The Mass Spectra of the 4,4-Dimethyloxazoline Derivatives of Some Conjugated Hydroxy Ene-yne C_{17} and C_{18} Fatty Acids[1]

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ABSTRACT: The mass spectra of the 4,4-dimethyloxazoline derivatives from various fatty acids with a hydroxy group in conjugation with conjugated double-triple bonds (7-hydroxy-*trans*-10-heptadecene-8-ynoic acid; 7-hydroxy-*trans*-10,16-heptadecadiene-8-ynoic acid; 8-hydroxy-*trans*-11-octadecene-9-ynoic acid; 8-hydroxy-*trans*-11,17-octadecadiene-9-ynoic acid) have been examined and compared with their analogous nonhydroxy derivatives. The position of the hydroxy group was unequivocally proven by characteristic odd-numbered fragment peaks, explainable by α-cleavage at the hydroxy group at the oxazoline end of the molecule. The weak ions produced by α-cleavage at the other side of the hydroxy group indicated that the hydroxy group must be in conjugation with the ene-yne system. Fragments that allow one to distinguish between ene-yne or yne-ene systems were absent. In conjunction, the weak molecular ion and the more intense M – 18 ion could confirm the molecular weight of each fatty acid.
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KEY WORDS: Acetylenic fatty acids, 4,4-dimethyloxazoline derivatives, GC/MS, hydroxy fatty acids.

Fatty acids (FA) with conjugated double-triple bonds (ene-yne systems) in C_{17} or C_{18} carbon chains have been reported only in plants of the families Santalaceae and Olacaceae, and they are therefore of chemotaxonomical importance (1,2). Some members of this type of FA contain an additional hydroxy group in conjugation with the acetylenic linkage and/or a terminal vinyl double bond. Until now, the following four members of the latter unusual compounds have been found in various seed oils: **A** = 7-HO-17:2(8*a*,10*t*); **B** = 7-HO-17:3(8*a*,10*t*,16); **C** = 8-HO-18:2(9*a*,11*t*); and **D** = 8-HO-18:3(9*a*,11*t*,17), where *a* = acetylenic bond; *t* = *trans* olefinic bond.

For the gas chromatography/mass spectrometry (GC/MS) characterization of HO–FA, various "on-site" or "remote-site" derivatization and chemical ionization methods have been tested in recent years and are reviewed elsewhere (3–5). A common method for the analysis of HO–FA is the preparation of the trimethylsilyl (TMS) ethers of the FA methyl ester (ME) derivatives before GC analysis. The products obtained show improved chromatographic properties (6) and, in most cases, their mass spectra can give information in terms of the position of the HO-group (3). When an additional double bond is present in the FA chain, the fragmentation pattern is strongly influenced by the position of the olefinic carbons in relation to that of the TMS ether group (3). For the TMS ethers of compounds **A–D**, fragment ions that enable the location of the HO-group and the unsaturated bonds adjacent to the HO-group have been observed (7,8). However, it was not possible to distinguish between an ene-yne or an yne-ene system, and the terminal vinyl group in the compounds **B** and **D** also could not be detected only by the fragmentation pattern (7,8).

To extend MS information on these unusual FA, mass spectra of the 4,4-dimethyloxazoline (DMOX) derivatives of the FA **A–D** have now been recorded. These derivatives show improved MS properties due to the enhanced charge stabilization on the oxazoline moiety and to the reduced tendency of bond migration; GC properties are also good (9). As was shown previously, the DMOX derivatives of miscellaneous unsaturated hydroxy FA (10,11) and conjugated ene-yne acetylenic FA (8,12,13) can produce mass spectra that enable the location of the position of the HO group and of the unsaturated systems.

The FA mixture used in this study was available from the seed oil of *Iodina rhombifolia* (Santalaceae), which is known to contain compounds **A** (7.3%), **B** (<0.1%), **C** (12.2%), **D** (1.3%), and their analogous nonhydroxy derivatives **A′–D′** (20.1, 0.7, 20.3, and 1.5%, respectively) (8).

EXPERIMENTAL PROCEDURES

Extraction and hydrolysis of the seed oil of *I. rhombifolia*, and the identification of each FA in the FA mixture have been

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described previously (8). The DMOX derivatives were prepared by condensation of the total free FA (obtained by hydrolysis of the oil with 1 N potassium hydroxide in 95% ethanol) with 2-amino-2-methylpropanol at 170°C for 0.5 h under nitrogen as described by Zhang *et al.* (9).

GC/MS analysis was performed with the Nermag Automass (Paris, France) operating with an ionization energy of 70 eV, a source temperature of 225°C, and an interface temperature of 220°C. The separation of the DMOX derivatives was carried out with a DB 23 (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25 mm, i.d. 0.25 μm) and the following temperature program: 160–230°C, 2°C/min. Helium was the carrier gas (0.5 bar).

RESULTS AND DISCUSSION

The spectra of the DMOX derivatives of **A**–**D** and their corresponding nonhydroxy derivatives **A′**–**D′** are presented in Figure 1. The spectrum of compound **B** was found to be noisy, explainable by its low concentration in the mixture.

All spectra of the HO–FA derivatives showed weak molecular ion peaks (**A**, *m/z* 333; **B**, *m/z* 331; **C**, *m/z* 347; **D**, *m/z* 345), accompanied by more intense M – $[H_2O]^+$ peaks (**A**, *m/z* 315; **B**, *m/z* 313; **C**, *m/z* 329; **D**, *m/z* 327). In the low-mass range, the typical DMOX derivative ions at *m/z* 113 and at *m/z* 126 (base peak) were observed. These are formed by a cyclization–displacement reaction and McLafferty rearrangement (9), respectively.

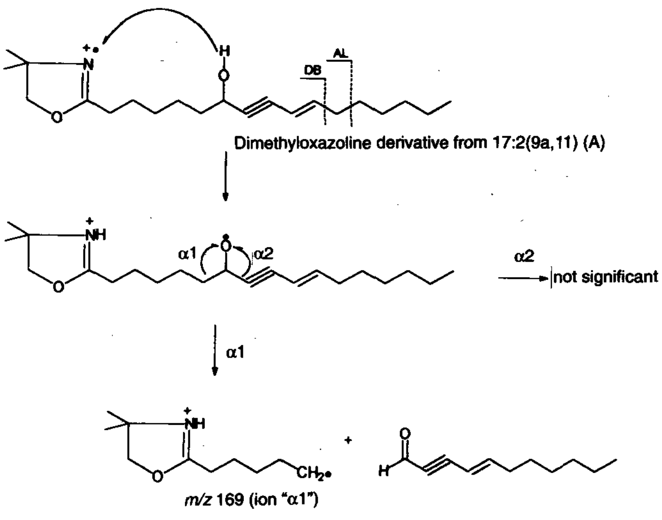
The positions of the HO groups were unequivocally indicated for all hydroxy FA. So, the even-mass homologous series *m/z* 126 + 14n, derived from subsequent cleavage at each methylene group with hydrogen transposition to the oxazoline moiety, was interrupted by prominent odd-numbered ions at *m/z* 169 (α1) for **A** and **B** and at *m/z* 183 (α1) for **C** and **D**. Assuming a mechanism similar to that proposed by Zhang *et al.* (10) for the DMOX derivatives of ricinoleic acid, these ions could be produced by α-cleavage at the hydroxylated carbon (for **A** and **B** between C_6–C_7; for **C** and **D** between C_7–C_8) with simultaneous rearrangement of the hydrogen of the HO group to the oxazoline moiety (Scheme 1).

Peaks due to α-cleavage at the other side of the HO group (α2) (*m/z* 198 for **A** and **B** and *m/z* 212 for **C** and **D**), which were observed in the DMOX derivative of 12-HO-18:1(9*c*) (ricinoleic acid) (10), were found to be weak. As for the mass spectrum of the methyl ester of the similar 8-HO-18:2(9*a*,11a), the latter finding is indicative of the HO-group being in conjugation with the unsaturated system (14).

The ions representing the cleavages on the unsaturated carbons of the hydrocarbon chain of the compounds **A**–**D** were difficult to interpret. As shown previously, the conjugated acetylenic and olefinic bonds in the DMOX derivatives are indicated by interruption of the *m/z* 126 + 14n series with characteristic 10 and 12 mu gaps, respectively (8,12,13). Thus, the ion series at *m/z* 198-208-222-234-248 for **A** and **B** and at *m/z* 212-222-236-248-262 for **C** and **D** could be expected. Figure 1 shows that the first three fragment ion peaks of these theoretical series do not appear, and only the two last ion peaks can be observed. In comparison, the mass spectra of compounds **A′** to **D′** showed the expected mass unit sequences at *m/z* 182-192-206-218-232 (**A′**/**B′**) and *m/z* 196-206-220-232-246 (**C′**/**D′**), proving the conjugated yne-ene linkage (see Fig. 1).

The ions for the cleavage on the double-bond side (DB: *m/z* 248 for **A** and **B**; *m/z* 262 for **C** and **D**) and those adjacent to the allylic methylene group (AL: *m/z* 262 for **A** and **B**; *m/z* 276 for **C** and **D**) in the spectra of **A**–**D** appeared with low abundance. In contrast, the equivalent ions in the DMOX derivatives of the nonhydroxy ene-yne FA were found to be significant (DB: *m/z* 232 for **A′** and **B′**; *m/z* 246 for **C′** and **D′** and AL: *m/z* 246 for **A′** and **B′**; *m/z* 260 for **C′** and **D′**) (see Fig. 1).

Obviously, conjugation of the OH group with the unsaturated system has a significant influence on the fragmentation mechanism. The structure-specific cleavages observable in the nonconjugated HO–FA or non-HO–FA derivatives are suppressed in the DMOX derivatives of **A**–**D**. A similar phenomenon was observed in spectra of the DMOX derivatives of the conjugated FA 18:3(9*c*,11*t*,13*t*) (α-eleostearic acid) and its corresponding 4-keto derivative (licanic acid) (15). The mass spectrum of the latter compound also showed diagnostic peaks only for the α-cleavage at the oxygenated carbon atom (4-keto), but lacked the fragments that enable the location of the unsaturated system, observable in the spectrum of α-eleostearic acid (15). In contrast, the DMOX derivative of 18-HO-18:3(9*c*,11*t*,13*t*) (α-kamlolenic acid) showed a mass spectrum that was in full agreement with the position of the HO-group and the conjugated double bonds in the chain (10). Thus, it can be concluded that two characteristics may influence the fragmentation pattern of the DMOX derivatives: the absolute position and sequence of the oxygenated carbon and the unsaturated system in the FA chain and the position of the oxygenated carbon in relation to the unsaturated system. It is



SCHEME 1

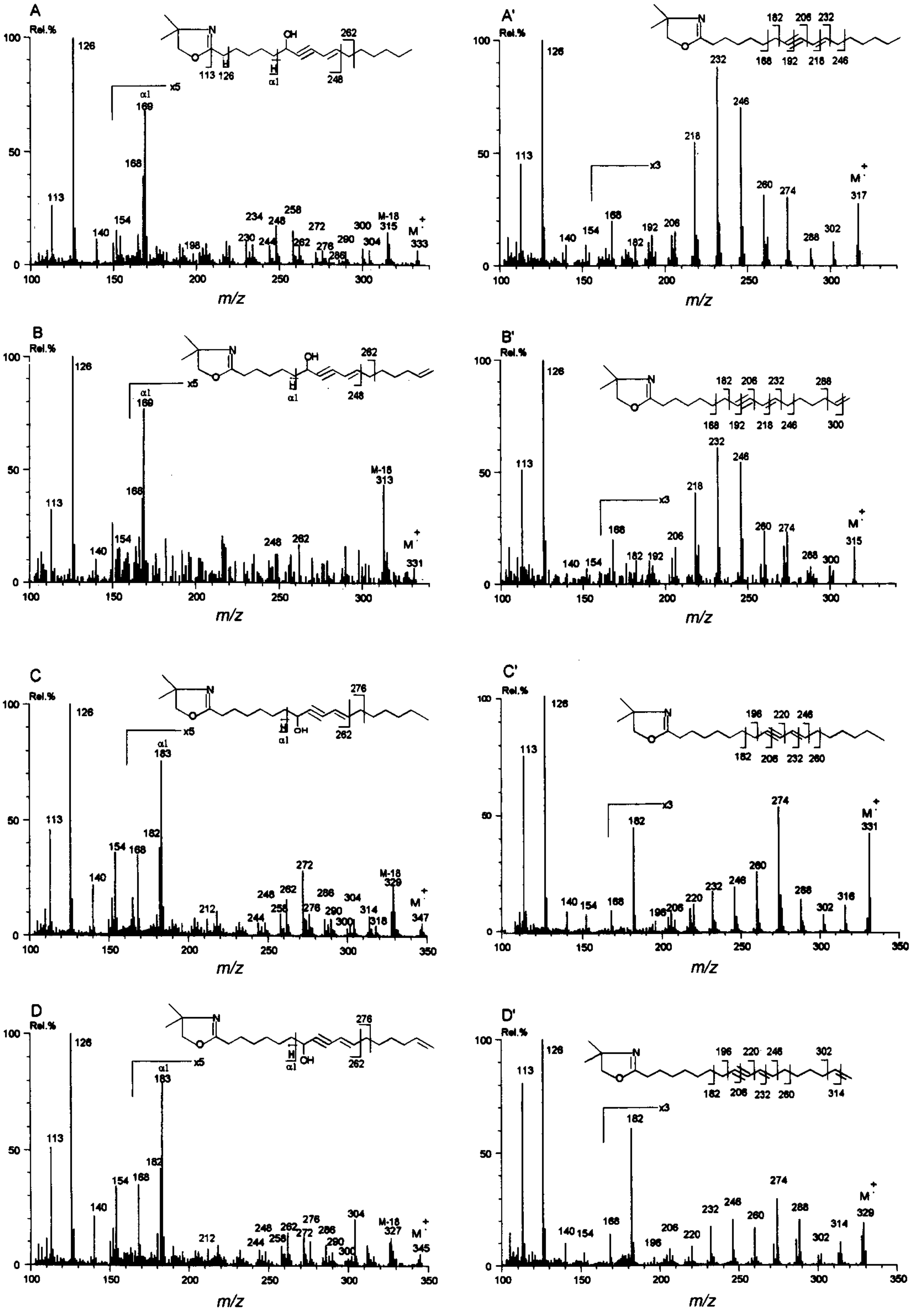


FIG. 1. The mass spectra of the 4,4-dimethyloxazoline derivatives of 7-HO-17:2(8*a*,10*t*) (A); 7-HO-17:3(8*a*, 10*t*,16) (B); 8-HO-18:2(9*a*,11*t*) (C); 8-HO-18:3(9*a*,11*t*,17) (D), and their analogous nonhydroxy derivatives (A′–D′). *a*, Acetylenic bond; *t*, *trans* olefinic bond.

worth noting that some of the peaks in the higher mass range of the mass spectra of **A–D** appeared as pairs of peaks with a mass difference of 4 mu (see Fig. 1). This observation can be explained by two competing fragmentation routes, where one is based on the DMOX derivative of the original FA molecule, and the other is based on the derivative after elimination of water ($[M - 18]^+$) in the ion source.

Ion peaks indicative of the terminal vinyl group in **B** and **D**, which appeared in the DMOX derivatives of the analogous nonhydroxy FA derivatives (see Fig. 1), could not be detected.

In comparison with the TMS ether derivatives of these FA (7,8), the DMOX derivatives have the advantage that the elution sequence and the separation of the FA on the GC column are identical with those of the ME derivatives. Moreover, a series of other types of FA occurring in a mixture can generally be better characterized by the mass spectra of the dimethyloxazoline derivatives than by the currently preferred FAME.

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