Identification of Conjugated Fatty Acids in the Seed Oil of *Acioa edulis* (Prance) syn. *Couepia edulis* (Chrysobalanaceae)

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The oil of *Acioa edulis* has the fatty acid composition: palmitic acid 28.3%, palmitoleic acid 1.3%, stearic acid 6.8%, oleic acid 26.5%, vaccenic acid 1.3%, linoleic acid 8.8%, arachidic acid 0.7%, α-eleostearic acid 7.3% and α-licanic acid 19%. The oil was analyzed and components were identified by ultraviolet (UV), infrared (IR), nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR), gas chromatography/mass spectrometry (GC/MS) and chemical methods. For transesterification the tetramethylguanidine reagent was used and it was shown that the method is very suitable for conjugated fatty acids. The equivalent chain lengths of α-eleostearic acid and α-licanic acid and their all-*trans* isomers were determined on DB 23 and OV-1. The electron impact (EI) and chemical ionization (CI) (with isobutane) mass spectra of licanic acid, eleostearic acid and the EI mass spectrum of 4-oxo-azelaic acid are presented and discussed. Furthermore, a comparison of literature data of seeds with the same common name is presented.

KEY WORDS: *Acioa edulis*, Chrysobalanaceae, conjugated fatty acids, eleostearic acid, gas chromatography/mass spectrometry (GC/MS), licanic acid, 4-oxo-azelaic acid.

Acioa edulis syn. *Couepia edulis* is a member of the family Chrysobalanaceae found in the basin of the Rio Purus and the central Solimoes of Amazonia, Brazil. The tree is 25 m tall and its stone-fruits are 8–9 cm long and 4.5-cm broad. The kernel is eaten raw by the habitants of the described region or is crushed and added to tapioca cakes. The oil of the seeds is used for cooking and soapmaking (1).

The common name *"Cotia Chestnut (Castanha de Cotia)"* obviously is not unique for this fruit. This curious designation is derived from the name of a tailless animal called *"Cotia,"* which feeds on these kernels (2). There are some early works about the botany and the oil of a fruit with the same name but they described different plants from different genera and families. We want to compare our data with some of the more important literature data to find out if one of these was identical with *Acioa edulis*.

In 1972 the botanist Prance (3) described *Acioa edulis* as a new species to name the *"Cotia Chestnut."* Three years later he classified the plant in the genus *Couepia* and called it *Couepia edulis* (4). Finally in 1989, the same author mentioned that it is best placed in the genus *Acioa* rather than *Couepia* (1).

Though the plant is of local economic importance, to our knowledge, no exact chemical examinations of the oil of these fruits have been reported until now. Thus we included *Acioa edulis* in our research project to find new fruits and seed oils of commercial value.

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The analyses of the conjugated fatty acids were also of chemotaxonomical interest, because Chrysobalanaceae species are known to contain substantial amounts of this group of unusual fatty acids (5). Especially licanic acid seems to be of taxonomical significance because it has been found only in members of this plant family.

In this paper the fatty acid composition and the spectroscopic data of the oil of *Acioa edulis* will be described for the first time.

MATERIALS AND METHODS

Material and oil extractions. The fruits were collected in the Amazonian rain forest in the region of Tefé (Brazil) in 1989, and the botanical identification was carried out in the Institute of Botany, Museu Goeldi, Belém (Brazil). The shells were separated from the kernels manually by sawing. Before oil extraction the thin brown perisperm was removed. To minimize autoxidation, butylated hydroxytoluene (BHT) (50 mg/L) was added to all solvents used in the following methods, and all experiments were carried out in the dark under nitrogen whenever possible. The white seeds were homogenized with a pestle under hexane. For quantitative determination of the oil content, the seed slurry was extracted with hexane in a Soxhlet apparatus for 8 hr. After extraction the hexane was removed by vacuum distillation at 30°C. For gas chromatographic and spectroscopic analyses the oil was extracted three times by shaking the slurry with hexane at room temperature (10 g homogenized seed/100 mL hexane). After drying the solution with anhydrous sodium sulfate, hexane was removed under the same conditions as above (6).

Basic analyses. Basic analyses were carried out following the established methods of the DGF (7). Density was measured with a CHEMPRO/PAAR DMA 46 at 18°C. The iodine value was calculated from the fatty acid composition obtained by gas chromatography (GC).

Preparation of the methyl esters. Transesterification of the oil was carried out according to a new method (8) with tetramethylguanidine as an alkaline catalyst. The advantages of the method are that it is mild and that free fatty acids are also converted to their methyl esters in contrast to other alkali-catalyzed reactions (8). In modification of this method the reaction was carried out in the dark, and the reaction time in the boiling water bath was reduced to one minute. Thus, isomerization and loss of conjugated fatty acids could be avoided, as shown by ultraviolet (UV) spectroscopy and GC. The boron trifluoride-methanol method was used for transesterification of the fatty acids obtained by oxidative splitting (9).

Preparation of the dimethyl disulfide (DMDS) adducts of the monoene methyl esters. The monoene fraction obtained by argentation thin-layer chromatography was converted to the *bis*-(methylthio) derivates as described previously (10).

Preparation of the picolinyl esters. Ten milligrams of the oil was hydrolyzed with 1 M solution of potassium hydroxide in 95% ethanol at room temperature for 12 hr. After recovery of the free fatty acids, the picolinyl derivates were prepared by the method described by Christie (11).

Hydrogenation, reduction with sodium borohydride, oxidative splitting, stereomutation, maleic adducts. The following reactions were carried out with the complete fatty acid mixture and with two conjugated fatty acids that were isolated from the seed oil (isolation procedure given below). Hydrogenation was done in methanol with H_2/PtO_2 (12). The products obtained were reduced with sodium borohydride in methanol solution (13). Both reaction products were examined by gas chromatography/mass spectrometry (GC/MS). The oxidative splitting was carried out by permanganate-periodate (14). The oxidation products were identified by GC/MS. Conversion to the all-*trans* isomers of the conjugated fatty acids was accomplished by treatment of the methyl esters with a trace of iodine in hexane solution (15). The products obtained were checked by infrared (IR) and ultraviolet (UV) spectroscopy and by GC. For preparation of the maleic anhydride adducts the methyl esters were refluxed for 5 hr under nitrogen in a maleic anhydride/toluene solution (16). The configuration of the double bonds of these derivates was examined by IR.

Argentation thin-layer chromatography (TLC). The separation of the methyl esters according to their degree of unsaturation was done in a preparative scale by using 0.5 mm thick, silver nitrate-impregnated silica gel plates and petroleum ether-diethyl ether (95:5) as developing solvent. The bands were detected with 2',7'-dichlorofluoresceine and eluted with diethyl ether (17). Monitoring of the fractions was carried out by GC.

Isolation of the conjugated fatty acids by high-performance liquid chromatography (HPLC). For structural analysis the conjugated fatty acids were isolated on a semimicro-preparative scale by HPLC (Varian 5000) with a spectrophotometric detector (Perkin-Elmer LC 55B) at 270 nm and a 25 m × 0.4 cm i.d. LiChrospher RP18 column, particle size 5 μm. The isocratic mobile phase was acetonitrile/water (80:20,v/v) with a flow rate of 1.5 mL/min. A solution (250 μL) of the methyl esters in hexane (3%,w/v) was injected repeatedly and the peaks were collected. To isolate the conjugated fatty acids from the acetonitrile/water mixture the acetonitrile portion was at first diminished at 30°C by vacuum distillation. Then the remaining liquid was extracted three times with the same volume of hexane, and the organic phase was dried over anhydrous sodium sulfate and concentrated with a stream of nitrogen. The purity of the isolated conjugated fatty acids was more than 95% as checked by GC.

Capillary GC. A Hewlett-Packard 5890 gas chromatograph with a 7673 A autosampler, flame ionization detector and a split/splitless injector with glass insert was used. For quantitative determination the fatty acid methyl esters (FAME) were separated on a DB 23 column from J&W Scientific Inc. (Folsom, CA) (30 m × 0.25 mm i.d., d_f 0.25 μm). The temperature program was 150-175°C, 1.4°C/min then 175-245°C, 40°C/min. For determination of the equivalent chain length (ECL) of the unusual fatty acids, the DB 23 and an OV-1 column (25 m × 0.25 mm i.d, d_f 0.25 μm) were used at 200°C and 210°C. Even-numbered fatty acid methyl esters from 14:0 to 22:0 were used as standards. Injector and detector temperatures were 250°C. The flow rate of nitrogen was 1 mL/min and the split ratio was 1:100. A 1-10% hexane solution (1 μL) of the FAME was injected. Calculation was done with a Shimadzu integrator model C-R4A.

Quantitation of the fatty acid methyl esters mixture. Quantitation was done with a nonadecanoic acid methyl ester as internal standard with an experimentally determined response factor for each component, except licanic acid. The response factor for licanic acid methyl ester was set to 1.00, because no pure standard was available.

Capillary GC/MS. A DANI 3800 gas chromatograph with PTV injector coupled directly with an AEI MS 30 mass spectrometer was used. The ionization energy was 25 eV. The source temperature was 225°C and the interface temperature was 220°C. Helium was the carrier gas (1,6 bar). Usually, 1 μL of the sample solution was injected in a cold splitless mode. The CI spectra of eleostearic and licanic acid methyl esters were obtained on a Delsi-Nermag GC-MS system Automass 120 with isobutane as reactant gas. For examination of the total fatty acid methyl esters and their reduction products, the DB 23 column was used. The DMDS derivates were analyzed on an OV-17 (36 m × 0.25 mm i.d., d_f 0.1 μm), and the picolinyl derivates and oxidation products of the unusual fatty acids were examined on the OV-1 column.

Spectroscopic procedures. IR spectra from the extracted oil and the FAME and their derivates were obtained with a Shimadzu IR-435 in a liquid film. Nuclear magnetic resonance ^{1}H-NMR and ^{13}C-NMR-spectra with proton noise decoupling were run in $CDCl_3$ on a Bruker AC 400 spectrometer with tetramethylsilane (TMS) as internal standard. The UV spectra of the oil and the methyl esters were run from 330-220 nm in purified hexane with a Perkin Elmer 554 UV/VIS spectrometer. The estimation of the amount of conjugated fatty acids was carried out following an established method (7).

RESULTS AND DISCUSSION

Characterization of the lipid fraction of Acioa edulis. Basic analytical data are given in Table 3. The UV data of the oil indicate a content of 27.56% conjugated fatty acids

$$\left(\text{using E } \frac{1\%}{1\text{ cm}} = 1710 \text{ at } 270 \text{ nm for calculation}\right)$$

with *cis, trans, trans* configuration (18). This was supported by the IR spectra, which are very similar to the IR spectra of oiticica oil (*Licania rigida*), known to contain α-eloestearic acid and α-licanic acid (19). Particularly significant are the keto absorption at 1720 cm^{-1} and the two bands at 962 cm^{-1} (w) and 990 cm^{-1} (s), typical for two *trans* and one *cis* double bond in conjugation (20). Besides the expected signals for normal fatty acids the ^{1}H-NMR spectra showed a complex multiplet with the most intensive signal at 6.1 ppm as the main feature. This is produced by olefinic protons of a conjugated *cis, trans, trans*, system (21).

The ^{13}C-NMR spectra strongly supported the IR and ^{1}H-NMR data and in addition allowed more detailed assignment of the keto group. Indicative for this oxygenated chain carbon atom was the signal at 208.25 ppm (22). The position of the keto group was analyzed by using the increment values of Tulloch and Mazurek (22) who

TABLE 1

Fatty Acid Composition and Content of Conjugated Fatty Acids of the Seed Oil of *Acioa edulis*

Peak no.[a]	Retention time (min)	Compound	Peak area (%)
1	4.85	BHT	—
2	10.20	C16:0	28.28
3	10.83	C16:1(n-7)	1.26
4	15.83	C18:0	6.78
5	16.65	C18:1(n-9)	26.45
6	16.82	C18:1(n-7)	1.30
7	18.15	C18:2(n-6)	8.84
8	18.89	C19:0[b]	—
9	19.92	C20:0	0.73
10	21.28	C18:3(c9t11t13)	7.34
11	27.39	C18:3-4-oxo(c9t11t13)	19.01
Concentration of conjugated fatty acids in %			
UV oil		27.56	
UV methyl esters		27.14	
Sum determined by GC		26.35	

[a]Peak no. corresponding to Figure 1.

[b]Internal standard.

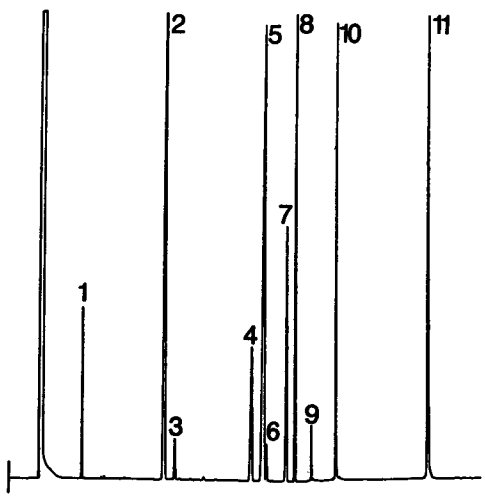

FIG. 1. Background-substracted capillary gas chromatogram (DB 23) of the fatty acid methyl ester mixture of *Acioa edulis*. For identification and retention times see Table 1.

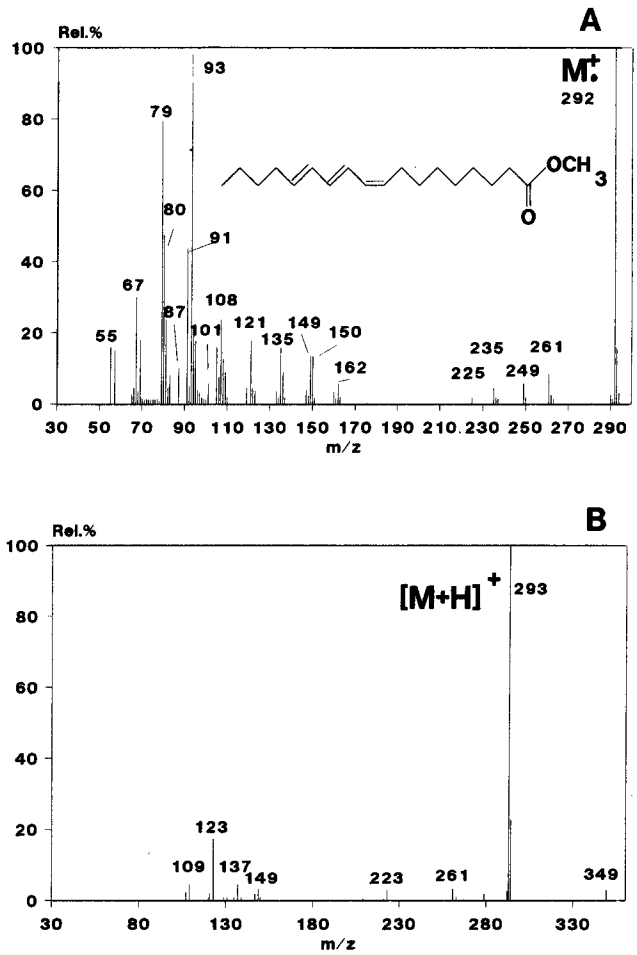

FIG. 2. Mass spectra of eleostearic acid methyl ester. (A) GC/EI-MS (25 eV). (B) GC/CI-MS (isobutane).

observed a large downfield displacement (13.1 ppm) of the α-carbon signals for oxygenated fatty acids in comparison to normal chain carbon atoms. This was fundamental for the following interpretation. For comparative calculations the published chemical shifts (23) for stearate, oleate, linoleate and some isomers 9,11,13-octadecatrienoates were used. In the glycerides of *Acioa edulis* two signals at 42.4 and 36.8 ppm were found in similar relative intensities. These carbon atoms could be assigned as CH_2-groups by DEPT experiment (Distortionless Enhancement by Polarization Transfer). The first signal (42.4 ppm) agrees theoretically with a carbon atom in α position to a keto group from position four to fifteen of the carbon chain (29 + 13.1 ppm $\approx$ 42.1 ppm), while the second signal (36.8 ppm) can be assigned to a carbon atom in α position to a keto group in position three of the chain (25 + 13.1 ppm $\approx$ 38.1 ppm). Calculation of all possible combinations showed that this interpretation is the only one in agreement with the experimental values. So, position four is very probable for the oxygenated carbon. The highfield C-17 (22.2 ppm) and C-18 (13.9 ppm) signals support the conjugated trienoic system (23).

Analysis of the fatty acids. The analyses of the fatty acids had the following objectives: the structural analyses of the conjugated fatty acids, the correct identification of the other fatty acids and the quantitative estimation of the fatty acid composition.

Transesterification, stability during GC analysis and fatty acid composition. Special care has to be taken in transesterification and GC analysis of seed oils containing conjugated fatty acids due to possible side reactions or decomposition (6,21,23,24). Corresponding to general recommendations (25) for the transesterification of conjugated fatty acids, a base-catalyzed reaction was used (8). This method, until now not used for derivatization of conjugated fatty acids, turned out to be very suitable for these kinds of analyses. Comparison of the UV spectra of the oil and the methyl esters showed that neither isomerization nor loss of these acids occurred during transesterification (see Table 1). Obviously, the DB 23 capillary

column is also appropriate for the analysis of methylated, conjugated fatty acids. In the gas chromatogram of the total fatty acid mixture no decomposition peaks could be found (Fig. 1). The quantitative composition of the fatty acids is presented in Table 1.

Isolation and structural characterization of the conjugated fatty acids. The GC analyses resulted in recognition of two unusual fatty acids which were subsequently identified as conjugated fatty acids. Because some workers were successful in determining the double bond positions of some conjugated dienes (26), long-chain alkatrienes (27) and olefins (28) by using EI-MS or CI-MS with isobutane, mass spectrometry in EI and CI mode was used first to get more structural information.

Peak no. 10 (Fig. 1), subsequently identified as α-eleostearic acid methyl ester showed a molecular ion(*m/z* 292) as the base peak in the EI spectrum (Fig. 2A). The fragmentation is similar to that of α-linolenic acid methyl ester according to Kubota *et al.* (29). Only slight differences in the intensities of the peaks were observed. This is caused by the well-known migration of the double bonds during the formation of the molecular ion in the ion source (3). Indeed, the allylic cleavage products, *m/z* 149 (13.3%) and *m/z* 249 (5.6%), appeared in the spectrum, but their diagnostic value for determination of the double bond position is low because these peaks were also detected in the spectrum of α-linolenic acid methyl ester with similar abundances. Even-numbered, significant ions due to a mechanism proposed (27) for long-chain 1,n,(n+2)-alkatrienes were not observed in noteworthy amounts.

The CI spectra (Fig. 2B) obtained with isobutane as reactant gas showed the quasimolecular ion $[M + H]^+$ as the base peak and *m/z* 261, due to $[M\text{-}OCH_3]^+$. Ions formed by addition of H^+ to the terminal double bonds with subsequent allylic cleavage accompanied by H-transfer, which has been observed for olefins (28) and for conjugated dienes (26), could not be detected. The ion *m/z* 149 could have originated from a regular allylic cleavage $[CH_3(CH_2)_3\ (CH{=}CH)_3CH_2]^+$. A first hint for the position of the double bonds were the ions *m/z* 123 $[[CH_3(CH_2)_3(CH{=}CH)_2CH]+H]^+$ and *m/z* 223 $[[COOCH_3(CH_2)_7(CH{=}CH)_2CH]+H]^+$. They could have arisen from cleavage of the terminal double bonds and concomitant addition of one hydrogen atom. Similar observations were made in the case of conjugated dienes (26).

For further structural determination we isolated the conjugated fatty acid methyl esters by HPLC. The methyl ester corresponding to peak no. 10, eluted after 24.8 min. Oxidative splitting gave azelaic acid as the only dibasic acid and pentanoic acid, identified as their methyl esters by GC/MS in comparison with standards. The formation of the maleic adduct gave a strong decrease in peak no. 10. The IR spectrum of the adduct did not show any absorption in the region between 965-990 cm^{-1}, hence the remaining double bond must be *cis*. Oxidation of the maleic anhydride adduct yielded azelaic acid. After catalytic hydrogenation, peak no. 10 was converted to octadecanoic acid methyl ester due to addition of six hydrogens to three double bonds.

All this indicated that peak no. 10 must be *cis*-9, *trans*-11, *trans*-13 octadecatrienoic acid, which is identical to α-eleostearic acid. This was supported by the spectroscopic data (see first section) and by comparison of ECL values (Table 2) with literature values (6,31) also

TABLE 2

Equivalent Chain Length of Different Conjugated Fatty Acid Methyl Esters on OV-1 and DB 23

Compound	ECL	
	OV-1	DB 23
α-eleostearic acid	18.99	22.07
β-eleostearic acid	19.36	22.49
α-licanic acid	20.37	27.81
β-licanic acid	20.80	28.30

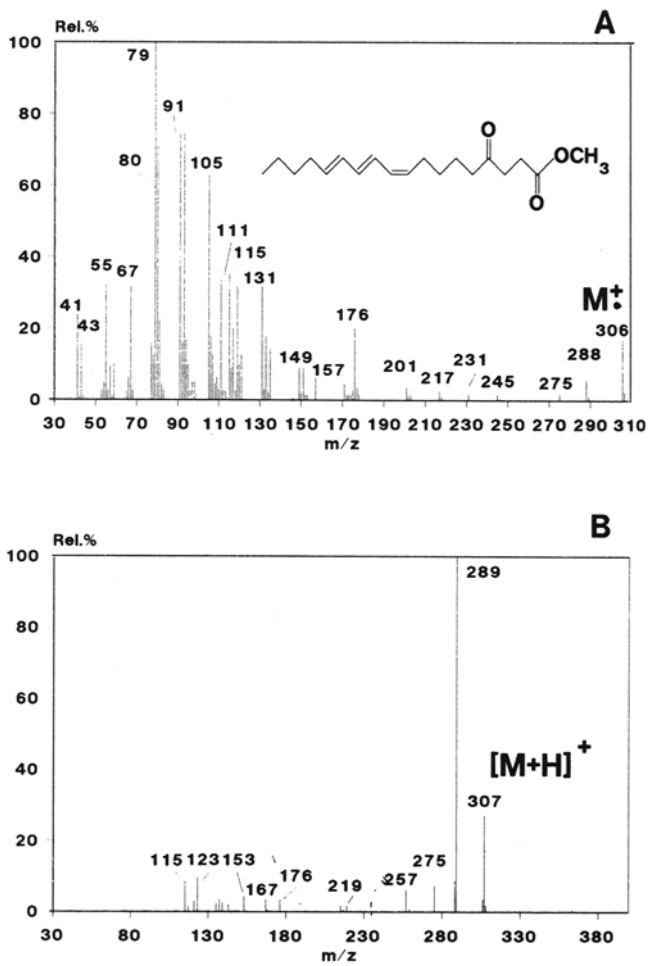

FIG. 3. Mass spectra of licanic acid methyl ester. (A) GC/EI-MS (25 eV). (B) GC/CI-MS (isobutane).

obtained on a OV-1 phase. To ensure the ECL assignment the all- *trans* isomer (β-eleostearic acid) was formed by iodine/light catalysis. The ECL values obtained for this compound were also identical with those published (6,31).

The EI spectrum (Fig. 3A) of peak no. 11 (Fig. 1), subsequently identified as α-licanic acid methyl ester, showed some interesting features. Obviously, the methyl ester easily eliminated one H_2O during ionization, thus yielding *m/z* 288, so that one part of the ions is formed by fragmentation of a system with four double bonds (see below). The peak *m/z* 275 (M-31) also originated from the molecular ion due to the loss of $CH_3O\cdot$. The other peaks

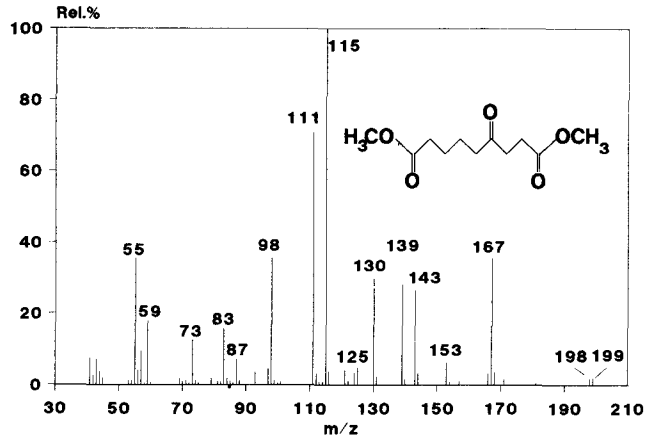

FIG. 4. EI mass spectrum (25 eV) of 4-oxoazelaic acid dimethyl ester.

with $m/z > 200$ can be explained as fragments proceeding from m/z 288 in the following manner: 245 = 288-43(C_3H_7·); 231=288-57(C_4H_9·); 217=288-71(C_5H_{11}·); 201=288-87(·$CH_2CH_2COOCH_3$) or 201=276-105(C_8H_9·).

The formation of highly unsaturated fragments is supported by the intensive m/z 91 (75%). The height of this peak, probably due to a tropylium ion (32), increases with the degree of unsaturation (33). Similar abundance of this ion was only observed in fatty acids with more than three double bonds. In addition, the series of ions due to m/z 91+n14 (n=1,2,3) that are typical for polyunsaturated systems (34) was detected. The ion m/z 115 could be explained as α-cleavage to the keto group $[COOCH_3(CH_2)_2C{\equiv}O]^+$. Also remarkable is the peak m/z 176, probably formed by a β-cleavage to the keto group, at which the alkyl chain is charged $[CH_3(CH_2)_3(CH{=}CH)_3CH_2CH{=}CH_2]^{+\cdot}$. The counterpart m/z 130 $[COOCH_3(CH_2)_2COH{=}CH_2]^{+\cdot}$ could not be detected.

Finally, it is noteworthy that the expected diagnostic important fragmentations due to α- and β-cleavage as known from monounsaturated oxygenated fatty acids (35,36) and conjugated oxodiene FAME (37) were strongly suppressed in the EI spectrum of licanic acid. In addition, loss of water has not been observed in the fragmentation patterns obtained (35-37).

The CI spectrum (Fig. 3B) of peak 11 supported the easy loss of one water molecule [m/z 289]. So, the spectrum is more similar to the published spectra of hydroxy fatty acids (38), which also showed [M+H-18] as base peak, than to the CI spectra of keto acids. Moreover the quasimolecular peak m/z 307 $[M+H]^+$ and the fragment ions m/z 275 $[M+H-CH_3OH]^+$, m/z 257 $[M+H-H_2O-CH_3OH]^+$ and m/z 219 $[M-H_2O-C_5H_9]^+$ were observed. The signals m/z 115 and m/z 176, already measured in the EI mode, were explained above. The intensity of these ions was low, which could be useful for localization of the keto group. This is in agreement with the low intensity of fragment ions typical for CI with isobutane (38). It is noteworthy that, similar to the EI spectrum, no other signals support this functional group. The peak m/z 123 could be significant for a (n-5)-terminus due to a mechanism proposed for α-eleostearic methyl ester described above. The signal m/z 153 and 167 can be explained as $[COOCH_3(CH_2)_2CO(CH_2)_4\text{-}H_2O]^+$ and $[COOCH_3(CH_2)_2CO(CH_2)_5\text{-}H_2O]^+$.

Finally both methods (EI/CI-MS) turned out to be not sufficient for structural assignment. So further methods were used to identify this fatty acid.

By catalytic hydrogenation of the fatty acid mixture, peak no. 11 (Fig. 1) was converted to 4-keto-octadecanoic acid methyl ester, identified by GC/MS, due to addition of six hydrogens to three double bonds. Here, in contrast to licanic acid, the expected α-(m/z 115,225) and β-cleavage (m/z 130,130-MeOH) products from the 4-keto group appeared in the mass spectrum with high abundance, so that the assignment was clear. Further reduction of the keto group with sodium borohydride yielded 4-hydroxy-octadecanoic methyl ester, also easily identified by GC/MS. This confirmed strongly the position of the keto group.

For correct identification of the stereochemistry and position of the double bonds, it was necessary to isolate the compound by HPLC. The methyl ester, corresponding to peak no. 11, eluted after 7.4 min. Oxidative splitting gave 4-oxoazelaic acid and pentanoic acid, identified as their methyl esters by GC/EI-MS. As far as we know, the mass spectrum of 4-oxoazelaic acid methyl ester (Fig. 4) has not been published until now, so it is discussed in more detail. α-Cleavage was observed to both sides of the keto group yielding m/z 115 $[COOCH_3(CH_2)_2C{\equiv}O]^+$ or $[COOCH_3(CH_2)_4]^+$ as base peak, m/z 143 $[COOCH_3(CH_2)_4C{\equiv}O]^+$ and m/z 87 $[COOCH_3(CH_2)_2]^+$. Furthermore, the ions m/z 130 $[COOCH_3(CH_2)_2COH{=}CH_2]^{+\cdot}$, probably produced by β-cleavage to the keto group, and m/z 98 $[130\text{-}CH_3OH]^{+\cdot}$ confirmed the 4-keto group. Also, typical peaks for dibasic methyl esters without an oxo group (39) were observed (m/z 167 $[M-[(OCH_3)_2]+H]^+$, m/z 139 $[M-[COOCH_3+OCH_3+H]]^+$ and m/z 125 $[M-COOCH_3CH_2+OCH_3+H]]^+$). The ions m/z 59, 73, 87 could be due to the series 59 + n14, while the signals m/z 41, 55, 83, 111, 125, 139, 153, 167 could be members of the series 27 + n14, known for dibasic acids (39). The molecular peak was absent. In contrast to dibasic acids without functional groups the signals m/z 199, 198 $[M-CH_3O\cdot,CH_3OH]$ were measured with low abundance.

The formation of the maleic adduct gave a strong decrease in peak no. 11 (Fig. 1). The IR spectrum of the adduct does not show any absorption in the region between 965-990 cm^{-1}, although the keto group was detectable (1720 cm^{-1}), hence the remaining double bond must be *cis*. Oxidation of the maleic anhydride adduct yielded 4-ketoazelaic acid.

All this indicated that peak no. 11 must be 4-oxo,*cis*-9, *trans*-11, *trans*-13 octadecatrienoic acid, which is identical with α-licanic acid. This also was supported by the spectroscopic data, described above. ECL values of the compound and its all-*trans* isomer are given in Table 2.

Identification of the usual fatty acids. The saturated acids were identified as their methyl esters by GC/MS in comparison with standards. To ensure the double bond position of the unsaturated compounds, two derivatization procedures were used. GC/MS of the picolinyl derivates enabled the clear identification of linoleic acid (fragmentation pattern, see (40)), whereas the GC peaks no. 10 and 11 diappeared after derivatization. Obviously it is not possible to prepare picolinyl derivates of conjugated fatty acids by this method, certainly due to double bond migration and polymerization caused by the trifluoroacetic acid, which is produced during this reaction as

TABLE 3

Comparison of Relevant Literature Data of *"Cotia Chestnut"* with the Data Obtained by Analysis of *Acioa edulis*

Parameter	Pelikan and Gerkins (2)	Pesce (41)	Cavalcanti (42)	Hopkins and Chisholm (43)	Data of *Acioa edulis*
Oil content	74.2%[a]	74.06%[b]	72.74%[c]	61%[d]	73.6%[a]
Protein nitrogen	—	2.67%	—	—	1.28%
Unsaponifiable	0.54%	—	1.3%	—	1.2%
Refractive index	—	1.4660(25°C)	1.4966(15°C)	—	1.4835(25°C)
Iodine value	114(Wijs)	—	192.3(Wijs)	—	115.6(GC)
Sapon. value	194.2	—	187.5	—	198.8
Density	—	0.9550(15°C)	0.9426(15°C)	—	0.9145(18°C)
UV (nm)	—	—	—	261/270/281	261/270/281
IR (cm^{-1})	—	—	—	957(w)/986(s)	962(w)/990(s)
Linolenic acid	0[e]	—	0[e]	—	0(GC)
Conjugated FA	supposed	—	supposed	24%(UV)	27.56%(UV)
Eleostearic acid	supposed	—	supposed	identified	identified
Licanic acid	—	—	—	—	identified
Hydroxy FA	—	—	supposed	not identified	not identified
Color	light straw	colorless	clear yellow	—	light yellow

[a]Solvent hexane.

[b]Extraction method unknown.

[c]Solvent diethyl ether.

[d]Solvent petroleum ether.

[e]Determined by hexabromate value.

by-product. Unfortunately, the isomeric monoenes could not be resolved completely with our GC equipment, so that the monoene fatty acids were isolated by argentation TLC, converted into their DMDS derivates and subsequently identified by GC/MS (10).

Comparison with literature data. In Table 3 a comparison of relevant literature data concerning *"Cotia Chestnut"* with our analytical data of *Acioa edulis* is presented in chronological sequence. It is remarkable that all the authors mentioned in Table 3 were considerably in doubt concerning the botanical classification of their samples.

Pelikan and Gerkens (2) first reported some chemical data from the oil of a so-called *"Cotia Chestnut."* Their data were similar to ours although incomplete, and the probability is high that they examined the same species. This presumption was supported by a photo of the fruits published in their paper.

In a report about "Oil Fruits from Amazonia" Pesce (41) described two varieties of *"Castanha de Cotia* or *Castanha de Anta,"* which are different in their geographic origin and their size. From the analytical point of view they appear to be different from *Acioa edulis* as shown in Table 3.

A paper from Cavalcanti (42) about the oil of *Castanha de Cotia* was more complete but the data are not in agreement with our values. The high iodine value and refraction index showed with a degree of certainty that they analyzed another tropical seed oil.

On the basis of the diene value and the drying properties, investigators (2,42) supposed that the oils of the kernels contained a conjugated fatty acid. Hopkins and Chisholm (43) identified α-eleostearic acid in a sample of the *"Cotia Chestnut"* and mentioned that there was no evidence of a hydroxy fatty acid, which had been proposed (42).

The most remarkable property of the *Acioa edulis* seed oil is its considerable content of eleostearic acid and licanic acid. Indeed, there are eleven families of plants that are known to contain conjugated fatty acids (15) but licanic acid only occurs in plants of the family Chrysobalanaceae. So, the above-mentioned chemotaxonomical significance of licanic acid was confirmed once more. To our knowledge *Acioa edulis* is the first example of a plant of the genus *Acioa* which was found to contain this oxo acid. Up to now only plants from the genus *Afrolicania, Chrysobalanus, Licania* and *Parinarium* are known to contain licanic acid in their seed oils (5,44). All of them are members of the family Chrysobalanaceae.

Though there are few investigations about the biological effects of higher amounts of conjugated fatty acids in seed oils, these seeds or their oils cannot be recommended for human or animal nutrition. Mammalian organisms are affected by feeding with those oils (45-47). In a recently published paper (48) about inhibition of prostaglandin biosynthesis by conjugated fatty acids, it was shown that the (n-5)-fatty acids, like eleostearic acid and licanic acid, are physiological active, though less than the (n-6)-type (e.g. jacarandic acid). Iketani *et al.* (49) proposed isolated glycerine esters from Cucurbitaceae, containing punicic acid (18:3,9=*cis*, 11*tr*=, 13=*cis*), which is similiar to eleostearic acid, as antithrombotic agents. This effect is surely caused by inhibition of the cyclooxygenase during prostaglandin synthesis. It is also known that conjugated fatty acids are unstable, even at ordinary temperature. Isomerization to all-*trans* compounds, spontaneous oxidation and polymerization are observed in less than an hour if exposed to air at room temperature. The result is a viscous oil with an unpleasant odor (15). During heating, β-eleostearic acid esters easily produce cyclic monomers (cyclohexene ring) which were found to be toxic to rats (50). Because of these effects, the seed oil of *Acioa edulis* can only be recommended for industrial purposes.

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