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# Cyclopropanoic Fatty Acids of Litchi (*Litchi chinensis*) Seed Oil. A Reinvestigation

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Semisynthetic cyclopropanoic fatty acids (CPFA), *cis*-9,10-methylenehexadecanoic acid, *cis*-7,8-methylenehexadecanoic acid, and dihydrosterculic acid, were obtained from the corresponding olefinic acids. Their chromatographic equivalent chain lengths on two liquid phases (Carbowax 20M and BDS), together with the mass spectral data of the methoxy derivatives, obtained using 50% boron trifluoride in dry methanol, were compared to the CPFA methoxy derivatives isolated from the lipid content of litchi (*Litchi chinensis* Sonn.) seeds. The fatty acid composition of the litchi seed lipids included palmitic acid (12%), oleic acid (27%), linoleic acid (11%), and CPFA (42%). The CPFA fraction contained dihydrosterculic acid (37%), *cis*-7,8-methylenehexadecanoic acid (4%), *cis*-5,6-methylene-tetradecanoic acid (0.4%), and *cis*-3,4-methylenedodecanoic acid (0.1%). These results show that CPFA from litchi seed lipids belong to the *n*-9 series as observed in *Euphoria longana*.

## INTRODUCTION

Cyclopropanoic fatty acids (CPFA) are widely distributed in bacteria (Asselineau, 1966; Christie, 1970). Lactobacillic acid (*cis*-11,12-methyleneoctadecanoic acid; 19:CA *n*-7, 1 Figure 1), first found in *Lactobacillus arabinosus* (Hofmann and Lucas, 1950), is associated with dihydrosterculic acid (*cis*-9,10-methyleneoctadecanoic; 19:CA *n*-9, 2, Figure 1) in *Streptococcus faecalis* (Teixeira et al., 1983). Dihydrosterculic acid is a major constituent of the phospholipid of trypanosomatid protozoa (Holz, 1985; Rahman et al., 1988). The C17 homologue of lactobacillic acid (*cis*-9,10-methylenehexadecanoic acid, 17:CA *n*-7, 5, Figure 1) has been found in the phospholipid fraction of *Escherichia coli* (Kaneshiro and Marr, 1961).

CPFA may occur in plants (Smith, 1970; Badami and Patil, 1981). Dihydrosterculic acid has been found in many plant families and orders, Malvales, Sapindales, Ebenales, and Rhamnales (Vickery, 1980, 1981), and cascarillic acid (*cis*-3,4-methylenedecanoic acid, 11:CA *n*-7, 11, Figure 1) has been characterized in the bark of *Croton eluteria*, Euphorbiaceae (Motl et al., 1972). Two species belonging to the Sapindaceae family are known to contain higher amounts of dihydrosterculic acid associated with lower homologues in their seed oils. *Litchi chinensis* Sonn. seed oil contains dihydrosterculic acid as major component (41–42%) and a minor component (4–8%) having the structure *cis*-9,10-methylenehexadecanoic acid (17:CA *n*-7, 5, Figure 1) according to Lie Ken Jie and Chan (1977) and Vickery (1980). These two fatty acids were separated as methyl esters using preparative GC and their structures determined by mass spectrometry (Lie Ken Jie and Chan, 1977). *Euphoria longana* was found to contain 17–19% dihydrosterculic acid (Kleiman et al., 1969; Ackman and Hooper, 1970) and three other lower homologues, which were tentatively identified as 17:CA *n*-9, 15:CA *n*-9, and 13:CA *n*-9 by Ackman and Hooper (1970) on the basis of retention data and degree of resolution of the pairs of monomethyl-branched fatty acids resulting from the hydrogenolysis of cyclopropane rings.

Continuing our research on cyclopropanoic and cyclopropanoic fatty acids (Bianchini et al., 1981; Ralaimanarivo

et al., 1982; Gaydou et al., 1983), we have reinvestigated the structure determination of 17:CA fatty acid of litchi seed oil. If the methylene group of the cyclopropane ring is across the 9 and 10 carbons numbered from the carboxylic function (17:CA *n*-7), as proposed by Lie Ken Jie and Chan (1977) and Vickery (1980), this fatty acid is not of considerable interest since its structure is the same as that of the 17:CA found in the microorganism, and its structure is in contradiction with that proposed by Ackman and Hooper (1970) in the case of *E. longana*. If we suppose that in plants, such *E. longana* and *L. chinensis*, the genesis of CPFA is similar, the structure of 17:CA in these two plants would be identical.

In this study, we have extracted CPFA from litchi seed oil and isolated the 19:CA and 17:CA fatty acids. The ring position has been determined using mass spectrometry of the derivatives obtained by boron trifluoride-catalyzed methoxylation. Furthermore, hemisynthesis of 17:CA *n*-7, 17:CA *n*-9, and dihydrosterculic acid (19:CA *n*-9), their gas chromatographic equivalent chain lengths (ECL), and their mass spectral data have been compared with the fatty acid extracted from litchi seed oil.

## MATERIALS AND METHODS

**Materials.** The shiny brown and large seeds were obtained from litchi fruits (*L. chinensis* Sonn.) collected on the east coast of Madagascar. Neutral lipids were obtained by Soxhlet extraction using light petroleum (40–60 °C). The lipid content was about 1.3–2.1%.

**Halphen Color Test on Oils.** The original method (Halphen, 1897) was used for characterization of cyclopropanoic fatty acid (CPEFA). Equal volumes (about 1–3 mL) of oil, 1-pentanol, and carbon disulfide containing 1% sulfur were placed in a test tube and warmed on a steam bath for 10–15 min. The samples did not give the characteristic red-pink color generally observed in the presence of CPEFA.

**Preparation of Methyl Esters.** Fatty acid methyl esters (FAME) were prepared from oils by base-catalyzed transesterification with sodium methoxide as previously indicated (Bianchini et al., 1981).

**Natural Cyclopropanoic Fatty Acid Extraction and Purification.** Urea (15 g) and litchi FAME (5.6 g) were dissolved in refluxed methanol. After cooling, the solid and liquid phases were separated by filtration. The non-urea inclusion complex fraction (2.5 g) contained 57.1% CPFA (by GC), while the urea inclusion complex fraction (2.9 g) contained 30.4% CPFA. The

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Compound	Name	Structure	Formula
1	lactobacillic	19:CA n-7	
2	dihydrosterculic	19:CA n-9	
3	dihydromalvalic	18:CA n-9	
4	cis-9,10-methyleneheptadecanoic	18:CA n-8	
5	cis-9,10-methylenehexadecanoic	17:CA n-7	
6	cis-7,8-methylenehexadecanoic	17:CA n-9	
7	cis-6,7-methylenepentadecanoic	16:CA n-9	
8	cis-5,6-methylenetetradecanoic	15:CA n-9	
9	cis-4,5-methylenetridecanoic	14:CA n-9	
10	cis-3,4-methylenedodecanoic	13:CA n-9	
11	cis-3,4-methylenedecanoic	11:CA n-7	

Figure 1. Structure of the principal cyclopropanoic fatty acids investigated.

Table I. Cyclopropanoic Fatty Acid Methyl Esters Obtained during the Oxidation Reaction of Dihydromalvalic Methyl Ester 3

compd <sup>a</sup>	ring location	ECL <sup>b</sup>	yield, %
9	14:CA n-9	14.32	0.2
8	15:CA n-9	15.24	1.7
7	16:CA n-9	16.25	14.9
6	17:CA n-9	17.24	83.2

<sup>a</sup> See Figure 1 for compound identification. <sup>b</sup> Equivalent chain length values on the Carbowax 20M column. <sup>c</sup> Relative area percentage.

CPFA-rich fraction (600 mg) was submitted to column chromatography (CC) (100 cm long  $\times$  0.7 cm i.d.) over Amberlite 15 (E. Merck) impregnated with silver nitrate (10%). Elution of pure CPFA (98.2% by GC) was carried out with methanol. This fraction (163 mg) was submitted to RP8 preparative HPLC chromatography (E. Merck): eluting solvent, acetonitrile; flow rate, 5 mL min<sup>-1</sup>. A first fraction was composed of 17:CA n-9 (82% purity by GC). The impurities were linear saturated fatty acids. The second fraction (124 mg) was composed of pure dihydrosterculic acid, 19:CA n-9 (98% by GC).

**Cyclopropanoic Fatty Acid Semisyntheses.** Dihydrosterculic acid (19:CA n-9) and a mixture of *cis*-methylenehexadecanoic acids (17:CA n-7 and 17:CA n-9) were obtained using oleic acid (E. Merck) and palmitoleic acid (E. Merck), containing 73.4% 16:1 n-7 and 26.6% 16:1 n-9 according to the stereospecific Simmons and Smith reaction (1958) as modified by Le Goff (1964). The better yields were obtained using an excess (half the amount) of zinc-copper catalyst and methylene iodide during 36 h. CPFA purification was achieved by CC as described above. The 17:CA n-7/17:CA n-9 ratio (3.05) was in the same order as that obtained from the starting 16:1 mixture (2.76).

Dihydromalvalic acid (3) was obtained (97% purity by GC) by Barbier Wieland oxidation using Grignard reagents (Bailey et al., 1965). A mixture containing CPFA 6-9 was obtained by using the same oxidation method, starting from dihydromalvalic acid.

**Methoxylation of Cyclopropanoic Fatty Acid.** Boron trifluoride-methanol (50% w/v) was prepared from BF<sub>3</sub> gas (E. Merck) and dry methanol. CPFA (10 mg) was dissolved in dichloromethane (1 mL) and treated in a Schlenk tube under nitrogen with 50% BF<sub>3</sub>-methanol reagent (1 mL) at 65 °C during 12 h. Fractionation of washed and dried products was achieved

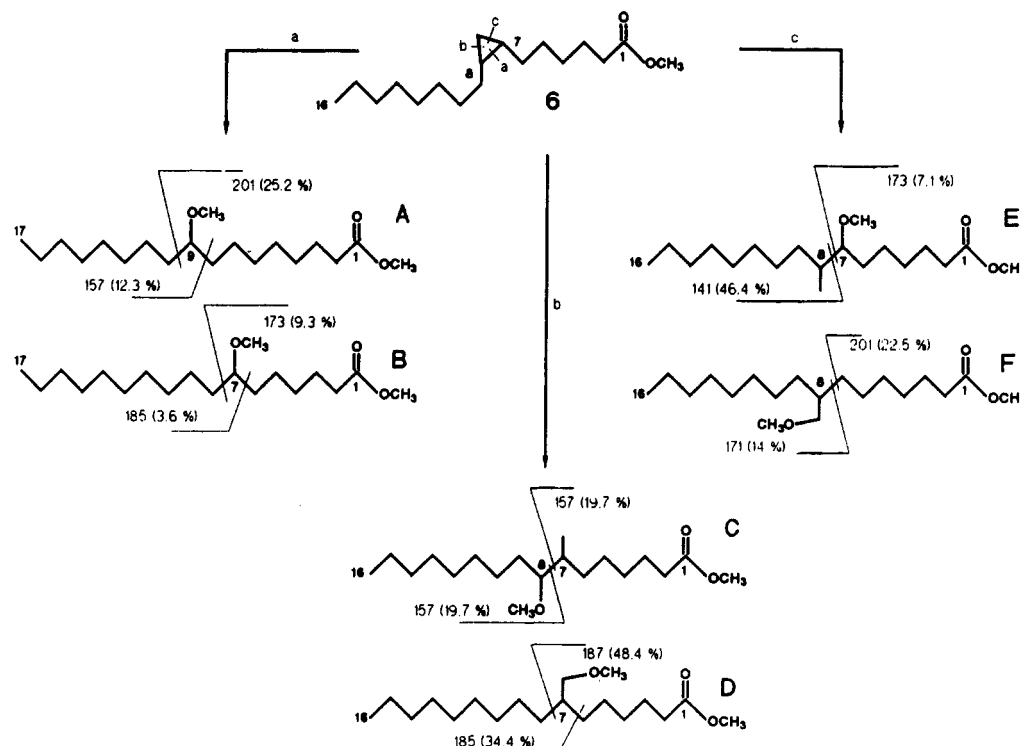
Table II. Fatty Acid Composition of Litchi Neutral Lipids

fatty acid	ECL <sup>a</sup>		fraction <sup>b</sup>			
	CW20M	BDS	I	II	III	IV
13:0			tr <sup>c</sup>		tr	
13:CA	13.40	13.36	0.1	0.4	tr	0.7
14:0			0.2		0.4	
15:0			0.1		0.1	
15:CA	15.24	15.29	0.4	0.7	0.1	1.3
16:0			11.9	0.1	21.9	0.2
17:0			0.2		0.3	
17:CA	17.25	17.28	4.0	7.7	1.4	12.9
18:0			3.1	0.9	4.6	1.4
18:1 n-9	18.21	18.27	26.7	15.1	38.0	
18:2 n-6	18.66	18.82	11.4	18.2	3.3	
19:CA	19.24	19.28	37.2	48.3	28.5	83.5
18:3 n-3	19.50	19.29	4.0	7.8	0.4	
20:0			0.4		1.0	
20:1			0.2	0.4	tr	
22:0			0.1	0.4	tr	
total CPFA			41.7	57.1	30.0	98.4

<sup>a</sup> Fused silica capillary column coated with either Carbowax 20 M or BDS. <sup>b</sup> I, starting neutral lipids; II, non-urea inclusion complex fraction; III, urea inclusion complex fraction; IV, saturated fraction of non-urea inclusion complex fraction obtained using column chromatography impregnated with silver nitrate. <sup>c</sup> tr, trace.

using preparative silica gel 60 TLC. Elution with *n*-hexane-ethyl ether (80:20 v/v) gave methoxylated esters (*R*<sub>f</sub> 0.41) and olefinic esters (*R*<sub>f</sub> 0.65), which were analyzed by GC and GC-MS.

**Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS).** A Delsi 300 gas chromatograph equipped with a flame ionization detector (FID) was used for FAME separation with a fused silica capillary column (0.32 mm i.d.) coated with either Carbowax 20 M (50 m, phase thickness 0.15  $\mu$ m, column temperature 170 °C) or BDS (25 m, phase thickness 0.25  $\mu$ m, column temperature 150 °C). Detector and inlet temperatures were 200 °C. Helium was used as carrier gas at an inner pressure of 0.7 bar. The injections averaged 1  $\mu$ L of a 2% solution of FAME in hexane. Combined GC-MS was recorded on a Delsi gas chromatograph linked to a Ribermag R-10-10C mass spectrometer and coupled with a Sidar data computer. The GC column was a 0.32 mm (i.d.)  $\times$  25 m fused capillary column coated with Wax 51 (0.20- $\mu$ m phase thickness).



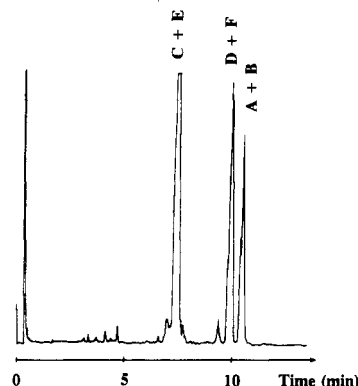
**Figure 2.** Methoxy derivative formation from 17:CA *n*-9 methyl ester isolated from litchi seed oil using 50% boron trifluoride reaction in dry methanol. The *m/z* values of the characteristic peaks are given (relative intensities).

The programmed column temperature was 150–220 °C at 3 °C min<sup>-1</sup>; carrier gas, helium; ion source, 220 °C; ionizing voltage, 70 eV.

## RESULTS AND DISCUSSION

**Semisynthesis of CPFA.** Dihydrosterculic acid (2) and methylenehexadecanoic acids 5 and 6 were obtained from oleic acid and a mixture of hexadecenoic acids using methylene iodide and zinc-copper catalyst according to the Simmon and Smith (1958) procedure. Compounds 2, 5, and 6 have equivalent chain lengths (ECL) of 19.24, 17.29, and 17.24 on the Carbowax 20M, respectively. Pure dihydromalvalic acid (3) was prepared by oxidation of 2 using the Barbier Wieland oxidation method (Bailey et al., 1965). Using CPFA 3 as starting material, compounds 6–9 were obtained in the same manner. The yields and ECL are given in Table I. During this experiment, CPFA 6, as expected, was the major component, showing the same ECL as that obtained from hexadecenoic reaction with methyl iodide.

**Extraction of CPFA from Litchi Neutral Lipids.** Seeds of litchi contained 1–2% neutral lipids that could be extracted with petroleum ether. The fatty acid composition of their methyl esters was prepared by transmethylation of the neutral lipids. The Halphen color test gave a negative red-pink color indicated on the absence of a cyclopropanoic group. This test was used since CPFA are frequently associated with sterculic and malvalic acids (Bohannon and Kleiman, 1978; Bianchini et al., 1981; Gaydou et al., 1983; Ralaimanarivo et al., 1982). As shown in Table II, four peaks representing 41.7% of the total fatty acids have ECL values on BDS (13.36, 15.29, 17.28, and 19.27, respectively) in the same range order as those found in the case of *E. longana* by Ackman and Hooper (1970). These ECL values on the Carbowax 20M column are also in agreement with 15:CA *n*-9, 17:CA *n*-9, and 19:CA *n*-9 obtained from hemisyntheses (Table I). These chromatographic data are evidence that the methylenehexadecanoic fatty acid found in litchi neutral lipids is



**Figure 3.** Gas chromatogram of methoxy derivatives of 17:CA *n*-9 methyl ester isolated from litchi seed oil obtained during 50% boron trifluoride reaction in dry methanol. Column, BDS at 170 °C; pressure, 0.4 bar; carrier gas, H<sub>2</sub>. Equivalent chain length (relative area): C + E, 18.36 (45%); D + F, 19.06 (40%); A + B, 19.20 (15%). Peak identification is as in Figure 2.

probably the 17:CA *n*-9 6. To confirm this structure, this compound was isolated in pure form in three steps from the litchi lipid mixture. An enrichment in CPFA was first achieved using urea clathration as shown in Table II (fraction II). A pure CPFA fraction (98.3%) was obtained using column chromatography with Amberlite resin impregnated with silver nitrate (fraction IV, Table II). Pure dihydrosterculate 2 (98% by GC) and 17:CA (82%) were isolated using RP8 liquid chromatography and analyzed by mass spectrometry.

**Mass Spectrometry of Methoxy Derivatives Obtained with 17:CA Extracted from Litchi Neutral Lipids.** Hydrogenation of cyclopropane esters yielding a mixture of isomeric methyl-branched and straight-chain esters is well established and can be used for ring location by mass spectrometry. In some cases, since both geometrical and positional isomers of cyclopropane fatty acid esters gave practically identical mass spectra, Minnikin (1972) has investigated another derivative method. This

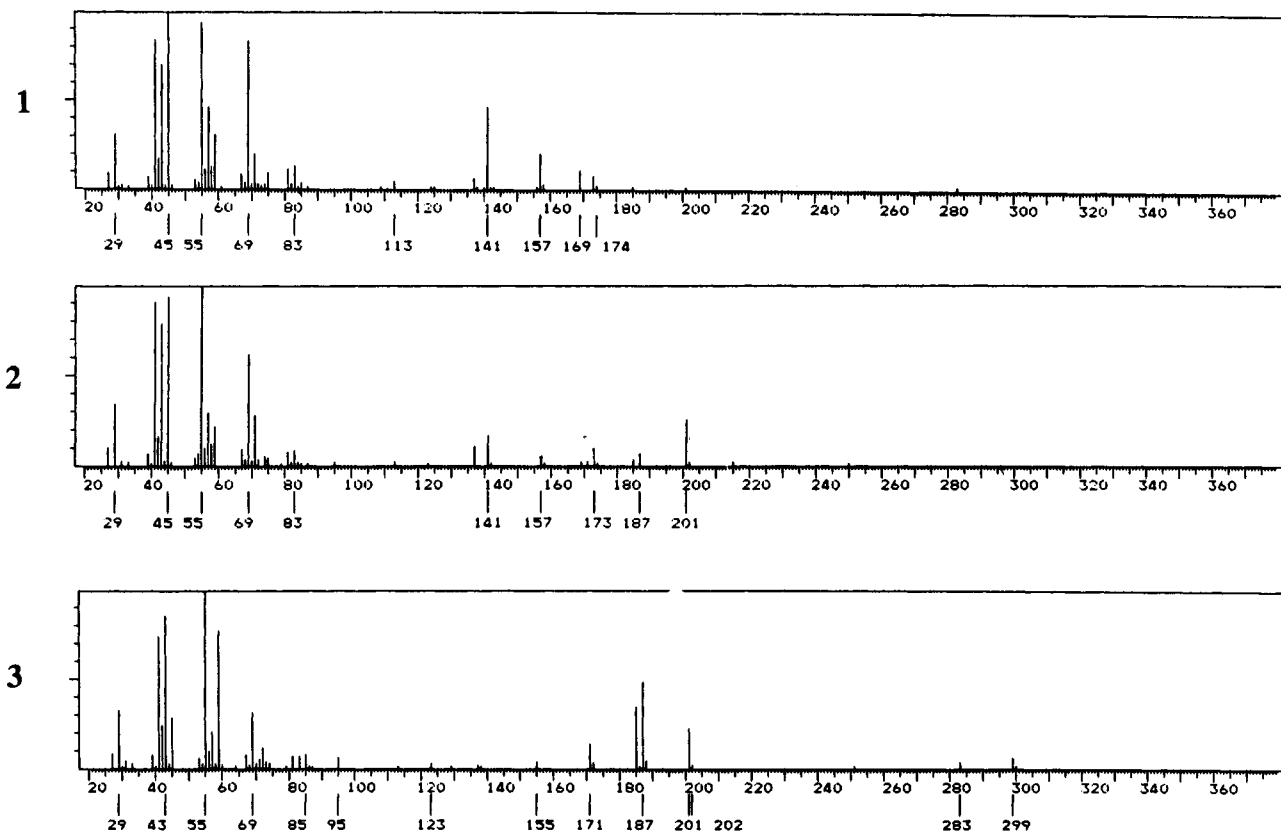


Figure 4. Mass spectra of the three main peaks (1: C + E; 2: A + B; 3: D + F) obtained by GC-MS of the methoxy derivatives of 17:CA *n*-9 methyl ester isolated from litchi seed oil treated by 50% boron trifluoride in dry methanol.

Table III. Equivalent Chain Length of Cyclopropanoic Fatty Acids Investigated

compd	structure	ECL			
		<i>E. longana</i> <sup>a</sup> BDS	<i>L. chinensis</i> CW20M	BDS	semisyntheses CW20M BDS
10	13:CA <i>n</i> -9	13.35	13.40	13.37	
9	14:CA <i>n</i> -9				14.24 <sup>b</sup>
8	15:CA <i>n</i> -9	15.24	15.24	15.30	15.23 <sup>b</sup> 15.30 <sup>b</sup>
7	16:CA <i>n</i> -9				16.25 <sup>b</sup> 16.29 <sup>b</sup>
6	17:CA <i>n</i> -9	17.24	17.25	17.28	17.24 <sup>b,c</sup> 17.28 <sup>b,c</sup>
5	17:CA <i>n</i> -7				17.29 <sup>c</sup> 17.32 <sup>c</sup>
2	19:CA <i>n</i> -9	19.18	19.23	19.27	19.24 <sup>d</sup> 19.27 <sup>d</sup>

<sup>a</sup> Ackman and Hooper (1970). <sup>b</sup> Obtained by oxidation of semi-synthetic dihydrostercularic acid, 19:CA *n*-9. <sup>c</sup> Obtained from a hexadecenoic fatty acid mixture (18:1 *n*-7 and 18:1 *n*-9) using the Simmons and Smith (1958) method. <sup>d</sup> Obtained from oleic fatty acid using the Simmons and Smith (1958) method.

author has observed in the case of *cis*- and *trans*-9,10-methyleneoctadecanoate methyl esters that reaction products with 50% boron trifluoride in dry methanol produce unsaturated and methoxy derivatives. Since the mass spectra of the methoxylated esters are characterized by intense peaks due to cleavage adjacent to the methoxy functions, which allows the position of the ring in the original cyclopropane ester, we have applied this method for the ring location in the case of the 17:CA methyl esters isolated from litchi seed lipids. As shown in Figure 2, six compounds (A-E) are expected, using 50% boron trifluoride in dry methanol. The GC reaction mixture of the methoxy derivatives (Figure 3) shows the presence of three main peaks on the BDS column. Using GC-MS, the mass spectra of these three main peaks (Figure 4) allowed the identification of the various isomers as shown in Figure 2. Pairs of CPFA unresolved by GC consisted of methyl 7- and 9-methoxyheptadecanoate A and B, methyl 7-methyl-8-methoxyhexadecanoate C and methyl 8-methyl-7-

methoxyhexadecanoate E, and methyl 7- and 8-methoxymethylhexadecanoate D and F. Major peaks due to fragmentations promoted by the methoxy group are in agreement with the 17:CA *n*-9 structure the fatty acid contained in litchi seed lipids. These results are in agreement with those proposed by Ackman and Hooper (1970) in the case of *E. longana*. Since litchi fruits are commonly found on world markets, the seeds are a convenient source of CPFA belonging to the series *n*-9. Table III summarizes the chromatographic behaviors of the various CPFA investigated.

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