Location of Triple Bonds in the Fatty Acids from the Kernel Oil of *Pyrularia edulis* by GC-MS of Their 4,4-Dimethyloxazoline Derivatives[1]

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Location of triple bond in long-chain fatty acids has been effected by mass spectrometry or gas chromatography-mass spectrometry of their 4,4-dimethyloxazoline (DMOX) derivatives. The position of acetylenic linkage, either isolated or conjugated with olefinic bond(s) is indicated by a clear separation of 10 atomic mass units between the highest peaks of two neighboring fragment clusters. The acetylenic acid components of the kernel oil of *Pyrularia edulis* were found to be 17:1(8a), 18:1(9a), 18:2(9a,11), 18:2(9a,17) and 18:3(9a,11,17).

Mass spectra of an almost complete series of methyl octadecynoates were investigated by Kleiman and coworkers (2). These authors found that, though each spectrum is unique, the spectra are complex and the complexity is greater when olefinic bonds accompany the acetylenic bonds. Derivatization at the triple bond by oxymercuration followed by $NaBH_4$-reduction led to the formation of hydroxy esters which, after O-silylation, gave diagnostic mass spectra indicative of the alkyne bond location (2). Later, in a study on the mass spectra of octadecynoic acid pyrrolidides, Holman's group (3) came to the conclusion that a mass interval of 10 atomic mass units (amu) between fragments containing n-2 and n-1 carbons of the acyl moiety indicates a triple bond at carbon n. Confirmation of the triple bond location is further provided by intense peaks at carbons n-2 and n+2. This appears to be an effective approach to the structure determination of acetylenic acids and is preferred for its simplicity compared to analysis based on methyl esters or their "on-site modification" products. However, owing to the great lability of the acetylenic linkage, the fragment ions displayed in the spectra are of low intensity and sometimes the position of the triple bond is hard to recognize. Moreover, the further the alkyne bond is moved from the middle of the fatty chain, the more the interval of 10 amu is obscured by other peaks in the diagnostic clusters.

In a search for a newer technique that would give more informative spectra under conventional GC-MS conditions (4), we found that 4,4-dimethyloxazoline (DMOX) derivatives of fatty acids were most revealing (5). The mass spectral behavior of certain long-chain fatty acid derivatives of this type was first reported by Gronowitz and collaborators (6,7) in the study of hydrozirconation and related reactions. We found that a clear-cut exhibition of the chain feature can usually be provided in the conventional electron impact mass spectra of these "hidden" carboxylic acids. This technique has been found well suited for the location of double bonds (5,8), methyl branching (9), cyclopropane rings (10), cyclopentene rings (1) and oxygenated groups (11,12). The advantages include: (i) high yield derivatization and easy clean-up; (ii) abundant diagnostic ions due to directed fragmentation [no difficulties in the structure elucidation of polyenoic acids having as many as six double bonds in the chain (5,8)]; (iii) good gas chromatographic characteristics [the volatility is comparable to that of simple esters and far better than the corresponding cyclic amides (5)], and (iv) the direct analysis of mixtures by means of in beam-electron impact (IBEI) ionization coupled with a linked scanning technique, keeping B/E constant (13).

The location of triple bonds in model compounds and the structure determination of component fatty acids in the kernel oil of *Pyrularia edulis* DC. (family Santalaceae) is described in the present paper.

MATERIALS AND METHODS

Mass spectrometry and gas chromatography-mass spectrometry. The mass spectrometry and gas chromatography-mass spectrometry measurements were carried out on MAT 711 and MAT 44S instruments under conditions similar to those described in previous works (5,8).

Derivatization. The general procedures used in the preparation of DMOX derivatives were the same as reported previously (5,8) [cf. also (6,7)].

Total fatty acids from kernel oil. The kernels of *Pyrularia edulis* DC, collected from the Tropical Botanical Garden, Xishuangbanna, were extracted with light petroleum to give a pale yellow oil. The source material and oil have the following physicochemical characteristics: oil, 59.32%; iodine value, 126.35; saponifiable content, 189.10; acid value, 1.78; n_D^{20} 1.4817; d_{40}^{40} 0.9309; and UV, λ_{max} 229 nm^{-1} [conjugated enynic system (14)]. Saponification gave the total fatty acids.

Preparation of acetylenic acids. The preparation of acetylenic acids was performed according to a literature method (15) by reaction of respective olefinic acids with Br_2 in diethyl ether at −10 to 0 C followed by dehydrobromination of the dibromo-acid with KOH in DMSO-PrOH at 100 C for two hr. Prepared this way were stearolic acid [18:1(9a)], m.p. 43-45 C [lit. (16), 47-48 C]; 13-docosanynic acid [22:1(13a)], m.p. 54-55 C [lit.(17), 57-57.5 C]; and 15-tetracosanynic acid [24:1(15a)], m.p. 61-62 C. All products were checked for authenticity and purity by mass spectrometry before use.

RESULTS AND DISCUSSION

Figure 1 shows the 70-eV mass spectra of the oxazoline derivatives of the three synthetic acetylenic acids. In the case of 18:1(9a) [Fig. 1(a)], the molecular ion, *m/z* 333 yields a McLafferty rearrangement product ion at *m/z* 113 (base peak) and a series of homologous ions *m/z* 126 + 14x. A mass interval of 10 amu occurs between *m/z* 196 (C_8) and *m/z* 206 (C_9), which indicates a triple bond at carbon 9

[1]Chemical Modification in Mass Spectrometry. 10. For preceding paper of this series, see (1).

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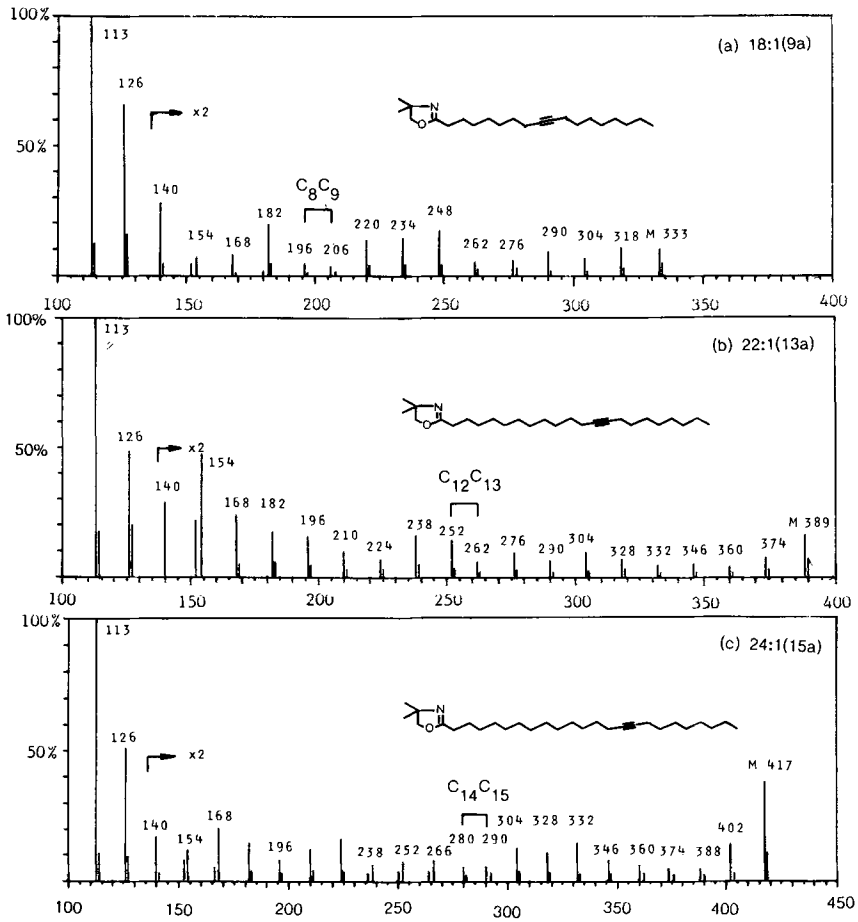

FIG. 1. Mass spectra of acetylenic acid oxazolines: (a) 18:1(9a); (b) 22:1(13a), and (c) 24:1(15a).

of the original fatty chain. For other isomeric acids, the 10-amu intervals between *m/z* 252 (C_{12}) and 262 (C_{13}) as well as *m/z* 280 (C_{14}) and 290 (C_{15}) indicate triple bonds at carbon 13 [Fig. 1(b)] and carbon 15 [Fig. 1(c)], respectively. Consequently, if a mass separation of 10 (rather than the usual 14) amu is observed between two neighboring even-mass homologous fragments involving n-1 and n carbon atoms of the original acid moiety, a triple bond exists between carbon n and n+1 in the chain. In addition, the characteristic ion pair is surrounded by two more intense peaks at carbon n-2 and carbon n+1 with a mass separation of 38 amu.

An extensive and varied array of conjugated acetylenes is known to occur in two plant families, the Olacaceae and Santalaceae. The C_{18} (or C_{17}) conjugated acetylenic fatty acids have certain common features that apply to almost the entire series. Their conjugated systems begin at the 9-position with one or more triple bonds. Usually the conjugated system is terminated with one or more double bonds, which may be *cis* or *trans*. Some members of the series have a double bond in the terminal position, a peculiar feature of conjugated acetylenic fatty acids (18,19).

The chromatogram of the fatty acids of the kernel oil of *Pyrularia edulis* DC, a species which has not been studied formerly, is given in Figure 2.

Five acetylenic acids, with isolated or conjugated double bonds, have been identified by interpretation of their mass spectra (Fig. 3). The results given in Table 1 conform well

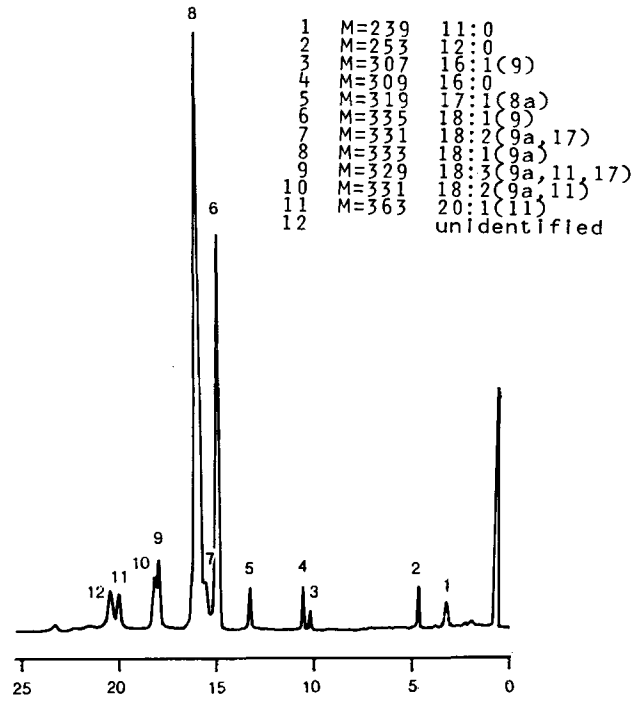

FIG. 2. Chromatogram of DMOXs of fatty acids from kernel oil of *P. edulis* using a SE-54 coated 28-m × 0.28-mm glass capillary column held at 170–240 C, programmed at 3 C/min.

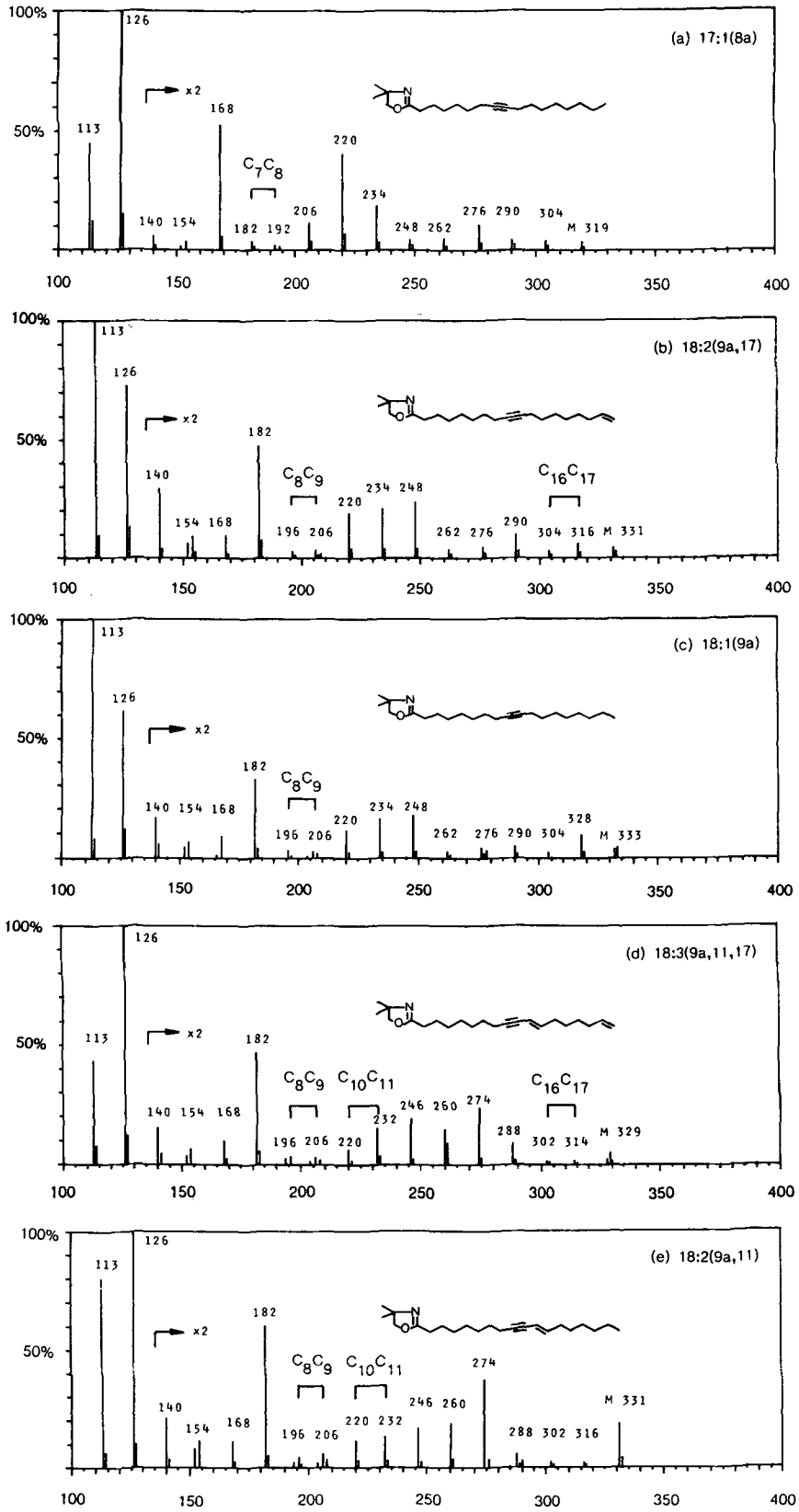

FIG. 3. Mass spectra of DMOX derivatives of component acetylenic acids: (a) 17:1(8a); (b) 18:2(9a,17); (c) 18:1(9a); (d) 18:3(9a,11,17), and (e) 18:2(9a,11).

with the common constituents found in the seed oils of Santalaceae species (Table 2). The configuration of the olefinic linkages was not determined.

ACKNOWLEDGMENT

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TABLE 1

Fatty Acid Composition of Kernel Oil of *P. edulis*

Peak number	% of total	MW of oxazoline	Assigned structure
1	0.98	239	11:0
2	1.24	253	12:0
3	0.88	307	16:1 (9)
4	1.46	309	16:0
5	2.05	319	17:1 (8a)
6	22.64	335	18:1 (9)
7	2.91	331	18:2 (9a, 17)
8	53.82	333	18:1 (9a)
9	2.88	329	18:3 (9a, 11, 17)
10	1.42	331	18:2 (9a, 11)
11	3.72	363	20:1 (11)
12	1.31		Unidentified

TABLE 2

Acetylenic Acids in *P. edulis*

Peak	Structure	Precedented origin
5	17:1(8a)	Previously not reported.
7	18:2(9a,17)	18% in *Acanthosyris spinescene* (Santalaceae) (20)
8	18:1(9a)	Stearolic acid, 20% in *P. pubera* and other Santalaceae species (21)
9	18:3(9a,11,17)	18:3(9a,11t,17) in *A. spinescens* (20), in *P. pubera* (22)
10	18:2(9a,11)	18:2(9a,11t), ximenynic acids, in Santalaceae species (21)

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