Occurrence of Conjugated Fatty Acids in the Seed Oil of *Couepia longipendula* (Chrysobalanaceae)

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The seeds of *Couepia longipendula* contain 74.2% oil. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) of the methyl esters and oxazoline derivatives of the fatty acids and ultraviolet (UV), infrared (IR), ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra of the oil showed the presence of palmitic (25.2%), palmitoleic (0.9%), stearic (6.2%), oleic (26.5%), vaccenic (0.4%), linoleic (7.4%), arachidic (0.3%), α-eleostearic (11.3%) and α-licanic (21.8%) acids. Licanic acid methyl ester was isolated by thin-layer chromatography (TLC) and the ^{13}C-NMR and ^{1}H-NMR data are presented.

KEY WORDS: ^{13}C-NMR, *Couepia longipendula*, α-eleostearic acid, GC/MS, ^{1}H-NMR, α-licanic acid, oxazoline derivatives.

Couepia longipendula Pilg. is a 5-30 m tree, which occurs in non-flooded forests in the lower Rio Negro region (Amazonia) in Brazil. The stone fruits are round to ellipsoid, 4-6 cm long, 4 cm broad, and contain a 2 × 3 cm seed. It is called *Castanha de Galinha.*

The species is cultivated around Manaus, Brazil, for consumption of raw, fried or roasted kernels. Also, the seeds are used to make a special meal with sun-dried seeds, manioc flour and sugar (1). Though the fruits are of local economic importance, there are no detailed studies about the fatty acid composition of the seed oil. Only a short report from Maravalhas *et al.* (2) with some physical and chemical data of the seeds and their oil has been published.

In a current research project about fruits and edible nuts from the Amazon, another species of the Chrysobalanaceae family, *Acioa edulis*, was examined. Its seed oil contains about 27% of conjugated fatty acids (3). So, the fruits of *C. longipendula* were examined for their suitability for human nutrition or for industrial purposes.

For this paper, the fatty acid composition was determined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), and the ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) spectroscopic data of the oil of *C. longipendula* are described for the first time.

EXPERIMENTAL

The fruits were collected in the Adolphe Ducke Reserve of the National Institute for Amazon Research (INPA), 27 km from Manaus, and the botanical identification was made in the herbarium of the Museu Goeldi, Belém (Brazil). Shell separation, quantitation of the oil content, preparation of the fatty acid methyl esters (FAME), determination of equivalent chain length (ECL) values and spectroscopy procedures were the same as described previously (3). The total lipids were extracted according to the method of Hara and Radin (4) with hexane/isopropanol (2:1) as the solvent system. Isolation of licanic acid methyl esters (ME) was carried out on 0.6 mm silica layers with ether/petroleum ether (40-60°C)(50:50 v/v) as solvent system. α-Eleostearic acid ME was isolated from the non-oxo-acids ME fraction obtained by the latter isolation procedure on 0.6 mm silica layers with 20% $AgNO_3$ and ether/petroleum ether (40-60°C)(25:75 v/v) as solvent system (5). For preparation of the maleic anhydride adducts, the conjugated FAMES were refluxed for 2 hr under nitrogen in a maleic anhydride/toluene solution (6). Oxidative splitting of the maleic adducts of the conjugated fatty acid ME was carried out by permanganate-periodate (7), and the methylated oxidation products were identified by GC/MS. Preparation of the oxazoline derivatives of the fatty acids was done as described by Zhang *et al.* (8). GC separation of the methyl esters and oxazoline derivatives of the fatty acids was carried out with a DB 23 (J&W Scientific, Folsom, CA) capillary column (25 m × 0.25 mm, i.d. 0.25 μm) under the same conditions as described previously (3). Gas chromatography-mass spectrometry (GC/MS) analysis was done with the AUTOMASS 120 (Delsi Nermag Instruments, Argenteuil, France) at 70eV.

RESULTS AND DISCUSSION

Oil and the methyl esters data. The seeds contained 74.2% of a yellow oil with a refractive index of n_D=1.4859 at 25°C. GC analysis of the fatty acid methyl esters (Table 1) showed a pattern similar to that observed in the oil of *Acioa edulis* (3). In addition to the usual fatty acids, two compounds, **A** and **B**, with the same mass spectra and ECL values on the DB 23 column as published for α-eleostearic ME and α-licanic acid ME (3) (OV1/DB23:**A** 18.99/22.07; **B** 20.37/28.30), respectively, were found. The UV spectrum of the oil (maxima at 261, 270 and 281

TABLE 1

Fatty Acid Composition of *Couepia longipendula* Seed Oil

Fatty acid[a]	Wt %[b]
C16:0	25.2
C16:1(n-7)	0.9
C18:0	6.2
C18:1(n-9)	26.5
C18:1(n-7)	0.4
C18:2(n-6)	7.4
C20:0	0.3
C18:3(c9 t11 t13) (**A**)	11.3
C18:3-4-oxo(c9 t11 t13) (**B**)	21.8

[a]Positions of the double bonds were determined by GC/MC analysis of the oxazoline derivatives (except for α-licanic acid).

[b]< 0.1% omitted.

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TABLE 2

^{13}C-NMR Chemical Shifts (ppm) of Conjugated Double Bond Carbons of Seed Oil of *Couepia longipendula* in Comparison with Those of α-Eleostearic Acid from Tung Oil (ref. 11)

Carbon	Compound **A** (α-eleostearic acid)	α-Eleostearic acid (ref. 9)	Compound **B** (α-licanic acid)
9	128.7	128.8	129.1
10	132.8	132.9	133.1
11	125.9	126.0	125.7
12	135.0	135.2	135.2
13	131.6	131.7	130.9
14	130.6	130.7	130.5

nm) indicated a conjugated trienoic acid (34.1% conjugated fatty acids calculated with $E^{1\%}/_{1cm}$ = 1710 as α-eleostearic acid) (5). The IR spectrum of the oil was identical with that of the oil of *Acioa edulis*, known to contain α-eleostearic and α-licanic acid (3). Particularly significant were the absorption values at 1720 cm^{-1} (keto) and at 962 cm^{-1} (w) and 990 cm^{-1} (s), the latter two bands indicative of conjugated *cis*, *trans*, *trans* systems (9). The isolated compounds **A** and **B** showed the same UV maxima and IR spectrum in the range from 900 to 1000 cm^{-1} as the oil, but only **B** contained the keto absorption.

The ^{1}H-NMR spectrum of the oil showed a complex multiplet at 6.1 ppm produced by conjugated double bonds (10). Furthermore, the presence of a keto acid was supported by signals due to methylenic groups in α position [2.7 ppm (*t*, J=6.5 Hz) and at 2.43 ppm (*t*, J=7.25 Hz)] and β position [2.53 ppm (*t*, J=6.5 Hz) and 1.6 ppm (m)] to a keto group. Beside the keto signal (208.2 ppm), the ^{13}C-NMR spectrum of the oil showed twelve characteristic signals for conjugated double bonds in the range of 125-136 ppm (Table 2).

The assignment of the signals in the double bond ranges from compound **A** in the oil was done by comparison with the data published by Tulloch and Bergter (11) for tung oil, which contains α-eleostearic acid, and by comparison with the shifts from the isolated compound **B**, subsequently identified as α-licanic acid ME. The ^{13}C-NMR and ^{1}H-NMR data obtained from pure compound **B** (α-licanic acid ME) are presented in Table 3. In addition, its ^{13}C-NMR spectrum was calculated by using the published data from α-eleostearic acid (11) and the increment values for oxo acids (12). The calculated shifts enabled a tentative assignment of the carbons 2-10. Assignments of the carbons 11-18 were done by comparison with the shifts of α-eleostearic acid.

After formation of the maleic adducts, both conjugated fatty acid ME **A** and **B** disappeared in the GC. Oxidative splitting of the adduct products yielded azelaic acid ME for compound **A** and 4-keto-azelaic acid ME for compound **B**, respectively, identified by GC/MS (3). Thus, the stereochemical structure (*cis*-9, *trans*-11, *trans*-13) for both compounds **A** and **B** (α-eleostearic ME and α-licanic ME) proposed by the GC data above was confirmed.

Mass spectra of the oxazoline derivatives. As published by Zhang *et al.* (8), the fragmentation pattern of 2-alkenyl-4,4-dimethyloxazoline derivatives facilitates location of double bond positions in long-chain acids with isolated double bonds (8) and up to two unsaturated

TABLE 3

NMR Spectral Data of Compound B (α-Licanic Acid ME)

Carbon	^{13}C-NMR	^{13}C-NMR (calculated)[a]	^{1}H-NMR
OCH_3	51.78	—	3.63 *s*
1	173.3	—	—
2	27.27	28.3	2.53 *t* (6.5)
3	37.03	37.8	2.7 *t* (6.5)
4	208.91	210.3	—
5	42.59	42.2	2.43 *t* (7.25)
6	23.4	23.4	1.6 (*m*)
7	29.21	29.3	1.3 (*m*)
8	27.59	27.6	2.15 (*q*)
9	129.11	128.6	5.3-6.3 (*m*)
10	133.12	132.8	5.3-6.3 (*m*)
11	125.78	126.0	5.3-6.3 (*m*)
12	135.41	135.2	5.3-6.3 (*m*)
13	131.05	131.7	5.3-6.3 (*m*)
14	130.54	130.7	5.3-6.3 (*m*)
15	32.52	32.6	2.05 (*q*)
16	31.43	31.6	1.3 (*m*)
17	22.25	22.3	1.3 (*m*)
18	13.96	14	0.89 (*t*)

[a]Calculation was done by using the chemical shifts of α-eleostearic acid (ref. 11) in combination with the increment values for oxo-acids (ref. 12).

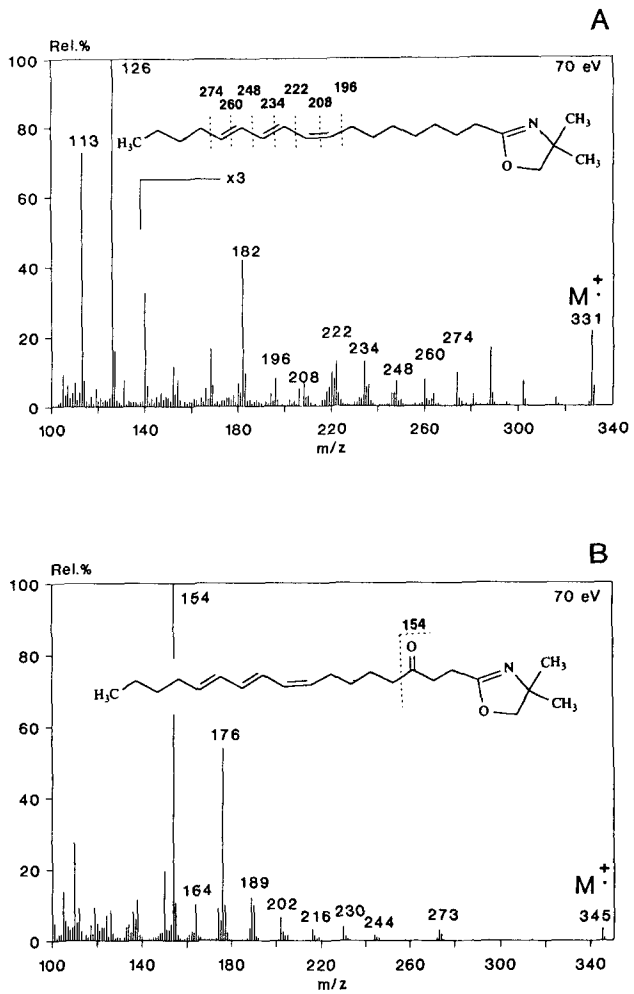

FIG. 1. Mass spectra of the oxazoline derivatives of α-eleostearic acid (A) and α-licanic acid (B).

bonds in conjugation (enyne-systems) (13). Migration of such double bonds in these derivatives during mass fragmentation is suppressed by preferential charge stabilization at the heterocyclic part (8). As expected, the mass spectra of the oxazoline derivatives of the usual fatty acids from *Couepia longipendula* were identical with the literature spectra (8) and clearly showed the double bond positions. The mass spectra of the oxazoline derivatives of compound **A** (α-eleostearic acid) and **B** (α-licanic acid) are presented in Figure 1.

The α-eleostearic acid derivative showed two intensive peaks at *m/z* 113, produced by McLafferty rearrangement and at *m/z* 126 (base peak) formed via a cyclization-displacement reaction (8). Furthermore, the even-mass homologous series *m/z* 126 + 14 mu, derived from cleavage at each bond, was observed. In the range of the olefinic bonds, the empirical rules at Zhang *et al.* (8) could be utilized successfully. A mass separation of 12 mu instead of the regular 14 mu (*m/z* 196 and 208) locates the first double bond between carbons 9 and 10. A regular 14 mu separation (*m/z* 208 and 222), followed by a 12 mu cleavage (*m/z* 222 and 234) locates the second double bond between carbons 11 and 12. This series n + (12 + 14)$_3$, (n=*m/z* 196), continues, thus locating the third double bond between carbon 13 and 14 (see Fig. 1A). The peaks above *m/z* 274 again showed the expected regular mass differences. The molecular ion (*m/z* 331) was also present.

The α-licanic acid derivative showed *m/z* 154 (base peak), produced by α-cleavage (see Fig. 1B), and *m/z* 176, probably formed by McLafferty rearrangement to the keto group, at which the alkyl chain is charged ($[CH_3(CH_2)_3(CH{=}CH)_3CH_2CH{=}CH_2]^{+\bullet}$). These ions enabled the determination of the keto group position. The molecular ion *m/z* 345 was also observed. The expected signals for the double bond range (analogous to α-eleostearic acid) do not appear in the spectrum. Obviously, the ionization of the keto group is strongly preferred.

For α-eleostearic acid, the oxazoline derivative is useful for locating the conjugated double bond system by mass spectroscopy. The oxo-group in the α-licanic acid derivative controls mass fragmentation and therefore information about double bond location can not be obtained by MS.

The composition of the oil of *Couepia longipendula* is very similar to that of the oil of *Acioa edulis*, also a member of the family Chrysobalanaceae. Due to the previously discussed (3) biological effects of fatty acids with conjugated double bonds, the seed oil can only be recommended for industrial purposes.

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