attached to the GC/MI interface.

A Sirius Model 100 FTIR 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 (25,26). The MI method involved adding argon (1.5% by volume) to the GC carrier gas (helium) and trapping the effluent onto the outer rim of a slowly rotating gold disk (at about 3 mm/min) held at cryogenic temperatures. During a run, helium was removed by the vacuum pumps, and the analyte molecules surrounded by an excess of argon atoms were frozen into a solid matrix on the gold disk. The IR-transparent argon matrix, containing the isolated analytes, was subsequently analyzed by IR spectroscopy. The position of each analyte peak on the Cryolect collection disk was indexed by its observed GC retention time. Procedures were previously described in detail (27) for reproducibly locating a peak maximum on the collection disk and for optimizing the performance of the system. These latter procedures, which include optical alignment, can minimize the extent of post-column peak broadening. Three hundred analyte interferograms were co-added (2 min, 43 s at 4 cm^{-1} resolution), and the background (300 scans) was usually collected either before or after the analyte peak.

Ultraviolet (UV) spectra in hexane were obtained with a Beckman DU-7 spectrophotometer (Beckman Instruments, Fullerton, CA).

Procedures. A test sample of oil was heated at 275°C under nitrogen for 12 h as described previously (14).

The procedures used in the present work 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 methyl esters by urea fractionation of the nonpolar FAME fraction were previously described (1,14). 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 in a microhydrogenator (Supelco, Inc.) as described earlier (15).

The method of Fay and Richly (28) for the oxazoline derivatization of CFAM methyl esters was modified as follows. About 150 μL of 2-amino-2-methyl-1-propanol was added to 24 mg of 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 of methylene chloride into a 250-mL separatory funnel that contained 40 mL of petroleum ether. The funnel contents were shaken, and the petroleum ether layer was washed with 40 mL of deionized water and then dried with sodium sulfate. The solution was evaporated under a stream of argon, and the residue was dissolved in isooctane. UV measurements of the methyl ester mixture of CFAMs were carried out after dilution in hexane.

RESULTS AND DISCUSSION

Oxazoline mass spectra for simple fatty acids that have only saturated hydrocarbon chains usually exhibit (i) a characteristic peak at *m/z* 113 attributed to McLafferty rearrangement (20), (ii) an even-mass homologous series, in which the most pronounced peak in each cluster is at *m/z* (126 + 14n, where

n = 0, 1, 2, ...), and (iii) the molecular ion. The first ion at the low-mass end of this series (*m/z* 126) is presumably formed *via* a cyclization-displacement reaction induced by the nucleophilic center (20). The homologous ion series with a pattern of peaks 14 atomic mass units (u) apart is due to the sequential cleavage of methylene groups. For oxazoline derivatives of CFAMs, this fragmentation at each skeletal carbon–carbon bond was interrupted when a double bond and/or a ring was present along the hydrocarbon chain. This structural tool was first applied to simple saturated fatty acid molecules with one ring along the hydrocarbon chain. Saturated CFAMs, obtained by hydrogenating a portion of the same mixture that was isolated from heated flaxseed oil, exhibited mass spectra in which the pattern of 14-u intervals was interrupted by a mass interval of 68 u or 82 u, thus confirming the identity and location of the cyclopentyl or cyclohexyl ring, respectively, in these cyclic fatty acid molecules. The mass spectra of two of the major compounds found in this saturated CFAM mixture were similar, as were the spectra observed for the pair of 10-(2′-*n*-propylcyclopentyl)decanoate cyclic stereoisomeric (*trans* and *cis* ring isomers) standards. These 1,2-disubstituted cyclopentyl CFAM standards were synthesized earlier as methyl esters (7), and derivatized to oxazolines in the present study. The observed EIMS spectra for saturated or unsaturated disubstituted 5- or 6-membered ring oxazoline species (*vide infra*) do not indicate that the rings in these CFAMs are ortho-disubstituted. However, the ring 1,2-disubstitution pattern was well established for several documented CFAM structures with 5- (8,11) or 6-membered (2,9,10,12,13) rings isolated from heated linseed oil, or else synthesized (6,7).

The FID profile for the methyl ester derivatives of the unsaturated CFAM mixture isolated from heated flaxseed oil was qualitatively similar to that of the same mixture of oxazoline derivatives (Fig. 1).

The mass spectrum for GC peak 1 exhibited successive mass intervals of 14 u with interruptions of 66 and 12 u (Figs. 2 and 3). This spectrum is due to a C_{18} CFAM structure with a cyclopentenyl ring having a 2′-*n*-butene substituent. As shown in Figure 2, the double bond is on C_1 of the butene group according to an empirical rule reported by Zhang *et al.* (20) that was applied to CFAMs in the present study. This rule states that if two consecutive even-mass homologous fragments, containing n-1 and n carbon atoms of the original fatty acid moiety, are separated by a 12-u interval, a double bond exists between carbons n and n + 1. The configuration of the double bond of this butene substituent was found to be *trans* (Table 1). Double bonds in 5- or 6-membered rings are usually stable in the *cis* configuration. In general, more than a single position is possible for a double bond with two *cis* hydrogen atoms in a 1,2-disubstituted 5- or 6-membered ring structure, which suggests that positional CFAM isomers may exist. The IR data listed in Table 1 are consistent with those previ-

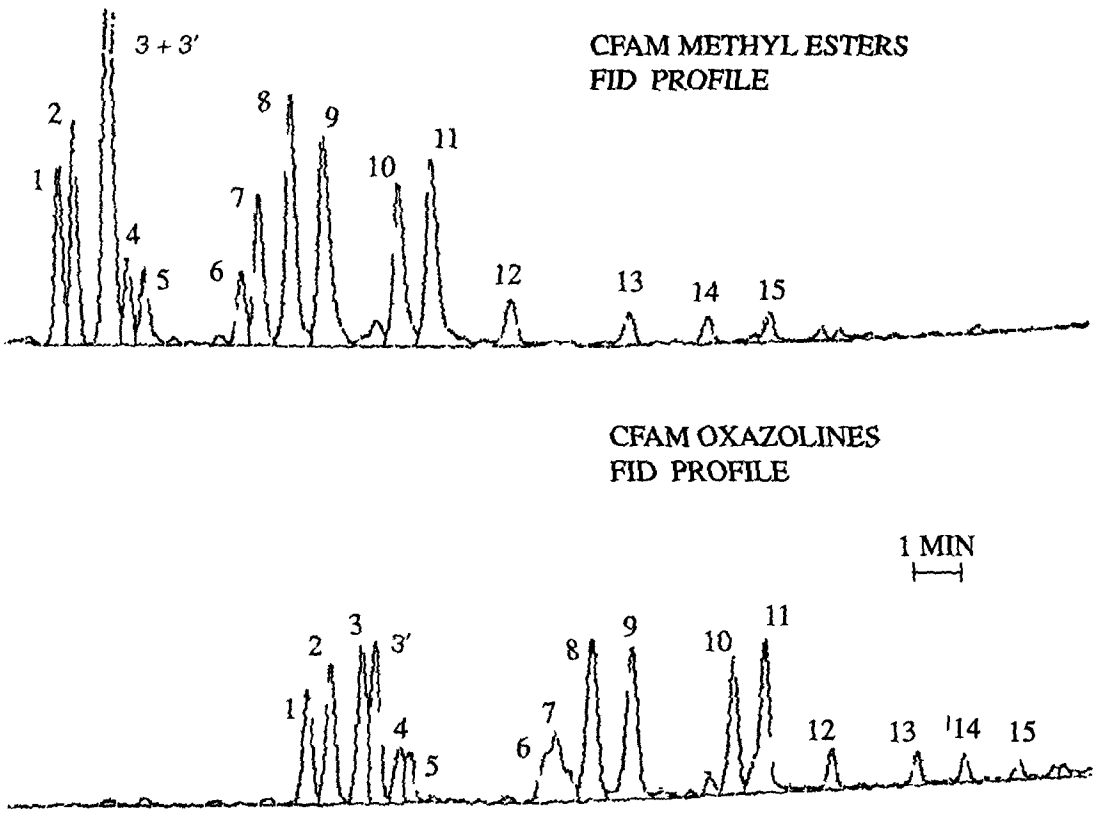

FIG. 1. Gas chromatograms for unsaturated mixtures of cyclic fatty acid monomer (CFAM) methyl esters (top) and CFAM oxazolines (bottom). FID, flame-ionization detection.

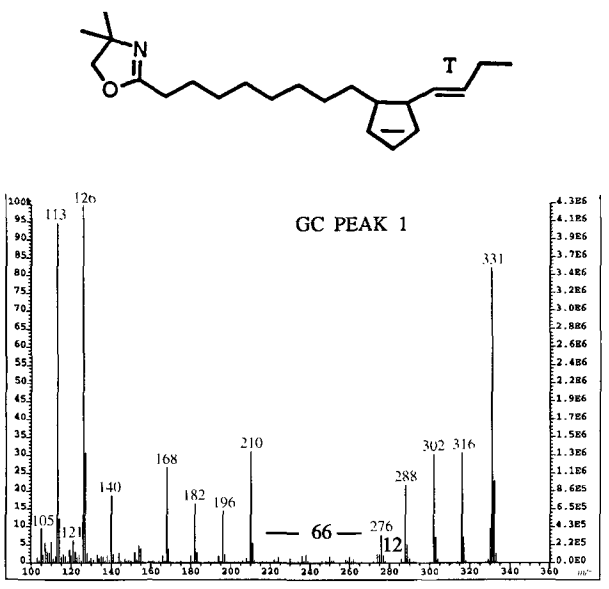

FIG. 2. Electron ionization mass spectrum for the eluates of gas chromatography (GC) peak 1: 2-[8-(2-but-*trans*-1-enyl-cyclopentenyl)octyl]-4,4-dimethyloxazoline.

ously reported by Sebedio *et al.* (29) for GC peaks 1 through 11 with a light-pipe GC/IR system.

The mass spectrum obtained for the compound corresponding to GC peak 2 exhibited an even-mass homologous series of 14-u intervals with interruptions of 12 and 66 u (Fig. 4), indicating the presence of a double bond between C_8 and C_9 and a cyclopentenyl ring with a 2′-*n*-propyl substituent in this molecule. The double bond on the chain of this CFAM had a *trans* configuration. Carbon atoms C_8 and C_9 along the 2-alkenyl chain of the oxazoline derivative correspond to carbons C_9 and C_{10}, respectively, in the original fatty acid chain.

The homologous series of ions, 126 + 14n, in the mass spectrum for GC peak 2 can be traced from *m/z* 126 to the 12-u interruption at *m/z* 196. The relative abundance of n = 2 in this series, *m/z* 154, is low in this spectrum and in the spectra of

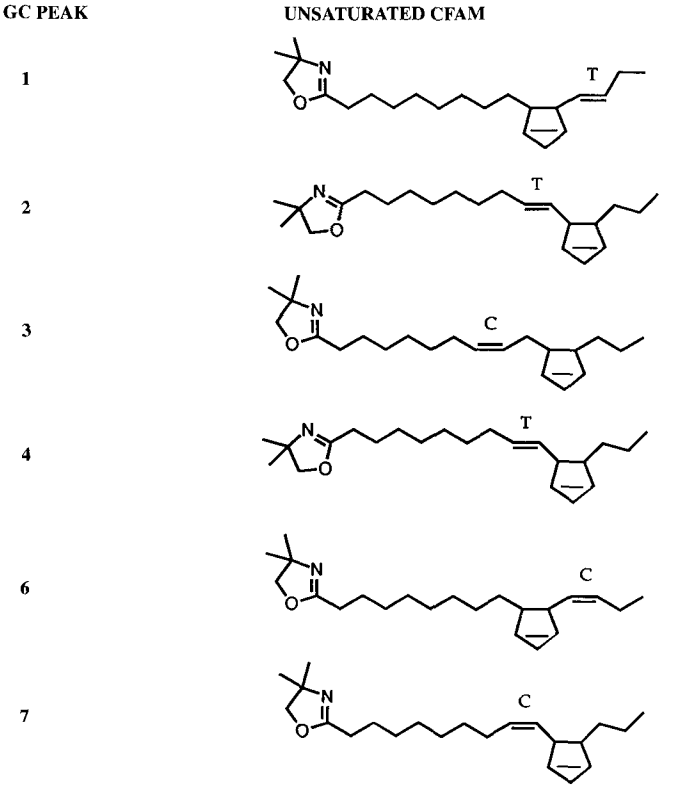

FIG. 3. Structures of CFAMs (containing 5-membered rings) in heated flaxseed (linseed) oil. Abbreviations as in Figures 1 and 2.

several other compounds in this mixture, including linoleic acid. The low relative abundance of n = 2 in this series has been previously reported (20) and does not interfere with the structural determination of unsaturated C_{18} CFAMs, because the more pronounced abundance of adjacent ions in the series reliably indicates the absence of a double bond in this portion of the molecule.

GC peaks 3 and 3′ are due to an unresolved mixture of at least two species (Fig. 3). Mass intervals of 66 u due to cy-

TABLE 1
Infrared Bands (cm^{-1}) Attributed to Unsaturation Sites in Cyclic Fatty Acid Monomers

Gas chromatographic peak	Ring (5-membered)	Chain *(trans)*	Ring (6-membered)	Chain *(cis)*	Chain *(trans)*	Chain *(trans)*	Ring (6-membered)	Ring (5-membered)	Ring (6-membered)
1	3061	3035			3003	970		719	
2	3061	3035			3003	970		719	
3 + 3′	3061			3005				716	
4	3063	3032			3005	979		716	
6	3063			3006				711	
7	3063			3006				711	
8			3032		3000	976			663
9			3032		3005	972			664
10			3032		3005	972			664
11			3032		3004	975			663
12			3031				723		664
13			3031				725		662
14			3025						
15			3021						

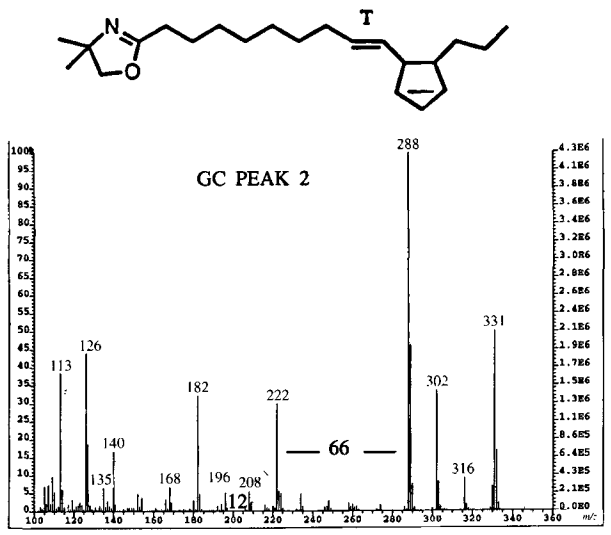

FIG. 4. Electron ionization mass spectrum for the eluates of GC peak 2: 2-[9-(2-propyl-cyclopentenyl)non-*trans*-8-enyl]-4,4-dimethyloxazoline. A double bond between carbons n and n + 1 in the original fatty acid chain corresponds to an unsaturation site between carbons n-1 and n, respectively, along the 2-alkenyl chain of the oxazoline derivative. Abbreviation as in Figure 2.

clopentenyl rings were found for both compounds. IR results also indicated that a double bond with *cis* configuration is located in the chain of these species. Because of the overlapping of GC peaks 3 and 3′, the mass spectrum obtained for the analyte of peak 3′ was less informative, and it was not possible to determine whether the ring substituent was a but-1′-ene or a but-2′-ene.

The mass spectrum for the compound corresponding to GC peak 4 was similar to the one obtained for the compound corresponding to GC peak 2. One difference between the two spectra was the much weaker *m/z* 222 peak in the latter case (GC peak 4). It is likely that this pair of CFAMs are cyclic stereoisomers in which only the configurations of the two ortho-substituents on the ring are different—*trans* and *cis* for the early- (GC peak 2) and late-eluting (GC peak 4) CFAMs, respectively. This elution sequence was previously determined (6,30) on the basis of the characterization of synthesized 1,2-disubstituted CFAM isomers. The peak at *m/z* 222, which is indicative of the location of the ring, may be of diagnostic value for distinguishing stereoisomers because its characteristic intensity difference was also found for the stereoisomeric pair of GC peaks 3 and 7.

The compound for GC peak 5 gave rise to a mass spectrum that is consistent with the structure of the oxazoline derivative of linoleic acid, which was not completely removed by urea fractionation.

Although GC peaks 6 and 7 were resolved as methyl esters, they overlapped in the chromatogram of the oxazoline mixture (Fig. 1). The oxazoline mass spectra collected at the leading edge of GC peak 6 and trailing edge of GC peak 7 were distinctly different and similar to those found for GC peaks 1 and 2, respectively, except that all the double bonds for GC peaks 6 and 7 had a *cis* configuration (Fig. 3).

The mass spectra obtained for the remaining CFAM oxazolines (GC peaks 8–15) were more complex than those of the cyclopentenyl CFAMs discussed so far. Fragments at *m/z* 208 and 288 with a mass interval of 80 u, consistent with a cyclohexenyl structure, were observed for the compounds corresponding to GC peaks 8 and 9 (Fig. 5). Unlike mass spectra of C_{18} CFAMs with a saturated 5- or 6-membered ring or a cyclopentenyl ring, which exhibit mostly large mass intervals, the mass spectrum of a compound containing a cyclohexenyl ring appears to have characteristic intense even- and odd-mass fragments at *m/z* 248 and 277, respectively (Fig. 5). The fragment at *m/z* 277 may arise *via* a retro Diels–Alder reaction, resulting in the loss of C_4H_6 from the cyclohexenyl ring. Subsequent shift of electrons in the conjugated product ion could give rise to *m/z* 248 *via* expulsion of C_2H_5. These spectra also suggest that these two CFAMs have a 2′-*n*-propyl substituent on the ring, as well as a double bond on the aliphatic chain between C_7 and C_8, with a *trans* configuration. The complexity of the observed cyclohexenyl CFAM mass spectra probably means that each GC peak may be due to more than one component. Hence, the *m/z* 248 peak may alternatively be due to the presence of a component with a different ring structure. The similarity of the mass spectra of the compounds for GC peaks 8 and 9 suggests that the corresponding CFAMs are probably ring stereoisomers.

The mass spectra obtained for the four compounds that correspond to GC peaks 10–13 (Fig. 6) showed a common fragmentation pattern that consists of a series of 14-u mass inter-

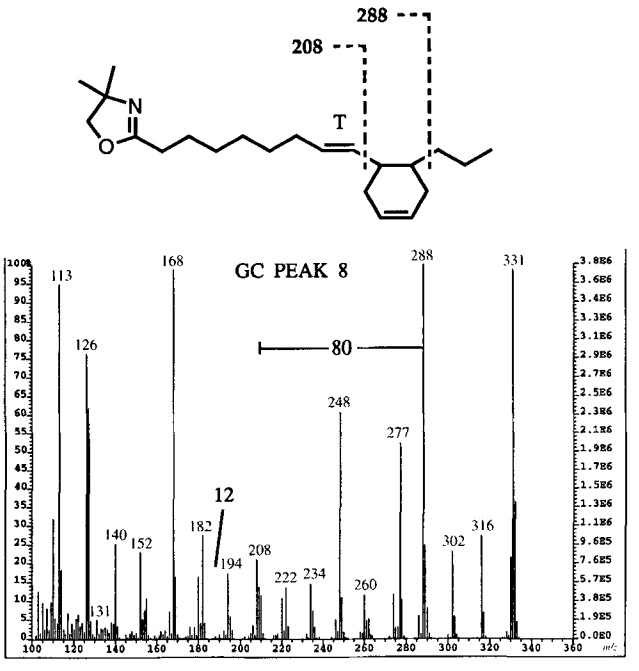

FIG. 5. Electron ionization mass spectrum for the eluates of GC peak 8: tentative structure 2-[8-(2-propyl-cyclohex-*cis*-4-enyl)oct-*trans*-7-enyl]-4,4-dimethyloxazoline; see text for details. Abbreviation as in Figure 2.

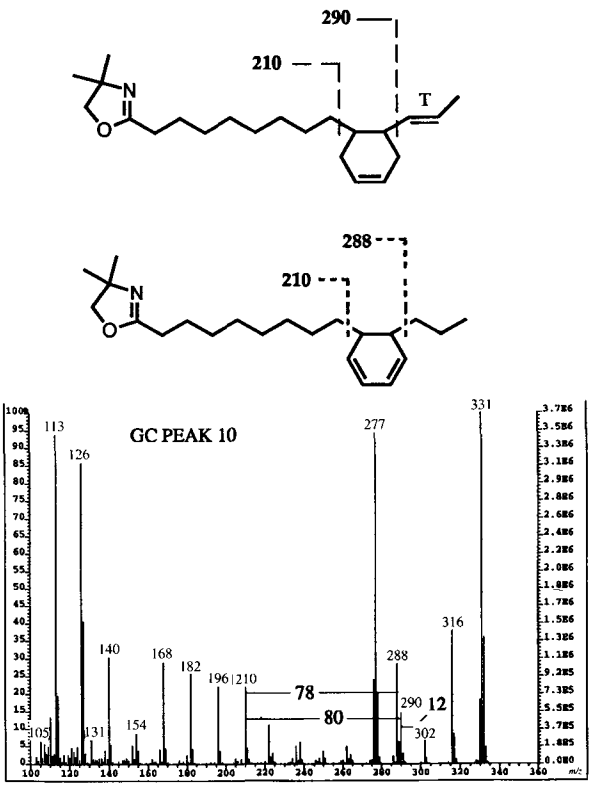

FIG. 6. Electron ionization mass spectrum for the eluates of GC peak 10: tentative structures 2-[8-(2-prop-*trans*-1-enyl-cyclohex-*cis*-1-enyl)octyl]-4,4-dimethyloxazoline (top) and 2-[8-(2-propyl-cyclohexa-*cis,cis*-3,5-dienyl)octyl]-4,4-dimethyloxazoline (bottom); see text for details. Abbreviation as in Figure 2.

vals and fragments at *m/z* 210 and 288 with a difference of 78 u, consistent with a CFAM that has a cyclohexadienyl ring with a 2′-*n*-propyl substituent. This cyclohexadienyl structure would preclude formation of *m/z* 277 by the pathway proposed for a cyclohexenyl ring. However, the MS data also provide support for a cyclohexenyl ring in these compounds (*m/z* 290 and 210) at reduced relative abundance (Fig. 6). These mass spectra point to the presence of more than a single CFAM for each GC peak, and do not necessarily exclude the presence of other CFAM structures.

IR data obtained for methyl esters of the same CFAM mixture indicate that each of the first two compounds (GC peaks 10 and 11) has a *cis* double bond on a cyclohexenyl ring and a *trans* double bond on a hydrocarbon chain. A similar conclusion was reached in an independent gas-phase GC/IR study (29) of CFAM methyl esters, isolated from heated linseed oil.

Each of the next two compounds (GC peaks 12 and 13) exhibited an IR band at 664 cm^{-1} characteristic of a double bond in a 6-membered ring, and another one near 723 cm^{-1} that is not due to a double bond in a hydrocarbon chain (31). This band is probably consistent with a second ethylenic bond in the ring. The relative location of the double bonds in the ring are not known, and of the several possible unsaturation sites, one could perhaps be at a substituted ring carbon. A similar IR band was also found in the published spectrum of 1,3-cyclohexadiene (32), and a UV band with a maximum at 233 nm [$E_{1\%}$ (absorptivity of a 1% solution) = 32.8], characteristic of conjugated dienes, was observed for the CFAM methyl ester mixture. Because the UV spectrum was obtained for the CFAM mixture, rather than for each of the compounds of concern, the available data may not be sufficient to confirm the presence of a conjugated diene system for the CFAMs of GC peaks 12 and 13.

We do not have an explanation for the presence of features that are indicative of both cyclohexenyl and cyclohexadienyl structures in the MS data (GC peaks 10–13), and bands that indicate the presence of either a cyclohexyl (GC peaks 10 and 11) or a cyclohadienyl ring (GC peaks 12 and 13) in the IR spectra. The previously proposed explanation of double bond migration during ionization (33) cannot be substantiated by the observed MS data in this case.

The mass spectra, observed for the last two minor compounds (GC peaks 14 and 15), showed a large gap of 120 mass units due to the loss of a sizeable fragment in each case. The identity of a methyl or an ethyl ring substituent, respectively, for these compounds is indicated by the mass intervals of 15 u (GC peak 14), and 14 and 15 u (GC peak 15) at the high-mass end of the corresponding spectra. These mass spectra are consistent with structures of bicyclic monoenoic fatty acid derivatives.

In published mechanisms (13,34) for the formation of cyclic and bicyclic fatty acids from heated linolenic acid, two possible bicyclic structures were proposed (13,34). Each structure consists of a 6-membered ring fused to a 5-membered ring, and has one double bond. This predicted bicyclic structure has a mass of 120 u and is consistent with the observed data for GC peaks 14 and 15. The two proposed structures (13,34) differ in the length of the ring substituent, ethyl or methyl; both were also observed (GC peaks 14 and 15, respectively). It was demonstrated that similar monounsaturated bicyclic fatty acid structures, originating from octadecatrienoic acids, are formed during tall oil distillation (35). These tentative structures are also consistent with the IR data. These two compounds exhibited a 2939 cm^{-1} band (asymmetric C–H stretch in a methylene group) that was diminished in intensity relative to the adjacent band at 2962 cm^{-1} (asymmetric C–H stretch in a methyl group). This decrease in intensity is consistent with bicyclization of the hydrocarbon chain in these molecules. Moreover, neither the characteristic bands for a *cis* double bond in a 5- (3061 and 719 cm^{-1}) or 6-membered (3032 and 663 cm^{-1}) ring nor those for a *cis* (3005 cm^{-1}) or *trans* (3032, 3005, and 970 cm^{-1}) double bond in a hydrocarbon chain were found. Instead, weak =C–H stretching vibrations at 3025 (GC peak 14) and 3021 cm^{-1} (GC peak 15) were observed. Because these bands are not sufficient to indicate the location of the double bond in this bicyclic system, no possibility can be ruled out, including the unsaturation site at a substituted ring carbon, such as a carbon shared by the two rings.

By using the complementary techniques of GC/MI–FTIR and GC–EIMS, it was possible to elucidate the structures of

diunsaturated monocyclic and monounsaturated bicyclic CFAMs: (i) Structures with a cyclopentenyl ring were the easiest to determine (shown in Fig. 3); (ii) structures with an unsaturated 6-membered ring were in two categories: first, those that had one *trans* double bond (between C_7 and C_8) in the oxazoline 2-alkenyl chain and a *cis* double bond in the 6-membered ring (e.g., GC peak 8), and second, those having MS and IR data that could not be reconciled and no double bonds in the oxazoline 2-alkyl chain [for compounds in the second category, the IR data indicated that either one double bond was in the 6-membered ring and the other *(trans)* was in the propenyl substituent of the 6-membered ring (e.g., GC peak 10, see Table 1), or the two double bonds were in the 6-membered ring (e.g., GC peak 12, see Table 1), whereas the MS spectra indicated that both possibilities existed (Fig. 6)]; (iii) the last type of CFAM found was monounsaturated; it had a bicyclic ring with one double bond and a short alkyl substituent on the ring (methyl or ethyl for GC peak 14 or 15, respectively).

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