The Structures of Cyclopentenyl Fatty Acids in the Seed Oils of Flacourtiaceae Species by GC-MS of their 4,4-Dimethyloxazoline Derivatives[1]

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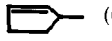
The structures of cyclopentenyl fatty acids in the seed oils of *Hydnocarpus anthelmintica* Pierre et Laness., *H. alpina* Wight, *H. hainanensis* (Merr.) and *Taraktogenos merrilliana* C.Y. Wu, all belonging to the family Flacourtiaceae, have been identified by GC–MS of the mixed 2-substituted 4,4-dimethyloxazoline (DMOX) derivatives. These variations are characterized by the presence of key fragments at (M-43), (M-55) and (M-67), while the location of a double bond in the chain is indicated by a 12-mass interval between the highest peaks of two adjacent homologous ion clusters. The unique fragmentation pattern coupled with the good gas chromatographic qualities of these derivatives enable convenient structure determination of cyclopentenyl fatty acids in complex mixtures.

Shrubs and trees of the plant family Flacourtiaceae occur in India, Senegal, Brazil and Southern China. The plants are largely underexploited. Cyclopentenyl fatty acids constitute up to 80% of the total fatty acids in the seed fats of the *Hydnocarpus* species (Table 1) and other genera of Flacourtiaceae that have been used in the treatment of leprosy (2).

In addition to the four most common cyclopentenyl acids, results of Cole and Cardoso (3) indicated the occurrence of some lower homologs of these cyclopentenes, but their structures were not completely determined. Increasing numbers of positional isomers of cyclopentenyl acids with double bonds at different positions became known with the advent of novel analytical methods. Thereafter, several new members were isolated from *H. anthelmintica* and related species and identified as oncobic acid [20:2(8,cy)], manoaic acid [16:2(6,cy)] and their isomers, such as 20:2(9,cy), 18:2(9,cy), 16:2(4,cy) and 16:2(9,cy) (4). The structures of these new acids were established on the basis of a multi-step reaction sequence followed by mass spectrometry (Scheme 1). The double bond in the chain was substantiated by GC analysis of the aldehydo-esters formed by ozonolysis.

TABLE 1

Cyclopentenyl Fatty Acids

		
n = 10,	16:1(cy),	hydnocarpic acid
n = 12,	18:1(cy),	chaulmoogric acid
n = 12, Δ^6,	18:2(6,cy),	gorlic acid
n = 14,	20:1(cy),	hormelic acid

The suffix "cy" denotes a cyclopentenyl structure.

[1]Chemical Modification in Mass Spectrometry 9. For preceding paper (Part 8) of this series, see (1).

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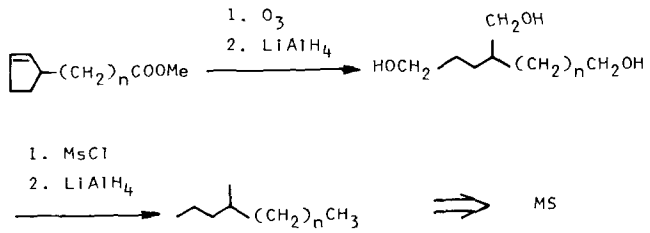
A much easier way to determine the structure of these cyclic acids was performed by Shukla and co-workers (5), who analyzed their N-acyl pyrrolidides by mass spectrometry; the key ion at M-67 (loss of $C_5H_7\cdot$) was attributed to the presence of a terminal cyclopentenyl ring.

Recently, a new derivatization technique for long-chain fatty acids has been developed in one of our laboratories. The carboxylic acid is first converted to the corresponding 2-substituted 4,4-dimethyloxazoline (DMOX) and then analyzed by mass spectrometry. These derivatives generally show rather abundant diagnostic ions and markedly improved volatility (comparable to that of simple esters), and are well suited for the location of olefinic linkage in polyunsaturated fatty acids having up to six double bonds in the molecule (1,6). In addition, this method has been successfully applied to the structure elucidation of alkynoic fatty acids (7), branched acids (8), cyclopropanoic fatty acids (9) and oxygenated acids (10,11).

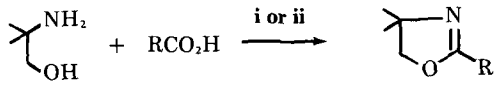
The fatty acid components of the seed oils obtained from *H. anthelmintica* Pierre et Laness., *H. alpina* Wight, *H. hainanensis* (Merr.) and *Taraktogenos merrilliana* (Li) C.Y. Wu, the latter being native to Xishuangbanna, Yunnan province (12), have now been investigated by GC-MS of their oxazoline derivatives. The analysis of lipid components of these plants, except *H. anthelmintica,* is reported here for the first time.

MATERIALS AND METHODS

Mass spectrometry. All electron-impact mass spectra were recorded on a MAT 711 mass spectrometer coupled with a SS 166 data system with operating parameters as follows: source temperature, 250 C; electron energy, 70 eV; emission current, 0.8 mA; accelerating voltage, 8 kV; resolution, 1000,



SCHEME 1



SCHEME 2. i. 170 C, 1 hr; ii. DCC, CH_2Cl, r.t., then $SOCl_2$.

and direct inlet probe temperature, 25-100 C, depending on the volatility of individual compounds.

Gas chromatography-mass spectrometry. All GC-MS analyses were carried out on a MAT 44S gas chromatograph-mass spectrometer equipped with a 28-m × 0.28-mm glass capillary column coated with SE-54 (crosslinked and bonded), carrier gas flow was one ml He/min, split ratio 1:10. Both separator and ion source were maintained at 250 C during the analysis. Column temperatures are indicated under each chromatogram (Fig. 1). Mass spectra were recorded at an ionization energy of 70 eV, while ion source was kept at 250 C during the analysis.

Methyl ester of hydnocarpic acid. The methyl ester of hydnocarpic acid, 16:1(cy), was isolated from the seed oil of *H. anthelmintica* according to a procedure described in the literature (13). The crude acid was converted to its methyl ester and then subjected to preparative GLC (PGLC) (column conditions: SE-30, 160-220 C, programmed at 5 C/min) to afford the pure methyl ester. ^{1}H NMR δ: 1.2-1.3 (18H,m), 1.6 (2H,m), 2.0 (1H,m), 2.2 (1H,m), 2.3 (2H,t), 2.6 (1H,m), 3.6 (3H,s) and 5.7 (2H,m). The ester was then saponified to give the pure acid, m.p. 58-60 C [lit.(13), m.p. 59-60 C].

Derivatization. The DMOX was prepared by a modification (6) of the literature method (14) (Scheme 2). A 10-μg sample of the fatty acid (or mixed acids) was heated with 50 μg of 2-amino-2-methylpropanol (AMP) in a micro-vial at 170 C for one hr. After cooling, the mixture was taken up in chloroform, washed with 5% aqueous sodium hydroxide, then water, and dried over anhydrous sodium sulfate. For products with higher purity, the following step is used: After concentration of the organic extract, the product was purified by passing it through a micro-column of silica gel (hexane, or 9:1 hexane-ethyl acetate for products with medium polarity). An aliquot of the product was introduced through

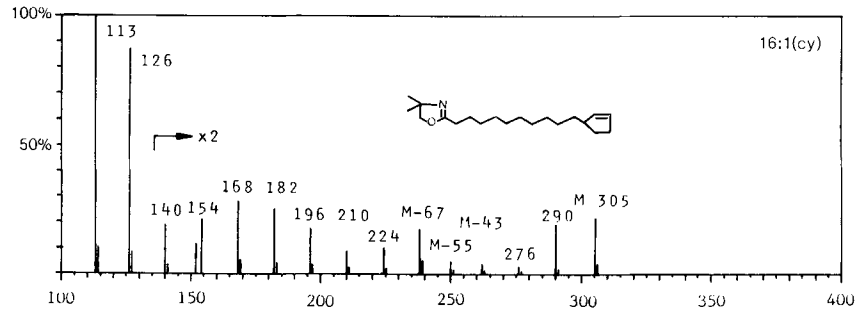


FIG. 1. The mass spectrum of 4,4-dimethyloxazoline derivative of 16:1(cy) obtained from *H. anthelmintica*.

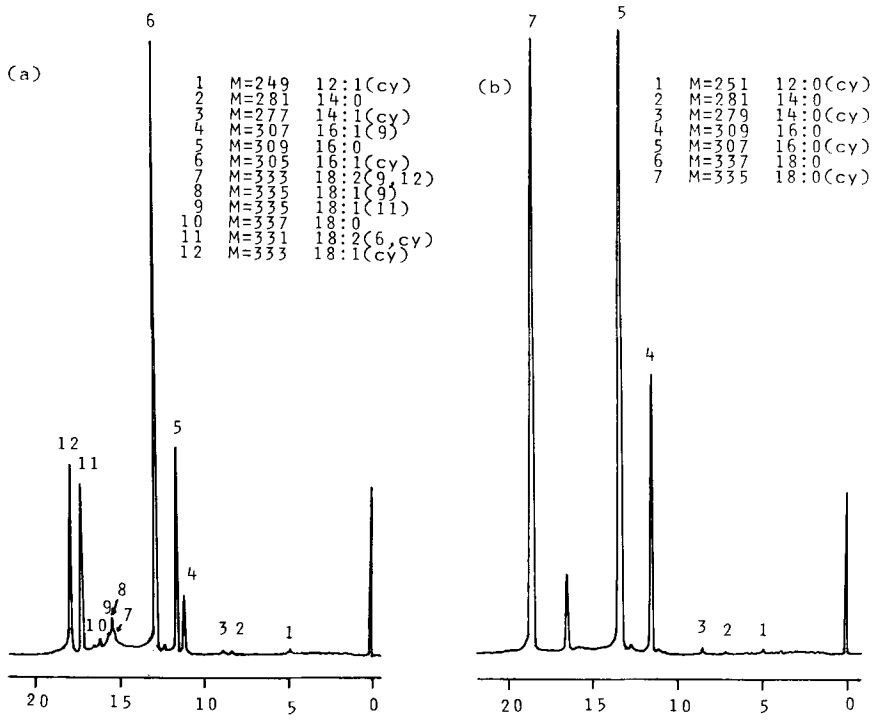


FIG. 2. Total ion current tracings of DMOX derivatives of constituent fatty acids from *T. merrilliana:* (a) before reduction, and (b) after reduction. Conditions: SE-54 glass capillary column, 28 m × 0.28 mm, 170–240 C at 3 C/min.

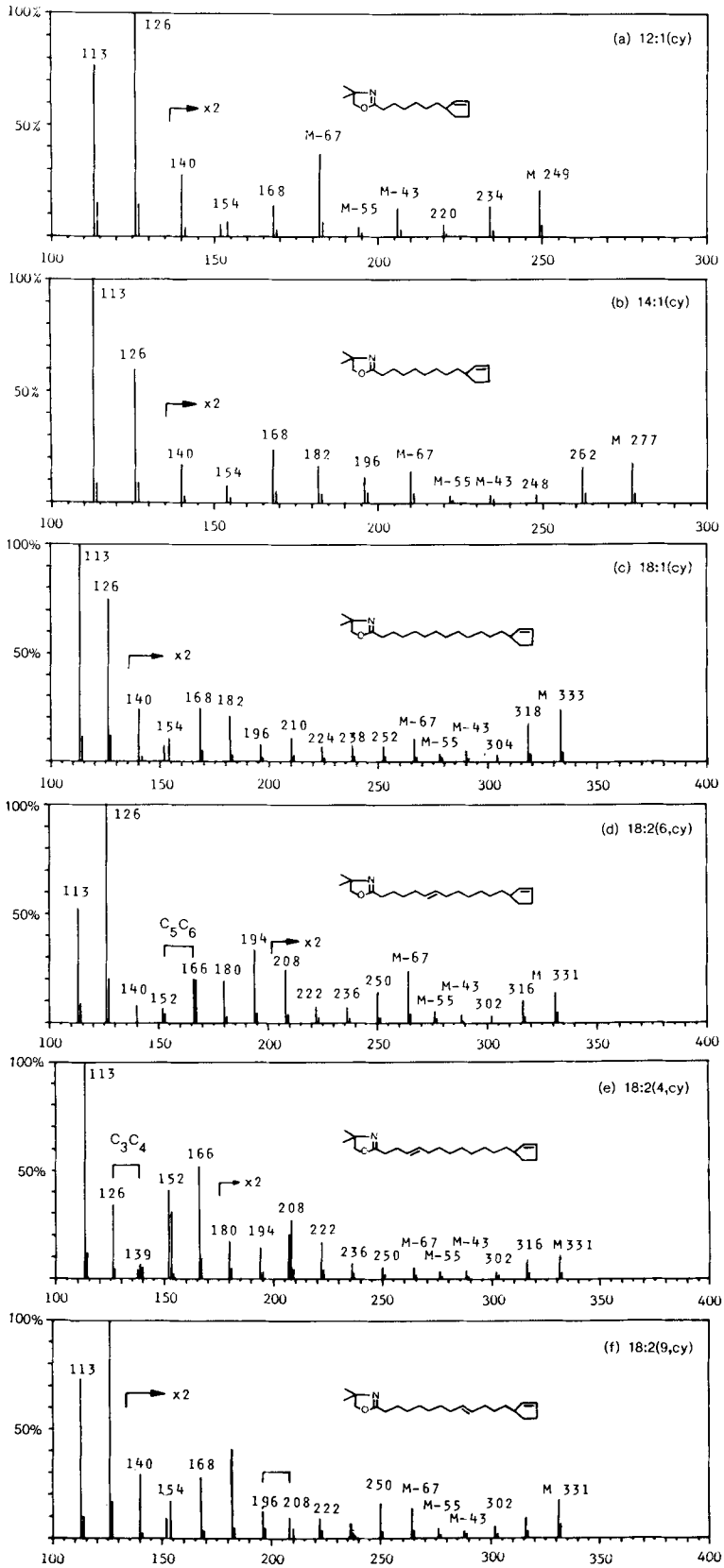


FIG. 3. Mass spectra of DMOX derivatives of cyclopentenyl fatty acids. (a), 12:1(cy) (*H. anthelmintica, H. hainanensis* and *T. merrilliana*); (b), 14:1(cy) (*H. anthelmintica, H. hainanensis* and *T. merrilliana*); (c), 18:1(cy) (*H. anthelmintica, H. alpina, H. hainanensis* and *T. merrilliana*); (d), 18:2(6,cy) (*T. merrilliana*); (e), 18:2(4,cy) (*H. anthelmintica, H. alpina*), and (f), 18:2(9,cy) (*H. hainanensis*).

the direct inlet system of the mass spectrometer or injected onto the column of the GC-MS system. Conversions, based on GC, were usually better than 95%. The reaction can be followed by TLC on silica gel GF_{254}.

Alternatively, the DMOX was prepared by mixing equimolar amounts of the fatty acid, AMP, and dicyclohexylcarbodiimide (DCC) in methylene chloride (20 C, 1-4 hr) and then treating with thionyl chloride (0 C, 0.5 hr). Yields are 90% (1,15).

Diimide reduction of double bonds. The reduction was performed as described by Cusack et al. (16). A solution of methyl esters (140 mg) of the total fatty acids from the seed oil of *T. merrilliana* and 2,4,6-trimethylbenzenesulfonyl hydrazide (228 mg, prepared from 2,4,6-trimethylbenzenesulfonyl chloride and hydrazine hydrate) in methyl alcohol (5 ml) was heated under reflux for one hr. After cooling, the mixture was diluted with water, extracted with ether (2 × 5 ml), and the ether layer evaporated. The residue was then saponified to afford the reduction product in an almost quantitative yield (132 mg). The completeness of the reduction was indicated by GC or GC-MS.

RESULTS AND DISCUSSION

Figure 2 shows the mass spectrum of the DMOX derivative of hydnocarpic acid prepared from the seed oil of *H. anthelmintica*.

The mass spectrum is similar to that of the corresponding N-acyl pyrrolidide reported by Shukla's group (5). A notable difference that can be observed is associated with the improved peak intensities in the former. The spectrum is dominated by ions containing the heterocyclic moiety: the McLafferty rearrangement product at *m/z* 113, and a homologous series starting from *m/z* 126. This series shows a local minimum at *m/z* 154 (C_5) typical of the mass spectra of 2-unsubstituted analogs. An interruption of the homologous series occurs at the site of unsaturation, if any, in the chain. The peculiar mass at M-67 represents the loss of cyclopentenyl radical ($C_5H_7\cdot$) from the C-terminus. Masses at two consecutive 12 amu intervals, M-67 vs. M-55 and M-55 vs. M-43, are unique neutral fragments that are common to all cyclopentenyl acids studied so far and can be regarded as diagnostic ions of these cyclic acids.

Fatty acid fractions from the seed fats of the four plants were subjected to GC-MS analysis, and the results are presented in Table 2.

A typical gas chromatogram is shown in Figure 1(a) for oxazolines from *T. merrilliana*. As a rule, within the series of same carbon number, DMOX derived from cyclic acids emerges after the straight chain members, either saturated or unsaturated. The mass spectra are given in Figure 3.

By applying the empirical rule for double bond location (6), the peaks No. 2, 4, 5, 7-10 in Figure 1(a) correspond to 14:0, 16:1(9), 16:0, 18:2(9,12), 18:1(9), 18:1(11) and 18:0, respectively. Peaks No. 1, 3, 11 and 12, all showing M-67, M-55 and M-43, represent cyclopentenyl acids with different chain lengths. DMOX derived from hydnocarpic acid (peak No. 6) has a spectrum identical to that depicted in Figure 2. The spectra of derivatives of two additional cyclic acids obtained from other species are given in Figure 3(e) and (f).

In all cases, the site of unsaturation can easily be recognized by the presence of a 12-mass separation between two neighboring clusters. In Figure 3(e), two clusters are spaced 12 amu apart at *m/z* 126 (C_3) and 138 (C_4). The latter is accompanied by a strong *m/z* 139, that is suggestive of a double bond at carbon 4. Analogously, the diagnostic peak *m/z* 154 (C_5) and 166 (C_6) in the spectrum of 18:2(6,cy) [Fig. 3(d)] is accompanied by an intense odd-mass *m/z* 167. In an earlier study on open-chain unsaturated acids (1,6), DMOXs of monoenoic acids with a double bond at carbons 4 and 6 exhibit doublets at *m/z* 138, 139 and 166, 167 respectively in their spectra, with abundant allylic cleavage peaks at *m/z* 166 or 194. The genesis of these diagnostic ion peaks remains uncertain. Nevertheless, the positional isomer of gorlic acid—18:2(4,cy) is a new cyclic acid which was not known previously.

The reduced cyclic acid mixture obtained by diimide

TABLE 2

Physicochemical Data of the Seed Oils of *Flacourtiaceae* Species Distributed in Xishuangbanna and Their Constituent Fatty Acids

	H. anthelmintica	*H. alpina*	*H. hainanensis*	*T. merrilliana*
Oil content, %	52.67	51.19	26.88	45.53
Iodine value	79.74	87.57	92.83	97.37
Saponification value	200.58	203.67	208.13	205.95
Acid value	0.65	0.36	1.30	17.52
Refractive index	1.4772(n_D^{20})	1.4737(n_D^{40})	1.4820(n_D^{20})	1.4809(n_D^{20})
Sp.gravity (d_{40}^{40})		0.9472	0.9492	0.9493
12:1(cy)	0.10		0.10	0.11
14:0	0.20			
14:1(cy)	0.22		0.24	0.20
16:0	10.90	5.82	3.64	6.70
16:1(9)	1.21	1.01	4.11	2.83
16:1(cy)	52.93	55.94	57.34	42.84
18:0	1.44	0.56	0.54	0.48
18:1(9)	7.35	3.88	2.27	1.61
18:1(11)			1.97	0.72
18:2(9,12)	0.94	0.83	1.27	1.20
18:1(cy)	19.11	25.04	21.62	20.31
18:2(4,cy)	2.17	3.43		
18:2(6,cy)				19.74
18:2(9,cy)			4.39	

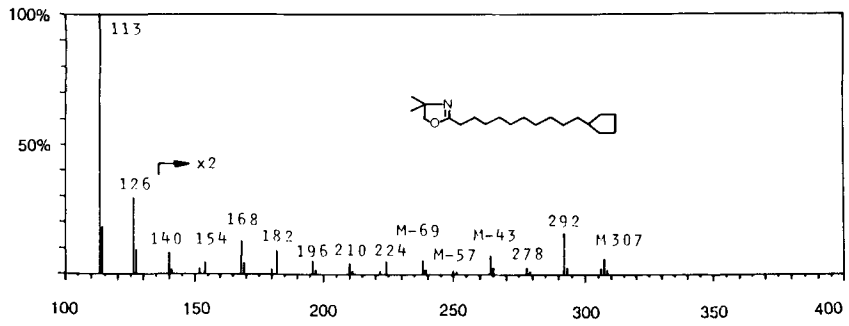


FIG. 4. Mass spectrum of DMOX derivative of the dihydrohydnocarpic acid obtained by diimide reduction.

reduction of the methyl esters with 2,4,6-trimethylbenzenesulfonyl hydrazide (16) and derivatized to DMOX was examined by GC-MS [Fig. 1(b)]. A close inspection of the chromatograms [Fig. 1(a) and (b)] reveals that the saturated fatty acids (16:0 and 18:0) were significantly enhanced with concurrent diminution of the unsaturated analogs [16:1(9), 18:1(9), 18:1(11), 18:2(9,12)]. All cyclopentanyl fatty acids were reduced to the corresponding cyclopentanyl acids as shown by characteristic ions at M-69, M-57 and M-43. The spectrum of the reduced 16:1(cy) derivative is given in Figure 4.

ACKNOWLEDGMENT

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