Electron Impact Mass Spectra of the Oxazoline Derivatives of Some Conjugated Diene and Triene C_{18} Fatty Acids

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The location of the double-bond systems of some conjugated diene and triene C_{18} fatty acids (C18:2[9,11], C18:2[10,12], C18:3[9,11,13] and C18:3[10,12,14]) derived from alkaline isomerization has been determined by gas chromatography/mass spectroscopy analysis of their 4,4-dimethyloxazoline derivatives. The positions of the double bonds were indicated by a characteristic mass separation of 12 atomic mass units for each olefinic bond. Furthermore, the structure assignments were supported by the presence of prominent formal allylic cleavage peaks.

KEY WORDS: Conjugated diene and triene C_{18} fatty acids, fatty acids, GC/MS analysis of 4,4-dimethyloxazoline derivatives.

Conjugated dienes C_{18} fatty acids are found in certain plant oils (1,2), foodstuffs, meats (3–9), human tissues, blood and body fluids (5,10,11). Anticarcinogenic and antioxidant properties have been reported (7,9,12), especially for conjugated linoleic acid (C18:2[9*c*,11*t*]). The conjugated triene C_{18} fatty acids are known to occur as different positional and geometrical isomers in the seed oils of some plant families (13). Various biological effects from certain triene derivatives have been discussed previously (14).

The mass spectra of the methyl ester derivatives of C_{18} unsaturated conjugated fatty acids are similar to those of the nonconjugated acids (10,14,15). During the ionization process of unsaturated fatty acid methyl esters, a migration of the double-bond system occurs, preventing the mass-spectroscopy detection of its original position in the chain (16). To overcome this problem, various derivatives, such as pyrrolidines (17), picolinyl esters (18) and 4,4-dimethyloxazolines (19), have been used for the mass spectroscopy (MS) characterization of unsaturated fatty acids. In these compounds, bond migration is suppressed by preferential charge stabilization at the heterocyclic part of the derivative.

The mass spectra of the pyrrolidine derivatives of conjugated C_{18} diene acids produced key fragments that showed the double bond closest to the carbonyl group, but the remote double bond was not indicated by the observed fragmentation pattern (10,17). Thus, these derivatives are not suitable for the identification of unknown compounds. In contrast, mass spectra of the oxazoline derivatives of α-eleostearic acid (C18:3[9*c*,11*t*,13*t*]) (20,21) and kamlolenic acid (18-OH-13:3[9*c*,11*t*,13*t*]) (22), both with a conjugated triene system in the 9,11,13-position, were found to be useful for the complete location of the conjugated double bonds in the fatty acid chain.

The present study discusses the mass spectra of the oxazoline derivatives of some conjugated diene and triene C_{18} fatty acids, derived from alkaline isomerization of linoleic acid (C18:2[9*c*,12*c*]) and α-linolenic acid (C18:3[9*c*,12*c*,15*c*]).

EXPERIMENTAL PROCEDURES

Twenty mg of C18:2[9*c*,12*c*] or C18:3[9*c*,12*c*,15*c*] (Sigma Chemical Co., St. Louis, MO) was heated at 180°C for 25 min in 3 mL of a solution of KOH in ethyleneglycol (6.5%) (23). The products were thoroughly extracted three times with 3 mL hexane. The extract was dried over Na_2SO_4 and evaporated with a stream of N_2. Preparation of the oxazoline derivatives was done as described by Zhang *et al.* (19). Gas chromatography (GC)/MS analysis of the reaction products in hexane solution was carried out with the Nermag Automass (France, Paris) at 70 eV ionization energy. GC separation was achieved on a DB 23 (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25 mm i.d., 0.25 μm) with a temperature program of 170–245°C at 2°C/min.

RESULTS AND DISCUSSION

The obtained mass spectra of all compounds show two intensive peaks in the low mass range at *m/z* 113, produced by McLafferty rearrangement, and at *m/z* 126, formed *via* a cyclization-displacement reaction (19). Furthermore, an even-mass homologous series at *m/z* 126 + 14 mu, derived from cleavage at each bond, is found. According with the empirical rule of Zhang *et al.* (19), these regular series are interrupted in the region of the double bond; instead of the 14-mu gap, 12-mu gaps are observed, representing the cleavages at each olefinic bond (see Figs. 1 and 2). The configuration of the double bonds could not be confirmed from the spectra.

Conjugated diene acids. As known from the literature, alkali isomerization of linoleic acid yields a mixture of C18:2[9*c*,11*t*] and C18:2[10*t*,12*c*] (24). According to such isomerization, the present GC/MS analysis of the products of linoleic acid showed two main peaks in similar proportions (Fig. 1). The mass spectra of both compounds (Fig. 1) exhibit a more intensive molecular ion at *m/z* 333, as found for the linoleic acid derivative. The even-mass homologous series *m/z* 126 + 14 mu is interrupted in the region of the double bonds. So the mass spectrum of the first peak shows a mass difference of 12 mu between *m/z* 196/208 and *m/z* 222/234, whereas the second peak shows 12-mu gaps between *m/z* 210/222 and *m/z* 236/248, according to conjugated double bonds in positions 9,11 and 10,12, respectively (see Fig. 1). This conclusion was further confirmed by two prominent peaks derived from a formal allylic cleavage at *m/z* 182/262 (compound 1) and *m/z* 196/276 (compound 2). The peaks above *m/z* 234 (compound 1) and *m/z* 248 (compound 2) again show the expected regular 14-mu differences.

In comparison with the mass spectra of the pyrrolidine derivatives of conjugated diene C_{18} fatty acids (17), the fragmentation patterns of the oxazoline derivatives are obviously unequivocally interpretable in the double-bond

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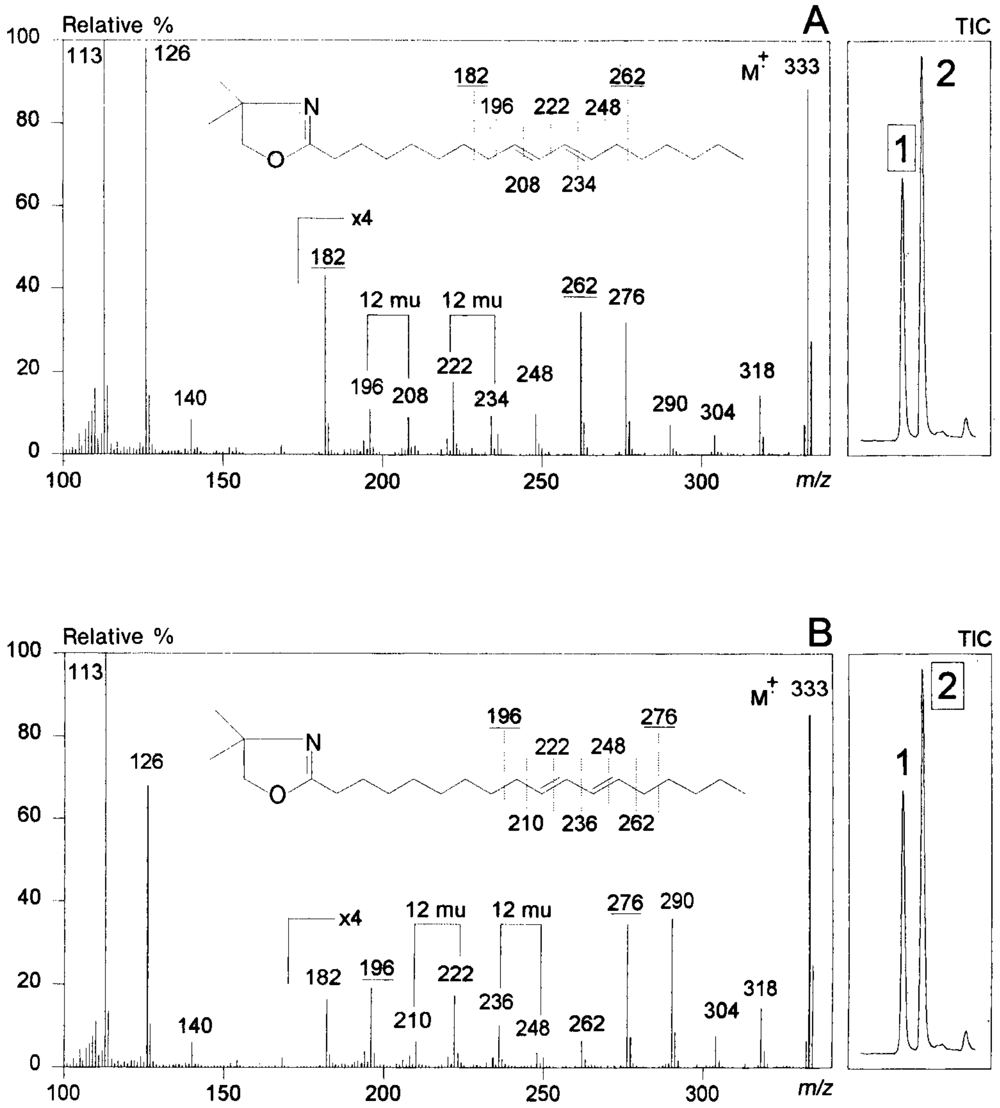

FIG. 1. Mass spectra (70 eV) of the oxazoline derivatives of (A) C18:2[9,11] and (B) C 18:2[10,12] with their corresponding total ion current curve (TIC) (peaks marked with a box). Allylic cleavage peaks are underlined.

region, and they show intensive allylic cleavage peaks, which support the structure assignments.

Conjugated triene acids. The reaction products of the alkali isomerization of triene fatty acids are more complex than those of the diene fatty acids. It is known that, under the used reaction conditions, α-linolenic acid yields a mixture of trienes with diene- and triene-conjugation (25), with 10,12,14-octadecatrienoic acid being the main reaction product (24). In this work, only the compounds with a conjugated triene structures will be considered in more detail.

The results of GC/MS analysis of the reaction products of α-linolenic are presented in Figure 2. According to their MS data, only compounds 1 and 5 (Fig. 2) could contain a conjugated triene structure. Both derivatives exhibit an intensive molecular ion at *m/z* 331. The mass spectrum of peak 1 (Fig. 2) is almost identical with that of the oxazoline derivative of α-eleostearic acid (20,21). Only slight differences in the peak intensities are observed. For this compound, the even-mass homologous series *m/z* 126 + 14 mu is interrupted with 12-mu gaps at *m/z* 196/208, 222/234 and 248/260, suggesting a 9,11,13-position of the conjugated double-bond system. In addition, two intensive formal allylic cleavage peaks are found at *m/z* 182 and *m/z* 288, which supports the proposed double-bond positions.

In the spectrum of peak 5 (main product) (Fig. 2), the 12-mu interruption of the series *m/z* 126 + 14 mu occurs at *m/z* 210/222, 236/248 and 262/274, which can be explained by a 10,12,14-triene structure. This assignment is also supported by the presence of the two intense allylic cleavage peaks at *m/z* 196 and *m/z* 302. The peaks above *m/z* 260 (compound 1) and *m/z* 274 (compound 5) again show the expected, regular 14-mu differences.

The present data show that GC/MS analysis of the oxazoline derivatives of conjugated C_{18} fatty acids can be a useful technique for both the determination of the number of olefinic bonds and their location in the hydrocarbon chain.

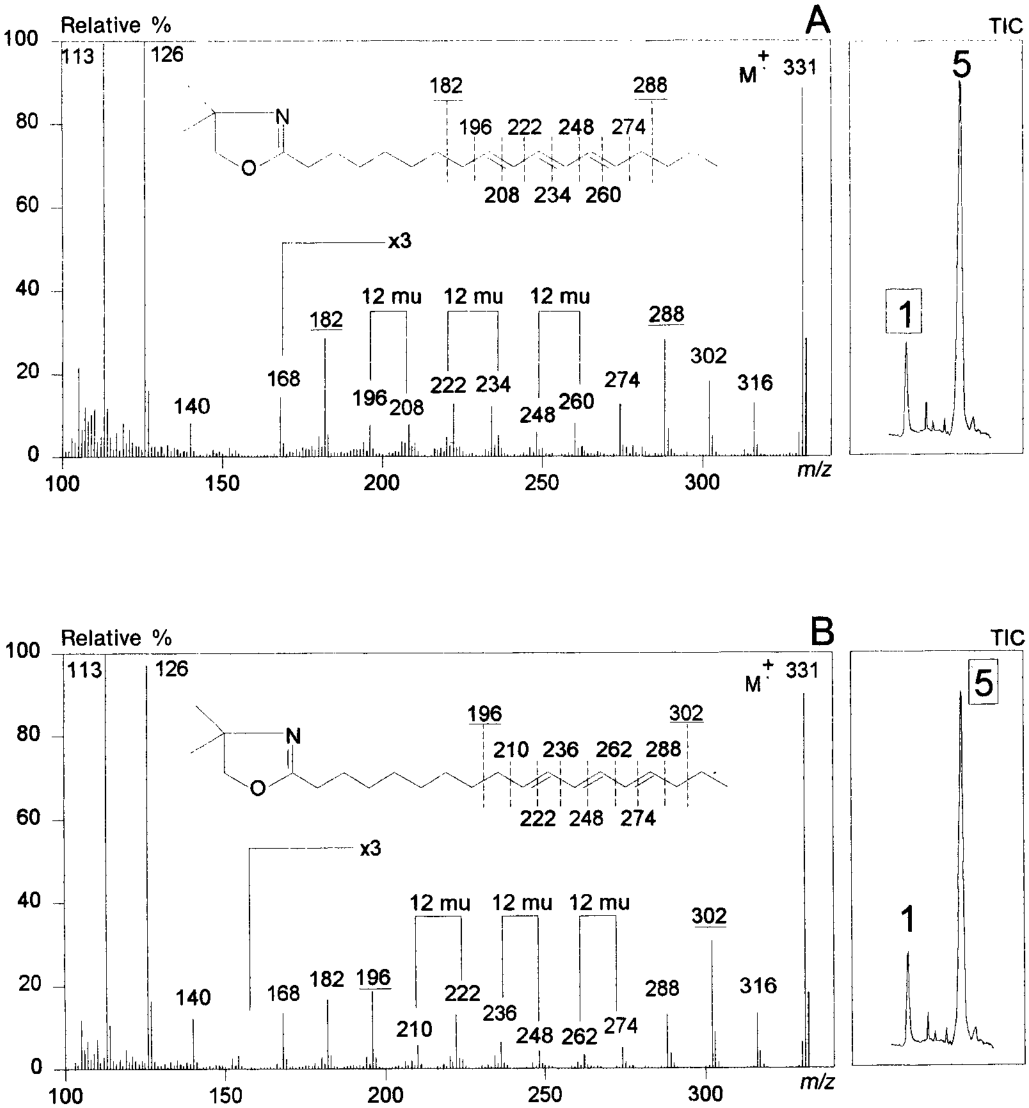

FIG. 2. Mass spectra (70 eV) of the oxazoline derivatives of (A) C18:3[9,11,13] and (B) C18:3[10,12,14] with their corresponding total ion current curve (TIC) (peaks marked with a box). Allylic cleavage peaks are underlined.

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