Identification of Nine Acetylenic Fatty Acids, 9-Hydroxystearic Acid and 9,10-Epoxystearic Acid in the Seed Oil of *Jodina rhombifolia* Hook et Arn. (Santalaceae)[1]

V. Spitzer[a,*], S.A. de L. Bordignon[a], E.P. Schenkel[a] and F. Marx[b]

[a]Faculdade de Farmácia, Curso de Pós-Graduação, Universidade Federal do Rio Grande do Sul, 90.610.000 Porto Alegre/RS, Brazil and [b]Lehrstuhl für Lebensmittelwissenschaft und Lebensmittelchemie, Rheinische Friedrich Wilhelms-Universität, Endenicher Allee 11-13, 53115 Bonn 1, Germany

In addition to some usual fatty acids, the seed oil of *Jodina rhombifolia* (Santalaceae) contains nine acetylenic fatty acids [9-octadecynoic acid (stearolic acid) (1.1%), *trans*-10-heptadecen-8-ynoic acid (pyrulic acid) (20.1%), 7-hydroxy-*trans*-10-heptadecen-8-ynoic acid (2.3%), *trans*-10,16-heptadecadien-8-ynoic acid (0.7%), 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoic acid (0.1%), *trans*-11-octadecen-9-ynoic acid (ximenynic acid) (20.3%), 8-hydroxy-*trans*-11-octadecen-9-ynoic acid (12.2%), *trans*-11,17-octadecadien-9-ynoic acid (1.5%), 8-hydroxy-*trans*-11,17-octadecadien-9-ynoic acid (1.3%), 9-hydroxystearic acid (<0.1%) and 9,10-epoxystearic acid (0.7%)]. The fatty acids have been analyzed by gas chromatography/mass spectrometry of their methyl ester and 4,4-dimethyloxazoline derivatives. The hydroxy fatty acid methyl esters have been examined also as trimethylsilyl ethers. Furthermore, the fatty acid methyl esters (FAME) have been fractionated according to their polarity (FAME-A: nonhydroxy; FAME-B: hydroxy fatty acids) and to their degree of unsaturation (FAME-A1/A2; FAME-B1/B2) by preparative thin-layer chromatography and argentation chromatography, respectively. All of these fractions have been analyzed by ultraviolet and infrared spectroscopy, and the fractions FAME-A and FAME-B have been analyzed further by nuclear magnetic resonance (^{1}H, ^{13}C, 2D H/C, attached proton test) spectroscopy and gas chromatography/mass spectrometry.

KEY WORDS: Acetylenic fatty acids, epoxy fatty acids, gas chromatography/mass spectrometry of 4,4-dimethyloxazoline derivatives of acetylenic fatty acids, hydroxy fatty acids, *Jodina rhombifolia*, NMR spectroscopy, Santalaceae.

Jodina rhombifolia is a tree (up to 4 m), native to southern Brazil, Uruguay, Argentina, Paraguay and Bolivia (1), and belongs to the plant family Santalaceae. The plant yields pulpy, yellow-pink and globose stone-fruits with a diameter of about 1.5 cm, containing one oily seed. The sweet-tasting fruits of *"cancorosa-de-três-pontas," "espinheira-de-três-pontas"* or simply *"cancorosa"* are eaten mainly by children, who confuse these fruits with the fruits of *Salacia* sp. (Hippocrataceae) (2). In popular medicine, the leaves are widely used, mainly against gastric troubles, cold and carcinoma (1). Though important as a plant drug and occasional foodstuff, up to now, only preliminary chemical data about the seed oil (3) and the components of the leaves (4) have been published.

Hopkins *et al.* (3) reported the presence of 55% acetylenic fatty acids (FA) with a conjugated eneyne structure, based on ultraviolet (UV) spectroscopy, of 16% of a C-17 FA [gas chromatography (GC) of the totally reduced fatty acid methyl esters (FAME)], and also of compounds with terminal vinyl unsaturation and hydroxy acids [infrared spectroscopy (IR)] in the seed oil.

In the present study, as a part of our current research project about seed oils of medicinal and edible plants from southern Brazil, the FA composition and nuclear magnetic resonance (NMR) spectroscopic data of the seed oil and of the FAME of *J. rhombifolia* are described for the first time. Furthermore, the mass spectra of some as yet undescribed derivatives of some acetylenic FA are discussed.

MATERIALS AND METHODS

The fruits from *J. rhombifolia* were collected in January 1994 in the region of Viamão, near Porto Alegre, in the state Rio Grande do Sul, Brazil, and a voucher specimen has been deposited in the Botany Department Herbarium (ICN 103 019) of the Federal University of the state Rio Grande do Sul (UFRGS) (Porto Alegre, RS, Brazil). The air-dried, crushed seeds were extracted in a Soxhlet apparatus for 6 h with petroleum ether (40–60°C). After extraction, the solvent was removed by vacuum distillation at 30°C, flushed with nitrogen and stored at −18°C until use.

Transesterification to the FAME was carried out overnight with 0.5 N sodium methoxide in anhydrous methanol at room temperature under nitrogen. The 4,4-dimethyloxazoline derivatives (OXFA) of the total FA were prepared after hydrolysis of the oil with 1N potassium hydroxide in 95% ethanol at room temperature, as described by Zhang *et al.* (5). Silylation of the hydroxy fatty acid methyl esters (OH-FAME) was done by an established method with BSTFA (6).

Thin-layer chromatography (TLC) of the FAME was carried out with 0.25-mm silica plates (Merck, Darmstadt, Germany) and petroleum ether/diethyl ether (80:20, vol/vol) as solvent system. Preparative separation of the FAME without hydroxyl groups (FAME-A) and the OH-FAME fractions (FAME-B) was carried out with 0.6-mm preparative silica layers with petroleum ether/diethyl ether (70:30, vol/vol) as solvent system. These fractions were subfractionated according to their degree of unsaturation on 0.6-mm silver nitrate-impregnated silica gel plates (10%) with petroleum ether/diethyl ether 80:20 (vol/vol) and 50:50 (vol/vol) for FAME-A and FAME-B, respectively. The bands of interest were scraped off [detected with UV (254 nm) and 2′,7′-dichlorofluorescein] and eluted with diethyl ether. Monitoring of each fraction was carried out by GC, IR and UV analyses.

A Hewlett-Packard 5890 GC (Palo Alto, CA) with a 7673 A autosampler, a flame-ionization detector (FID) and a split/splitless injector with glass insert was used to analyze the FAME. Separation of the compounds was achieved with a DB 23 (J&W Scientific, Folsum, CA)

[1]This work is dedicated to the 65th birthday of Prof. Dr. K. Pfeilsticker, Institut of Food Science, University Bonn (Germany).

*To whom correspondence should be addressed at Faculdade de Farmácia UFRGS, Curso de Pós-Graduação, Avenida Ipiranga, 2752, 90.610.000 Porto Alegre/RS, Brazil.

capillary column (30 m × 0.25 mm i.d., 0.25 μm) and the following temperature program: 130–230°C, 2°C/min. The equivalent chain length (ECL) values were measured at 190°C (FAME-A) and 210°C (FAME-B) with a reference saturated FAME mixture from C14:0 to C26:0 for calibration. GC/mass spectrometry (GC/MS) analysis was done with the NERMAG AUTOMASS (Paris, France) operating with an ionization energy of 70 eV, a source temperature of 225°C and an interface temperature of 220°C. The separation of the FAME derivatives was carried out with the same DB 23 column and temperature program as used in the GC analysis. For the OXFA derivatives the temperature program was 160–230°C; 2°C/min. Helium was used as carrier gas (0.5 bar).

IR spectra of the oil and the methyl ester fractions were obtained with a Shimadzu FTIR-8101 (Tokyo, Japan) in a liquid film. UV spectra of the oil and the methyl esters were recorded from 400–200 nm in cyclohexane solution with a Shimadzu UV-2201 spectrophotometer. The UV estimation of the amount of conjugated fatty acids was carried out following an established method (7).

The ^1H NMR and ^{13}C NMR spectra were run in deuteriochloroform solution on a Varian VLX-200 spectrometer (Palo Alto, CA) with trimethylsilyl as internal standard.

RESULTS AND DISCUSSION

Spectroscopic data on the oil. The mature seeds of *J. rhombifolia* contain 30.8% of a pale-yellow oil with a refraction index of n_D = 1.4865 at 25°C. The UV spectrum of this oil showed a maximum at 229.5 nm with E(1%/1 cm) = 332.2, indicating the presence of 59.6% FA with a conjugated *trans*-eneyne structure [calculated as ximenynic acid with E(1%1 cm) = 557] (3). No absorption above 230 nm was observed; hence there are no appreciable contents of enediyne , dieneyne or similar groups of higher conjugation.

Beside the usual vegetable oil absorptions, the IR spectrum of the oil showed characteristic bands at 3490 cm^{-1} (*m*, broad) for a hydroxyl-group, 2359 cm^{-1} (*vw*), 2336 cm^{-1} (*vw*) and 2214 cm^{-1} (*w*) for acetylenic bonds, 910 cm^{-1} (*w*) for a terminal vinyl group, and 1642 (*w*) and 955 cm^{-1} (*s*) for a double bond of a *trans*-eneyne linkage (8).

The ^1H NMR and ^{13}C NMR spectral data [proton noise decoupling and attached proton test (APT)] of the oil were in good agreement with the UV and IR data, confirming the presence of FA with conjugated eneyne systems, hydroxy groups and terminal vinyl bonds. As additional information, the pairs of ^{13}C NMR peaks with similar intensities observed at 145.36:145.31 ppm (CH) and 109.69: 108.78 ppm (CH), which all were accompanied by small satellite signals, indicated the presence of more than one FA with a double bond in conjugation with a triple bond (9). Furthermore, the pairs of ^{13}C NMR signals at 62.77: 62.68 ppm (CH-O-) and 37.78:37.62 ppm (CH_2) suggested the occurrence of at least two FA with a -C≡C-CH(OH)- structure (10). This assignment was supported by the ^1H NMR signal at 4.4 ppm (*t*), which can also be attributed to the latter type of structure element (10,11). Further details of the assignments of the NMR data are described in the following section.

Identification of the FA of J. rhombifolia. To identify the FAME GC peaks (Fig. 1, Table 1), various analysis and separation procedures were utilized. The peak numbers of the FA in the following text correspond with numbers in Figure 1 and Table 1. Chromatograms obtained with the DB 23 column did not show noticeable decomposition of the conjugated FA, which was observed by other investigators (12).

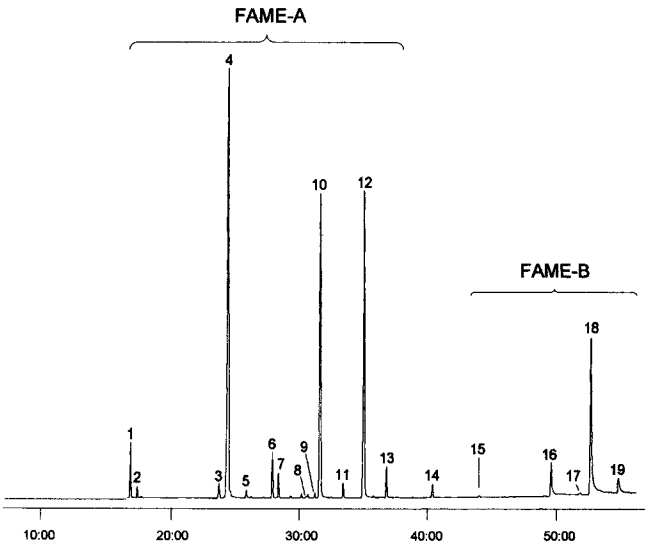

FIG. 1. Capillary gas chromatogram (DB 23 column; 130–230°C, 2°C/min) of the fatty acid methyl esters (FAME) of *Jodina rhombifolia*. For identification and equal chainlength values, see Table 1.

Identification of the common FA (Peaks 1-6, 8-9). FAME with the usual ECL-values were identified by GC and GC/MS against authentic standards. In addition, the OXFA derivatives were examined by GC/MS, which enables the unequivocal location of the double bond(s) (5).

Identification of the unusual FA (Peaks 7, 10-19). TLC analysis of the total FAME showed two main bands under UV (FAME-A with R_f = 0.81 and FAME-B with R_f = 0.32). Argentation TLC of the fractions FAME-A and FAME-B, separated by preparative TLC, yielded two additional bands for each fraction (FAME-A1/A2 and FAME-B1/B2). The UV maxima (229 nm) from all of these fractions suggested the presence of conjugated eneyne systems. The fractions FAME-A1 and FAME-A2 lacked the IR absorption for the OH group, and the typical terminal vinyl absorption band (910 cm^{-1}) was absent in FAME-A1/FAME-B1 but was strongly enhanced in FAME-A2 and FAME-B2.

Comparison of the NMR spectral data of the fractions FAME-A and FAME-B with the literature data for usual FAME (13,14), acetylenic FAME (9,15,16), conjugated acetylenic-hydroxy FAME (10,11) and hydroxy FAME (17), together with the APT and 2D H/C NMR experiments, allowed the assignments of the signals to individual hydrogen and carbon atoms (Table 2). The ^1H NMR spectrum of both fractions showed a doublet at 5.43 ppm (*J* 15 Hz) and a doublet-triplet at 6.1 ppm (*J* 15; 7 Hz) in equal intensities. These signals can be assigned to the hydrogens of a conjugated eneyne system (see Table 2), and the high value for the coupling constants indicated *trans*-configuration of the double bond (9,18). The corresponding close pairs of ^{13}C NMR signals (2D H/C) at 109.82:109.89 ppm and 143.25:143.31 ppm for FAME-A, and 108.97:108.91 ppm and 145.31:145.27 ppm for FAME-

TABLE 1

Fatty Acid Composition of the Seed Oil of *Jodina rhombifolia*[a]

Peak number	Compound	Peak area % (ECL)	Diagnostic mass fragments (in *m/z*) of the unsaturated and unusual fatty acids (relative abundance)
1	16:0	2.3	
2	16:1(*c*9)	0.3	OXFA: 307(6.2) (M), 196(4.1), 208(3.8)
3	18:0	0.8	
4	18:1(*c*9)	32.4	OXFA: 335(5.1) (M), 196(4.5), 208(5.2)
5	18:2(*c*9,*c*12)	0.3	OXFA: 333(3.1) (M), 196(2.7), 208(3.1), 236(4.0), 248(2.2)
6	18:3(*c*9,*c*12,*c*15)	2.1	OXFA: 331(17.2) (M), 196(6.4), 208(4.0), 236(9.9), 248(2.8), 276(7.4), 288(4.1)
7	18:1(9a)	1.1(19.4)	OXFA: 333(5.1) (M), 196(2.1), 206(2.3)
8	20:0	<0.1	
9	20:1(*c*11)	0.3	OXFA: 363(3.8) (M), 214(2.6), 226(1.9)
10	17:2(8a,10*t*)	20.1(20.4)	see Figure 2
11	17:3(8a,10*t*,16*t*)	0.7(20.9)	see Figure 2
12	18:2(9a,11*t*)	20.3(21.4)	OXFA: 331(17.1) (M), 196(1.1), 206(2.5), 220(3.7), 232(8.7)
13	18:3(9a,11*t*,17*t*)	1.5(21.9)	OXFA: 329(6.4) (M), 196(1.2), 206(1.8), 220(2.9), 232(7.1), 302(1.4), 314(2.9)
14	9,10-epoxy-C18:0	0.7(23.3)	FAME: 55(100), 69(65), 74(70), 87(34), 97(25), 109(36), 127 (21), 139(17), 155(86), 171(29), 199(32), 263(0.7), 281(1.3), 294(1.2), 312(0.2) (M)
15	9-OH-C18:0	<0.1(24.4)	FAME: 55(45), 74(42), 87(58), 115(19), 129(7.3), 155(100), 158(35), 187(27), 222(8.4), 264(35), 296(1.5); FAME-TMS: 229(45), 230(29), 259(100), 355(4), 371(2.3) (M-CH_3)
16	7-OH-C17:2(8a,10*t*)	2.3(26.1)	FAME-TMS: 73(100), 237(48), 265(2.2), 279(3.1), 281(7.4), 293(2.3), 295(1.7), 366(0.8)(M); OXFA: 113(29), 126(100), 140(12), 154(2.5), 168(8.2), 169(14), 258(3.2), 315(2.9), 333 (1.4) (M)
17	7-OH-C17:3(8a,10*t*,16*t*)	<0.1 (26.8)	FAME-TMS: 73(100), 235(36), 263(1.3), 277(2.1), 281(4.2), 291(1.2), 364(0.5) (M); OXFA: 113(32), 126(100), 140(13), 154(2.3), 168(8.8), 169(15), 258(2.1), 313(1.8), 331(1.3) (M)
18	8-OH-C18:2(9a,11*t*)	12.2(27.1)	FAME-TMS: see Figure 2
19	8-OH-C18:3(9a,11*t*,17*t*)	1.3(27.8)	FAME-TMS: 73(100), 235(43), 263(1.6), 277(2.7), 295(5.2), 378(0.5) (M); OXFA: 113(51), 126(100), 140(22), 154(7), 168 (6.5), 182(8.8), 183(17), 272(3.2), 327(1.4), 345(0.9) (M)

[a]FAME = fatty acid methyl ester, OXFA = 4,4-dimethyloxazoline derivative of the fatty acid; FAME-TMS = trimethylsilyl derivative of the hydroxy fatty acid methyl ester; "a" as in 18:1(9a) = acetylenic bond; ECL = equivalent chainlength.

B suggested the occurrence of at least two FA with olefinic carbons of this type of conjugated linkage in each fraction (9). The small differences between the chemical shifts of these signal pairs implied that these FA could be positional isomers. The *trans*-configuration of the conjugated double bond(s) was confirmed again by the downfield shifted ^{13}C signal for an allylic carbon atom at 33 ppm (14). Furthermore, the presence of FAME with a terminal vinyl group was confirmed in both fractions by the corresponding weak ^{1}H (4.94 and 5.76 ppm) and ^{13}C (114.7 and 138.4 ppm) signals (see Table 2). Beside these similarities, the NMR spectral data revealed also some characteristic differences between FAME-A and FAME-B.

So, the ^{13}C NMR spectrum of fraction FAME-A showed two signal pairs at 79.25:79.33 ppm and 88.31: 88.44 ppm, attributable to the acetylenic carbons of a conjugated eneyne system (9,18), which confirmed the presence of at least two compounds with the same unsaturated system but a small difference in its position in the FA chain. The conjugation of a triple bond with a double bond was supported by the fact that only one methylene carbon signal in equivalent intensity at 19.3 ppm beside an acetylenic carbon was observed (see Table 2) (9). For isolated or conjugated triple bonds, two separated signals at about 18–19 ppm for the methylene groups beside the acetylenic bond would be expected (16).

The ^{13}C NMR spectrum of fraction FAME-B showed signals at 62.65:62.73 ppm and 37.57:37.73 ppm, indicating an additional hydroxyl group in conjugation with a *trans*-eneyne system (Table 2) (10). As in fraction FAME-A, the fraction FAME-B showed these characteristic signals also in pairs, proving the occurrence of at least two FA with this structure. The cross signal in the 2D H/C-NMR spectrum at 4.4:62.65, 62.73 ppm supported the assignment to a -CH=CH-C≡C-CH(OH) structure (10). The absence of a methylene signal at about 19 ppm, indicated that the hydroxyl group must be in conjugation with the acetylenic bond.

TABLE 2

Chemical Shifts of the H and ^{13}C Atoms (in ppm relative to TMS) of the Fractions FAME-A and FAME-B of the Seed Oil of *Jodina rhombifolia* and Their Tentative, Partial Assignments[a]

FAME-A			FAME-B		
^{13}C-Shift	^{1}H-Shift	Assignments	^{13}C-Shift	^{1}H-Shift	Assignments
14.06:14.09	0.89 (*t*)	CH_3-	14.02	0.89(*t*)	CH_3-
19.26[b]:19.30	2.28 (*m*)	-C≡C-CH_2	22.52	1.3 (*m*)	CH_3-CH_2-
22.59:22.68	1.3 (*m*)	CH_3-CH_2-	24.78:24.94	1.6 (*m*)	-$COCH_2CH_2$-
24.81:24.89:24.94	1.6 (*m*)	-$COCH_2CH_2$-	28.3–28.6 (six signals)	1.3 (*m*)	-$(CH_2)_n$-
25.7[b]	2.8[b] (*t*)	-CH=CH-CH_2-CH=CH-	31.58	1.3 (*m*)	CH_3-CH_2-CH_2-
27.15:27.20	2.02 (*m*)	-CH=CH-CH_2-	33.00	2.05 (*q*)	-CH_2-CH=CH-(*trans*)-
28.3–29.8 (13 signals)	1.3 (*m*)	-$(CH_2)_n$-	33.91:33.99	2.28 (*t*)	-$COCH_2$-
31.68:31.92	1.3 (*m*)	CH_3-CH_2-CH_2-	37.57:37.73	1.6 (*m*)	-CH(OH)-CH_2-
32.96	2.02 (*m*)	-CH_2-CH=CH-(*trans*)-	51.45	3.61 (*s*)	-OCH_3
34.02:34.06	2.28 (*t*)	-$COCH_2$-	62.65:62.73	4.4 (*t*)	-C≡C-CH(OH)-
51.50	3.61 (*s*)	-OCH_3	83.51:83.57	—	-CH=CH-C≡C-CH(OH)-
79.25:79.33	—	-CH=CH-C≡C-CH_2-	88:53:88.63	—	-CH=CH-C≡C-CH(OH)-
88.31:88.44	—	-CH=CH-C≡C-	108.97:108.91	5.43 (*d*, 15 Hz)	-CH=CH-C≡C-
109.82:109.89	5.43 (*d*; 15 Hz)	-CH=CH-C≡C-	114.7[b]	4.94[b] (*d*)	CH_2=CH-
114.7[b]	4.94[b] (*d*)	CH_2=CH-	138.4[b]	5.76[b] (*m*)	CH_2=CH-
127–132 (eight signals)	5.3 (*m*)	-CH=CH-	145.331:145.27	6.1 (*dt*, 15, 7 Hz)	-CH=CH-C≡C-CH(OH)-
138.4[b]	5.76[b] (*m*)	CH_2=CH-	174.32	—	-$COOCH_3$
143.25:143.31	6.1 (*dt*, 15, 7 Hz)	-CH=CH-C≡C-			
174.21	—	-$COOCH_3$			

[a]Data were obtained by ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, Attached Proton Test and 2D H/C NMR experiments. TMS, trimethylsilyl; FAME, fatty acid methyl esters.
[b]Weak signals.

GC analysis demonstrated that the FAME-A fraction consists of the normal FA (Peaks 1–6, 8–9) and contains furthermore two major (Peaks 10, 12) and three minor FA with unusual ECL-values (Peaks 7, 11, 13). The five unusual FA 15–19 with high ECL values belong to the FAME-B fraction (Fig. 1). After silylation of the latter fraction, all GC peaks appeared at lower retention times, confirming the presence of a hydroxyl group. According to the weak signals in the IR and NMR spectra for the terminal vinyl group, it was concluded that only the small unknown peaks of the GC (Peaks 7, 11, 13, 15, 16, 18) could come into question for the FA with this type of unsaturation. FA 14 appeared only in the GC of the total FAME, suggesting different structural properties.

All these data indicated that the oil of *J. rhombifolia* must contain at least four types of unusual FA, with a conjugated *trans*-eneyne structure, whereas the FA of the fraction FAME-B contain a -CH=CH-C≡C-CH(OH) structure. The FA of the fractions FAME-A2/FAME-B2 must have an additional terminal vinyl group. To get more detailed information about the unknown compounds, the mass spectra of different derivatives were examined.

GC/MS characterization of stearolic acid [Peak 7 (Fig. 1)]. The mass spectra of the FAME and the OXFA derivative of FA 7 were identical with those of the corresponding derivatives of 9-octadecynoic acid (stearolic acid) in the literature (19,20).

Characterization of the conjugated acetylenic FA without terminal vinyl and without hydroxyl groups [Peaks 10 and 12 (Fig. 1)]. The difference of 1.0 between the ECL values of FA 10 and FA 12 and molecular ions *m/z* 292 and 278 in their mass spectra suggested that these FA must be structural homologues differing by one methylene group. Their mass spectra were identical with those of *trans*-10-heptadecen-8-ynoic acid methyl ester (21) and *trans*-11-octadecen-9-ynoic acid methyl ester (9,18), respectively. In accordance with the cyclic fragmentation and rearrangement mechanism proposed for FAME with acetylenic bonds (22), the position of the eneyne linkage is here indicated by the fragments *m/z* 164 $[H\text{-}(CH{=}CH)_2\text{-}CH{=}CH\text{-}(CH_2)_5CH_3]^+$ and *m/z* 150 $[CH_2{=}C{=}CH\text{-}CH{=}CH\text{-}(CH_2)_5CH_3]^+$. There were no cleavage products to support either an 8-ene-10-yne-structure (theoretically *m/z* 208 and 222) or a 9-ene-11-yne-structure (theoretically *m/z* 222 and 236). To confirm this structural information, the OXFA derivatives of these compounds were also examined by GC/MS. These derivatives were useful for the location of conjugated double-triple bonds in some long acetylenic chain FA (9,20). The mass spectrum of the OXFA derivative of FA 12 was identical with that published for the OXFA derivative of ximenynic acid (9,20). The mass spectrum of the OXFA (Fig. 2) of the peak corresponding to FA 10 revealed similar characteristics. In the low mass range, *m/z* 113 and *m/z* 126 (base peak) appeared as the most intense peaks. These fragment ions are formed by cyclization–displacement reaction and McLafferty rearrangement (5), respectively. The even-mass homologous series at *m/z* 126 + 14n, deriving from cleavage at each saturated methylene bond (5), was interrupted by a mass separation of 10 mu between *m/z* 182/192, pointing to a acetylenic bond at C-8. The double-bond position of the conjugated system was indicated by a 12-mu gap between *m/z* 206:218. In addition, these characteristic ion pairs were accompanied by two other intense peaks at *m/z* 168:246, derived from cission allylic to the conjugated system, following the empirical rules of Zhang *et al.* (20) proposed for OXFA derivatives of this type of compounds. The 14n series was then continued regularly after the double bond up to the molecular ion at *m/z* 317. Considering the MS, NMR, IR and UV data

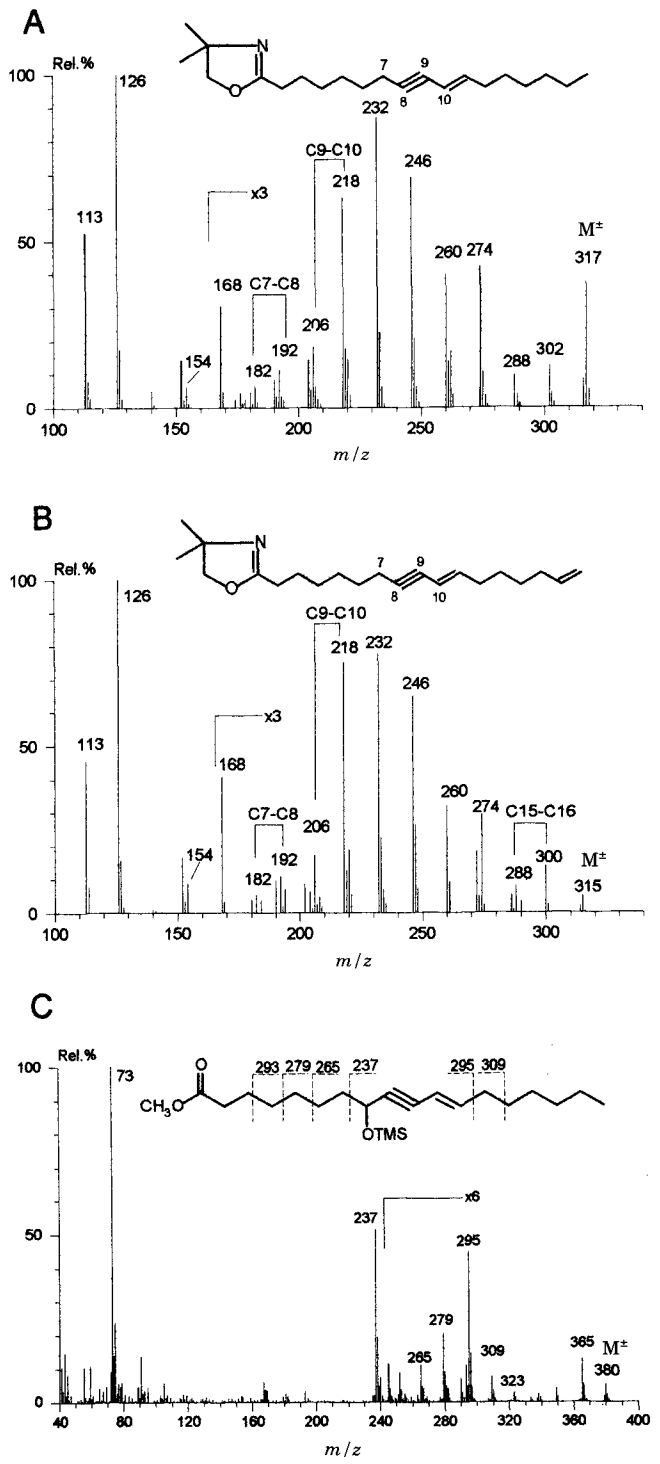

FIG. 2. Mass spectra (70 eV) of the 4,4-dimethyloxazoline derivatives of *trans*-10-heptadecen-8-ynoic acid (A), *trans*-10,16-heptadecadien-8-ynoic acid (B), and the trimethylsilyl derivative (OTMS) of the methyl ester of 8-hydroxy-*trans*-11-octadeca-9-ynoic acid (C).

of FAME-A, the FA 10 and 12 can be identified as *trans*-10-heptadecen-8-ynoic acid (pyrulic acid) and *trans*-11-octadecen-9-ynoic acid (ximenynic acid).

GC/MS characterization of the conjugated acetylenic FA with a terminal vinyl and without a hydroxyl group [Peaks 11 and 13 (Fig. 1)]. The mass spectra of FAME 11 and 13 showed a series of hydrocarbon fragments [*m/z* 41, 55, 67, 79, 91 (base peak), 105, etc.], which is typical for highly unsaturated FAME (23) and FAME with acetylenic bonds (24). Intensity of McLafferty ions (*m/z* 74) were very weak, and the molecular ions were not detectable for either ester under the experimental conditions. In contrast to the FAME, the OXFA derivatives of these compounds showed molecular ions at *m/z* 315 (peak 11) and *m/z* 329 (peaks 13), suggesting unsaturated C-17 ($C_{17}H_{28}O_2$) and C-18 ($C_{18}H_{30}O_2$) FA, respectively. The difference of 1.0 between the ECL values of FAME 11 and FAME 13 implied that these FA must be structural homologues that differ by one methylene group.

By comparison with the OXFA mass spectra published by Zhang *et al.* (20), peak 13 was identified as C18:3(9a, 11,17). The OXFA spectrum of the FA corresponding to peak 11 (Fig. 2) showed principally the same characteristics as the OXFA spectrum of C18:3(9a,11,17), but the characteristic gaps in the 14-mu series were found between *m/z* 182:192, 206:218 and 288:300, indicating C17:3(8a, 10,16). Because in the ^1H NMR spectrum of the fraction FAME-A, only signals for the hydrogens of a *trans*-ene-yne system were observed (see Table 2), it is probable that both compounds have a conjugated system with a *trans* olefinic bond.

Characterization of the FA with a hydroxyl group [Peaks 15–18 (Fig. 1)]. GC analysis showed that FAME-B consists of 0.6% FA 15, 10.4% FA 16, 0.5% FA 17, 77.7% FA 18 and 8.4% FA 19. FA 15 was identified as 9-hydroxystearic acid by comparison of its mass spectra of the FAME and the corresponding TMS derivative with the literature data (10). Differences of 1.0 ECL unit for FA 18/16 and for FA 19/17 suggested that these compounds could be structural homologues differing by one methylene group. The mass spectra of the TMS derivatives of FAME 16, 17 and 19 (Table 1) were identical with those published for the TMS derivatives of 7-hydroxy-*trans*-10-heptadecen-8-ynoate, 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoate and 8-hydroxy-*trans*-11,17-octadecadien-9-ynoate (25), respectively (see Table 1). Kleiman and Spencer (25) examined these acids isolated from the seed oil of *Acanthosyris spinescens* and demonstrated by GC/MS the suitability of the TMS derivatives for locating the OH group and the position of the conjugated unsaturated system. Unfortunately, it is not possible to distinguish between an ene-yne or an ynene system with this method (25). Thus, the MS data gave the first hint that the FA 16 and FA 17 must contain a 7-OH-10-ene-8-yne or a 7-OH-8-ene-10-yne system, whereas the FA 17 must have one additional double bond in the chain. For FA 19, an 8-OH-11-ene-9-yne or an 8-OH-9-ene-11-yne system with one additional olefinic bond can be proposed. The mass spectrum of the TMS FAME 18 (Fig. 2) was similar to that of the TMS FAME 16, but the molecular ion peak was found at *m/z* 380, suggesting a homologous C-18 OH-FAME with one double and one triple bond. The position of the OH group at C-8 and of the conjugated unsaturated system between C-9 and C-12 was here indicated by the intense peaks at *m/z* 237 and *m/z* 295, which arise from alpha-cleavage at the TMS group and at the double bond, respectively (25). Other characteristic peaks of the TMS derivative of FA 18, arising from cleavage at the methylene groups, are explained in Figure 2. As for FA

from the MS data that the FA 18 must contain an 8-OH-11-ene-9-yne or 8-OH-9-ene-11-yne structure. Because the mass spectra of the MS derivatives of this type of compounds neither distinguish between eneyne and ynene systems nor give unequivocal information about the nonconjugated double bond in FA 17 and 19, the other spectroscopic and chromatographic data of FAME-B were considered for complete structure elucidation.

As described above, the ^{1}H and ^{13}C NMR data of FAME-B (see Table 2) do not show signals attributable to a -C≡C-CH=CH-CH(OH) structure, but support a *trans*-CH=CH-C≡C-CH(OH) structure. Thus, FAME 16 and 18 could be identified as methyl 7-hydroxy-*trans*-10-heptade-cen-8-ynoate and 8-hydroxy-*trans*-11-octadecen-9-ynoate, respectively. The intense IR absorption at 910 cm^{-1}, observed in fraction FAME-B2, demonstrated that at least one of the FA 17 and 19 must contain a terminal vinyl unsaturation. As the ^{1}H NMR and ^{13}C NMR spectra of FAME-B showed only signals for the double bond of a *trans*-CH=CH-C≡C-CH(OH) structure and for a terminal vinyl double bond (which is absent in FA 15, 16 and 18), it follows that both compounds must contain the latter type of olefinic linkage. This interpretation was supported by the fact that the difference in the ECL values between FA 19-FA 17 was 1.0, showing that the compounds must be homologous. On the basis of all these data, FA 17 and FA 19 can be identified as 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoic and 8-hydroxy-*trans*-11, 17-octadecadien-9-ynoic acid.

GC/MS characterization of 9,10-epoxystearic acid [Peak 14 (Fig. 1)]. The mass spectrum of the methyl ester of peak 14 (see Table 1) was identical with that of 9,10-epoxystearic acid methyl ester (25). Saturated epoxy esters produce mass spectra, the interpretation of which is so straightforward that epoxidation and MS form an established procedure to locate double bonds (25).

Chemotaxonomical considerations. The oil of *J. rhombifolia* contains about 60% of acetylenic FA, which (with the exception of stearolic acid) all contain a conjugated eneyne system at the n-7 terminal end of a C-17 or C-18 chain. Some of these compounds have an additional terminal vinyl unsaturation and/or an OH group in conjugation with the acetylenic bond. Up to now, this type of FA was only found in the two closely related plant families Santalaceae and Olacaceae (both members of Santalales), and is therefore of chemotaxonomic importance (26). The Olacaceae show a preponderance of FA with two acetylenic groups (8). The 9,10-epoxystearic acid identified here, which could be an intermediate product during the biosynthesis of higher unsaturated FA starting from oleic acid (27), has been detected as a minor compound in three seed oils of the family Asteraceae (28), in one of the Euphorbiaceae (28), in one of the Olacaceae (29), and in the oils of certain fungi (27). In contrast, 9-hydroxystearic acid has been found only in isano oil (*Ongokea gore*, Olacaceae), where it also occurs as a minor constituent (10). To decide whether this saturated OH-FA could be of chemotaxonomic importance, more oils of these families have to be investigated. Because the main papers about the Santalales were published from 1960–1970, it would also be interesting to reinvestigate the seed oils with the analytical equipment available today.

ACKNOWLEDGMENTS

The authors thank Dr. V.U. Costa and Mônica Zucolotto (IQ/UFRGS, Brazil) for performing the NMR experiments, H. Fabricius (Institute of Food Science, University of Bonn, Germany) for GC/MS analysis, Sandra Mendonça Silveira for laboratory work and the reviewer for improving language deficiencies. The first author is grateful to the German Academic Exchange Service (DAAD), Bonn, Germany, for the scholarship as visiting professor and the Bundesministerium für wirtschaftliche Zusammenarbeit, Bonn, Germany (GTZ), for financial support (PN 87.2061.7-02.300).

REFERENCES

1. Simões, C.M.O., L.A. Mentz, E.P. Schenkel, B.E. Irgang and J.R. Stehmann, in *Plantas da Medicina Popular no Rio Grande do Sul*, Editora da Universidade Federal do Rio Grande do Sul, 3ª ediç o, 1989, p. 38.
2. Pio Correa, M., in *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas, Ministerio da Agricultura*, Rio de Janeiro, 1969, Vol. VI, p. 134.
3. Hopkins, C.Y., M.J. Chisholm and W.J. Cody, *Phytochemistry 8*:161 (1969).
4. Sobottka, A.M., in Estudo Químico de *Baccharis ochraceae* Spreng. e *Jodina rhombifolia* Hook et Arn. e Efeito de Seus Extratos Aquosos sobre a Reprodução de Ratas, Master of Science Thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, 1992, p. 103.
5. Zhang, J.Y., Q.T. Yu, B.N. Liu and Z.H. Huang, *Biomed. Env. Mass Spectrom. 15*:33 (1988).
6. Pierce, A.E., *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, 1968, pp. 20–25.
7. *DGF-Einheitsmethoden*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1987, CIV 6 (68).
8. Hopkins, C.Y., A.W. Jevans and M.J. Chisholm, *J. Chem. Soc.*:2462 (1968).
9. Spitzer, V. F. Marx, J.G.S. Maia and K. Pfeilsticker, *Fat Sci. Technol. 93*:169 (1991).
10. Miller, R.W., D. Weisleder, R. Kleiman, R.D. Plattner and C.R. Smith, Jr., *Phytochemistry 16*:947 (1977).
11. Powell, R.G., C.R. Smith, Jr., C.A. Class and I.A. Wolff, *J. Org. Chem. 31*:528 (1966).
12. Holman, R.T., and K. Fontell, *J. Lipid Res. 1*:412 (1960).
13. Christie, W.W., in *Gas Chromatography and Lipids*, The Oily Press, Ayr, 1989, p. 149.
14. Tulloch, A.P., and L. Bergter, *Lipids 14*:996 (1979).
15. Purcell, J.M., and H. Susi, *Anal. Chem. 40*:571 (1968).
16. Bus, J., I. Sies and M. S.F. Lie Ken Jie., *Chem. Phys. Lipids 17*:501 (1976).
17. Tulloch, A.P., *Org. Magn. Reson. 11*:109 (1978).
18. Vickery, J.R., F.B. Whitfield, G.L. Ford and B.H. Kenneth, *J. Am. Oil Chem. Soc. 61*:890 (1984).
19. Kleiman, R., M.B. Bohannon, F.D. Gunstone and J.A. Barve, *Lipids 11*:599 (1976).
20. Zhang, J.Y., X.J. Yu, H.Y. Wang, B.N. Liu, Q.T. Yu and Z.H. Huang, *J. Am. Oil Chem. Soc. 66*:256 (1989).
21. Mass Spectroscopic Library (based on the NBS/NIST) from the Nermag Automass, Paris, No. 39200; CAS *16714-85-5.
22. Bohlmann, F., D. Schumann, H. Bethke and C. Zdero, *Chem. Ber. 100*:3706 (1967).
23. Hallgren, B., R. Ryhage and E. Stenhagen, *Acta Chem. Scand. 13*:845 (1959).
24. Kohn, G., A. Vierengel, O. Vandekerkhove and E. Hartmann, *Phytochemistry 26*:2101 (1987).
25. Kleiman, R., and G.F. Spencer, *J. Am. Oil Chem. Soc. 50*:31 (1973).
26. Hegnauer, R., in *Chemotaxonomie der Pflanzen*, Vol. VI, Birkhäuser Verlag, Basel, 1973, pp. 261–271.
27. Pohl, P., and H. Wagner, *Fette Seifen Anstrichm. 74*:541 (1972).
28. Badami, R.C., and K.B. Patil, *Prog. Lipid Res. 19*:119 (1981).
29. Morris, L.J., *J. Chem. Soc.*:5779 (1963).

[Received June 1, 1994; accepted September 14, 1994]