GC-MS Characterization (Chemical Ionization and Electron Impact Modes) of the Methyl Esters and Oxazoline Derivatives of Cyclopropenoid Fatty Acids[1]

Volker Spitzer
Lehrstuhl für Lebensmittelwissenschaft und Lebensmittelchemie, Rheinische Friedrich-Wilhelms-Universität, D-5300 Bonn 1, Germany

The CI-(CH_4) mass spectra of the methyl esters and the EI mass spectra of the oxazoline derivatives of three cyclopropenoid fatty acids (malvalic, sterculic and α-hydroxy-sterculic acid) from the seed oil of *Pachira aquatica* were found to be useful for structure elucidation of such compounds. Furthermore, some hitherto unknown minor fatty acids were identified and the nuclear magnetic resonance data of the oil and α-hydroxy-sterculic acid methyl ester are presented.

KEY WORDS: Bombacaceae, chemical ionization mass spectrometry, cyclopropenoid fatty acids, NMR spectroscopy, oxazoline derivatives, *Pachira aquatica*.

Cyclopropenoid fatty acids (CPeFA) are found in seed lipids of many plant families, *e.g.*, in Sterculiaceae, Malvaceae, Bombacaceae, Sapindaceae and Tiliaceae, and they may be accompanied by small amounts of their saturated cyclopropane analogs (CPaFA). The natural occurrence of these fatty acids has been reviewed by Badami and Patil (1). Until now, only four natural CPeFA have been characterized in higher plants—malvalic (8,9-methylene-heptadec-8-enoic acid), sterculic (9,10-methyleneoctadec-9-enoic acid), D-2-hydroxy-sterculic and sterculynic acid (8,9-methyleneoctadeca-8-en-17-ynoic acid) (2). These fatty acids produce numerous physiological effects, *e.g.*, disturbance of the lipid metabolism (3–5), carcinogenic and co-carcinogenic activities (6–8) in animals. Thus, the identification and quantitation of CPeFA are of considerable interest. Besides the Durbetaki method (9) and the Halphen test (10), other spectroscopic methods, such as infrared (11) and proton nuclear magnetic resonance spectroscopy (12) (^{1}H-NMR), can be used to analyze those compounds.

Mass spectra of the CPeFAME (methyl esters of CPeFA) have been published by Pawlowski *et al.* (13). These spectra are different from the spectra of most other unsaturated esters, but they do not demonstrate the presence nor the location of the cyclopropene ring. Other authors (14,15) used mass spectrometry (MS) to determine the molecular weight of new CPeFAs, and they obtained more structural information by a combination of spectroscopic examinations before and after chemical modification of the original ring. Typical derivatives for ring location by MS are produced by oxidation (ozonolysis or permanganate-periodate) of the CPeFAME to the 1,3-diketo-FAME (16), by addition of methanethiol to the cyclopropene ring (16,17), or by reaction with anhydrous methanol/silver nitrate to the corresponding methoxy- and keto-derivatives (18). Most analysts prefer the latter reaction to prepare stable derivatives prior to quantitative gas chromatographic (GC) analysis, because the CPeFAMEs tend to decompose or rearrange on GC columns (19). Appropriate methods for quantitation of CPeFA by GC or reversed-phase high-performance liquid chromatography (HPLC) are described by Christie (19,20).

This study describes a new method for detection and ring location of CPeFA by GC/MS. The oil of *Pachira aquatica* (Aubl.), Bombacaceae, was chosen for examination because it is known to contain malvalic, sterculic and α-hydroxy-sterculic acid (21–23). Interpretation of the electron impact (EI) mass spectra of the 4,4-dimethyloxazoline derivatives of the CPeFA and chemical ionization (CI, methane) spectra of the CPeFAME will be discussed. Furthermore, hitherto unknown minor fatty acids of *P. aquatica* are identified, the ^{13}C-NMR and ^{1}H-NMR data of D-2-hydroxy-sterculic acid ME are presented, and possible quantitation of cyclopropenoid acids by ^{13}C-NMR is discussed.

MATERIALS AND METHODS

The fruits from *P. aquatica* were collected near Belém, Brazil, in 1991, and the botanical identification was made in the herbarium of the Museu Goeldi, Belém.

Total lipids were extracted from the ground kernels with hexane/isopropanol (2:1, v/v) (24). Transesterification to the FAME was carried out with 0.5N sodium methoxide in anhydrous methanol at room temperature. Preparation of the oxazoline derivatives of the total FA, obtained after hydrolysis of the oil with 1N potassium hydroxide in 95% ethanol at room temperature, was done as described by Zhang *et al.* (25). Isolation of the OH-FAME fraction was carried out on 0.6 mm preparative silica layers with ether/petroleum ether (40–60°C) (30:70; v/v) as solvent system. Acetylation of the OH-FAMES was done as described by Christie (26). Hydrogenation was achieved with H_2/PtO_2 (26), oxidation to the diketo-derivatives was carried out with permanganate-periodate (16), and a part of the FAME was treated with absolute methanol saturated with silver nitrate (27).

A Hewlett-Packard 5890 gas chromatograph with a 7673 A autosampler, a flame ionization detector and a split/splitless injector with glass insert (Hewlett-Packard, Norwalk, CT) was used to analyze the methyl esters and oxazoline derivatives of the fatty acids. Separation of the compounds was achieved with a DB 23 (J&W Scientific, Folsum, CA) capillary column (30 m × 0.25 mm, I.D. 0.2 μm) by using the following temperature program: 130–170°C, 1.5°C/min, then 170–245°C, 2°C/min (FAME), and 150–245°C, 2°C/min (Oxazolines). ECL (Equivalent Chain Length) values were measured at 160 and 190°C. GC/MS analysis was done with the Nermag Automass (France, Paris). EI and CI spectra were obtained with 70 eV ionization energy and with methane as reactant gas, respectively.

[1]This work is part of the author's doctoral thesis.

*Address correspondence at Lehrstuhl für Lebensmittelwissenschaft und Lebensmittelchemie, Rheinische Friedrich-Wilhelms-Universität, Endenicher Allee 11-13, D-5300 Bonn 1, Germany.

^{1}H-NMR and ^{13}C-NMR-spectra (proton noise decoupling) were run in $CDCl_3$ on a Bruker AC 400 spectrometer (Bruker, Karlsruhe, Germany) with tetramethylsilyl (TMS) as internal standard.

RESULTS AND DISCUSSION

Identification of the fatty acids of P. aquatica. In order to identify the FAME GC peaks observed on the DB23 column (Table 1), various derivatization procedures were used.

Identification of fatty acids without unusual functional groups. The saturated FA were identified as their FAME by GC/MS against authentic standards. The unsaturated FA were identified by GC/MS of their oxazoline derivatives, which enables the location of the double bond(s) (25). In addition to FA identified by other authors (21–23,28), the following minor compounds were identified: 15:0, 16:1(n-7), 17:1(n-9), 17:2(n-6,9), 18:1(n-7), 20:1(n-9), 21:0, 22:0, 23:0, 24:0 and 25.0 (see Table 1).

Identification of dihydrosterculic acid. This acid (ECL 19.12) was identified by comparison of the mass spectrum of its oxazoline derivative with that of the oxazoline derivative of the synthetic dihydrosterculic acid published by Zhang *et al.* (29), who demonstrated previously that these derivatives are useful for the determination of the cyclopropane ring positions in long-chain CPaFA.

TABLE 1

Composition of the Methyl Esters and Oxazoline Derivatives of the Fatty Acids of *Pachira aquatica*

Fatty acid	Weight % methyl ester (ECL)	Weight % oxazoline (mol. wt.)
12:0	<0.1	<0.1
14:0	0.25	0.22
15:0	<0.1	<0.1
16:0	63.3	61.03
16:1(n-7)	0.23 (16.31)	0.26
17:0	0.14	0.18
17:1(n-9)	0.12 (17.21)	0.12
17:2(n-6,9)	<0.1 (17.61)	<0.1
iso-16:0?	0.18 (17.77)	1.29 (309)
Malvalic acid	1.63 (17.91)	1.55
18:0	2.22	2.43
18:1(n-9)	8.21 (18.20)	8.57
18:1(n-7)	0.74 (18.25)	0.84
18:2(n-6,9)	6.21 (18.62	6.52
Sterculic acid	8.16 (18.91)	8.03
DH-sterculic acid	0.64 (19.12)	0.87
18:3(n-3,6,9)	<0.1 (19.18)	<0.1
20:0	0.12	0.13
20:1(n-9)	<0.1 (20.21)	<0.1
?	0.33 (20.68)	0.78 (333)
21:0	<0.1	<0.1
18:1-2-OH	0.1 (21.96)	<0.1
22:0	<0.1	<0.1
18:2-2-OH	<0.1 (22.59)	<0.1
α-OH-sterculic acid	6.5 (22.69)	6.08
23:0	<0.1	<0.1
?	0.41 (23.64)	0.56 (363)
24:0	<0.1	<0.1
25:0	<0.1	<0.1

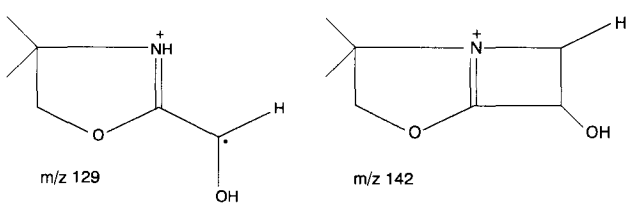
Identification of the fatty acids with a cyclopropene ring. The mass spectra of the compounds with ECL values of 17.91 and 18.91 were identical with those published (13) for methyl malvalate and methyl sterculate. After reaction with $AgNO_3$/MeOH, these FAME disappeared in GC, and the mass spectra of the resulting oxidation products were identical with those for the methoxy and keto derivatives of methyl malvalate and methyl sterculate published by Eisele *et al.* (18).

Identification of the fatty acids with an α-hydroxy-group. The FAME were separated into two bands and isolated as fraction 1 with R_f = 0.79 (non-oxo FAME) and fraction 2 with R_f = 0.34 (R_f identical with that of authentic methyl ricinoleate) by preparative thin-layer chromatography (TLC) GC analysis of fraction 2 showed two small peaks (**OH1, OH2**) and one main peak (**OH3**) with high ECL values (21.96, 22.59, 22.69), and their ECL values increased after acetylation. These data (R_f and ECL) suggest that these compounds each must contain a hydroxy group. In order to get more structural information, compounds **OH1, OH2** and **OH3,** subsequently identified as α-OH-oleic acid, α-OH-linoleic acid and α-OH-sterculic acid, were examined by GC/MS.

The mass spectra of these FAME showed molecular ions at *m/z* 312 (**OH1**), *m/z* 310 (**OH2**) and *m/z* 324 (**OH3**), and the ions at M-18 (loss of H_2O) could be observed in the spectra of **OH1** and **OH2.** The peaks at *m/z* 90 and *m/z* 103, indicative of an α-hydroxy group (30), were found in all OH-FAME spectra. Reaction of the OH-FAME with $AgNO_3$/MeOH and $KMnO_4/KIO_4$, followed by GC/MS, showed that only **OH3** was converted to the corresponding methoxy (molecular ion *m/z* 356), ketone (molecular ion *m/z* 340) and 9,11-diketo (molecular ion 356) derivatives, respectively. The MS fragmentation patterns of the $AgNO_3$/MeOH reaction products were analogous to those published by Eisele *et al.* (18) for methyl sterculate, and the diketo derivative showed the same diagnostic peaks as found for the oxidation product of α-OH-sterculic acid ME (16). Thus, it was concluded that **OH3** was α-OH-sterculic acid, confirming data reported by others (21–23).

GC/MS of the H_2/PtO_2 reduction products showed four new peaks: The mass spectrum of the first (MW 314) was identical with that published for 2-OH-octadecanoic acid ME (30), obviously formed by addition of two and four hydrogens to **OH1** (MW 312) and **OH2** (MW 310), respectively. The three other peaks (MW 328, 328 and 326) must be derived from reduction of α-OH-sterculic acid and will not be discussed here.

The mass spectra of the oxazoline derivatives of **OH1** and **OH2** (Fig. 1) and their corresponding reduction product, **OH1,2R,** showed *m/z* 142 as base peak and *m/z* 129 as the second most intense peak in their spectra. These ions:



SCHEME 1

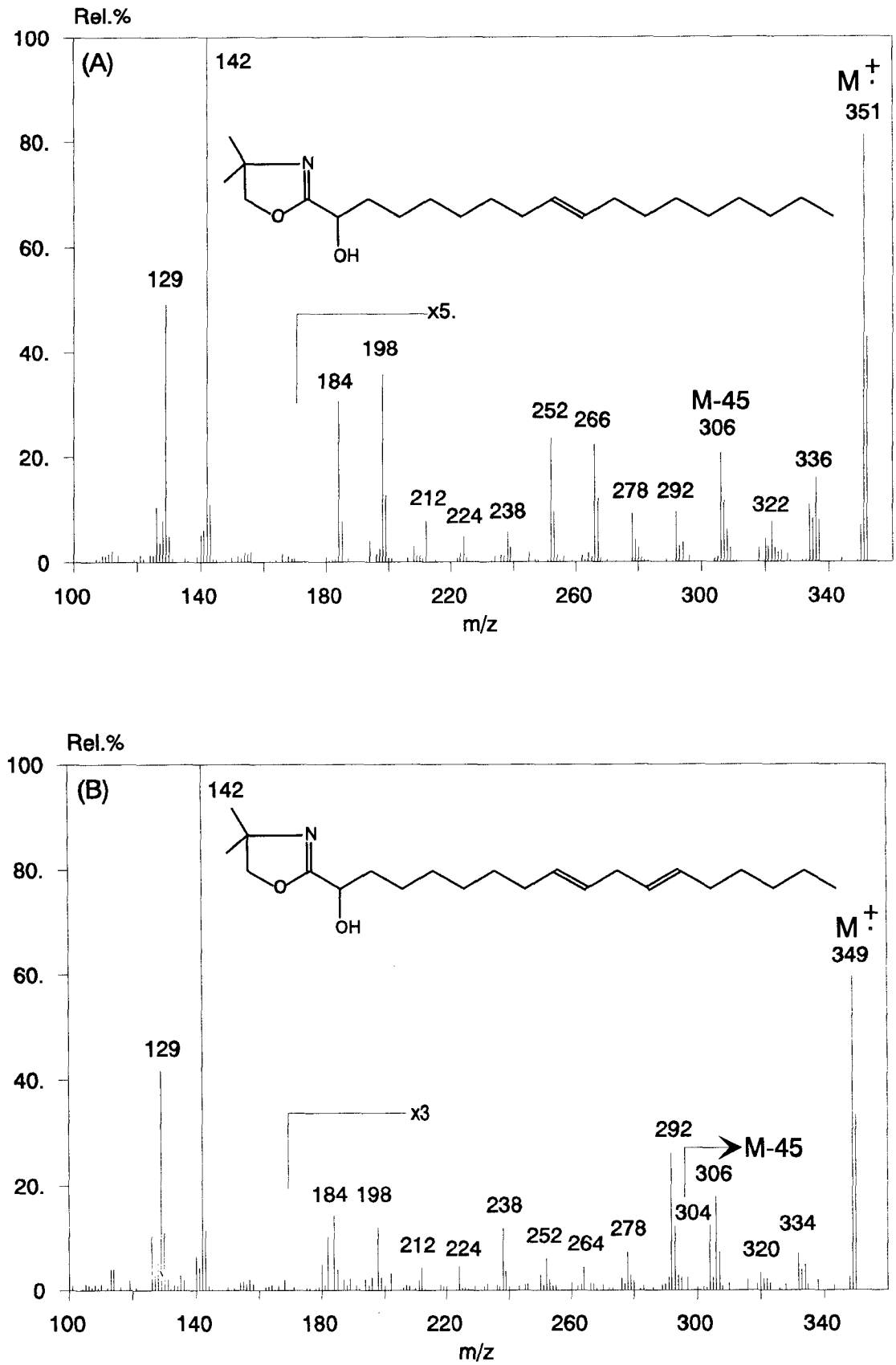


FIG. 1. Mass spectra of the oxazoline derivatives of (A) α-OH-oleic acid, and (B) α-OH-linoleic acid.

strongly support position 2 for the OH-group on the FA chain, and are formed by cyclization-displacement reaction and McLafferty rearrangment (25), respectively. The fragmentation pattern of the oxazoline derivative of **OH1,2R** was identical with that published for α-OH-stearate (31). Zhang *et al.* (31) demonstrated previously that the latter spectrum is strongly influenced by preferential loss of $C_2H_5O^{\cdot}$ (M-45) *via* an α-OH assisted extrusion mechanism and that the fragment ion abundances are influenced by the 2-position of the OH-group. Moreover, the homologous ion series (*m/z* 142 + 14n), deriving from simple cleavage of the carbon chain, is not so discernible in α-OH-derivatives as in, *e.g.*, 12-OH-18:1(9c).

In the spectrum of **OH1** (α-OH-Oleic acid) (Fig. 1A) a mass interval of 12 instead of the usual 14 u for saturated chains occurred between *m/z* 212 and 224, indicating a double bond between C9 and C10 (25). This was supported by the intense allylic cleavage products at *m/z* 198 and 252. In the higher mass range, the spectrum was obviously influenced by the loss of $C_2H_5O^{\cdot}$ (M-45 = *m/z* 306), and the fragmentation signals, clearly defined in unsaturated chains with OH groups more remote from the carboxyl terminus (31), showed irregular mass intervals of 12 u (*m/z* 266 and 278) and 16 u (*m/z* 306 and 322).

In contrast to that, the spectrum of the oxazoline derivative of **OH2** (α-OH-linoleic acid) (Fig. 1B) was unmistakable. The signal due to the loss of $C_2H_5O^{\cdot}$ (M-45 = *m/z* 304) was strongly suppressed. Mass separations of 12 u occurred between *m/z* 212 and 224 and between 252 and 264, due to two double bonds at position C9 and C12 in the chain. This was supported by the intense allylic cleavage ions *m/z* 196, 238 and 292, corresponding to those observed for linoleic acid (25). Thus, it can be concluded that the loss of $C_2H_5O^{\cdot}$ (M-45) in α-OH-derivatives decreases with the degree of unsaturation of the hydrocarbon chain.

CI mass spectra of the cyclopropenoid compounds. A CI mass spectrum is obtained by reaction of neutral FAME with ions derived from the reactant gas. The methane reactant ions are mainly CH_5^+, $C_2H_5^+$ and $C_3H_5^+$, and these may act as either Brønsted (proton transfer) or Lewis (hydride abstraction) acids (32), forming quasi-molecular ions $[M+H]^+$ and $[M-H]^+$, respectively. Interaction of a reactant ion with the carboxy function leads initially to formation of the protonated ester ($[M+H]^+$), while interaction of the reactant ion with the hydrocarbon chain results preliminarily in H^- abstraction ($[M-H]^+$) (33). Tsang and Harrison (33) demonstrated that the importance of the hydrocarbon chain increases rapidly with the alkyl chainlength and they found $[M-H]^+$ to be the base peak in methyl stearate. In contrast, other authors (34–37) found $[M+H]^+$ as base peak in several long-chain FAME derivatives, and $[M-1]^+$ was found to be less intense.

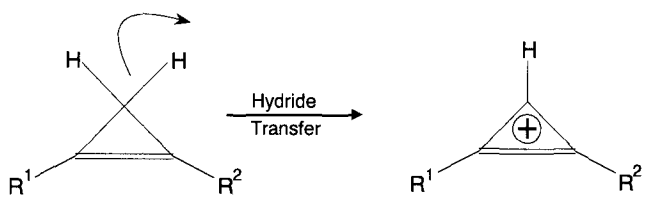
Under the CI measuring condititions, the FAME of *P. aquatica* without a cyclopropenoid ring showed $[M+1]^+$ ions as base peak, and the intensity of the $[M-1]^+$-ions was maximal 45%. In contrast, the CI-(CH_4) mass spectra (see Table 2), obtained from malvalic, sterculic and α-OH-sterculic acid ME, exhibited $[M-1]^+$ (*m/z* 293, 307 and 323) as base peak and a less intense $[M+1]^+$-ion (<15%). The preferential formation of the $(M-1)^+$-ion in the spectra of these CPeFAME can probably be explained by preferred hydride abstraction from the cyclopropenoid ring, according to the Hückel (4n+2) rule, which predicts special electronic stability for the resulting cyclopropenium ions due to their special aromatic character. Other peaks observed in the CI spectra are not discussed here.

TABLE 2

Intensities of the Diagnostic Peaks (Molecular Weight Range) in the Chemical Ionization Mass Spectra of the Cyclopropenoid Acids from *P. aquatica*[a]

Cyclopropenoic acid ME	M-1	M	M+1
Malvalic acid ME	100 (293)	47.1 (294)	13.0 (295)
Sterculic acid ME	100 (307)	49.6 (308)	13.7 (309)
2-OH-sterculic acid ME	100 (323)	48.2 (324)	19.1 (325)

[a]*m/z* In parentheses.



SCHEME 2

In conclusion, the distinct difference in the intensities of the $[M+1]^+$ and $[M-1]^+$ ions in the CI spectra of FAME with and without cyclopropenoid rings could be helpful to detect CPeFAME when ECL values and EI mass spectra are considered, too.

EI mass spectra of the oxazoline derivatives of the cyclopropenoid compounds. Due to the known instability of the cyclopropenoid compounds, the FA composition after formation of the oxazoline derivatives was compared with those of the FAME. As shown in Table 1, the composition was not affected significantly under the reaction conditions, and these derivatives of CPeFA (OXCPeFA) were found to be suitable for GC/MS measurement.

The mass spectra of the OXCPeFA are given in Figure 2. Base peak in the spectra of the OXCCPeFA without a hydroxy group was *m/z* 126, formed by cyclization-displacement reaction (25). Peak *m/z* 113, formed by McLafferty rearrangement (25), was the second most intense ion. The most abundant ions in the α-OH-sterculic acid derivative (OXOHCpeFA), *m/z* 142 and *m/z* 129, were produced by the same mechanism discussed above. Molecular ions were detected in all spectra. The expected even-mass homologous series at *m/z* 126 + 14n for the OXCPeFA, and at 142 + 14n for the OXOHCpeFA, deriving from cleavage of the carbon chain, was interrupted by one mass separation of 10 u. For malvalic acid this 10 u cut was observed between *m/z* 182 (C7) and 192 (C8), for sterculic acid between *m/z* 196 (C8) and 206 (C9), and for α-OH-sterculic acid between *m/z* 212 (C8) and 222 (C9), according to ring location (see Fig. 2). Furthermore, diagnostic abundant ions, explained by beta cleavage to the cyclopropene ring (remote from the oxazoline ring), could be observed in all OXCPeFA spectra (*m/z* 234 for malvalic acid, *m/z* 248 for sterculic acid and *m/z* 264 for α-OH-

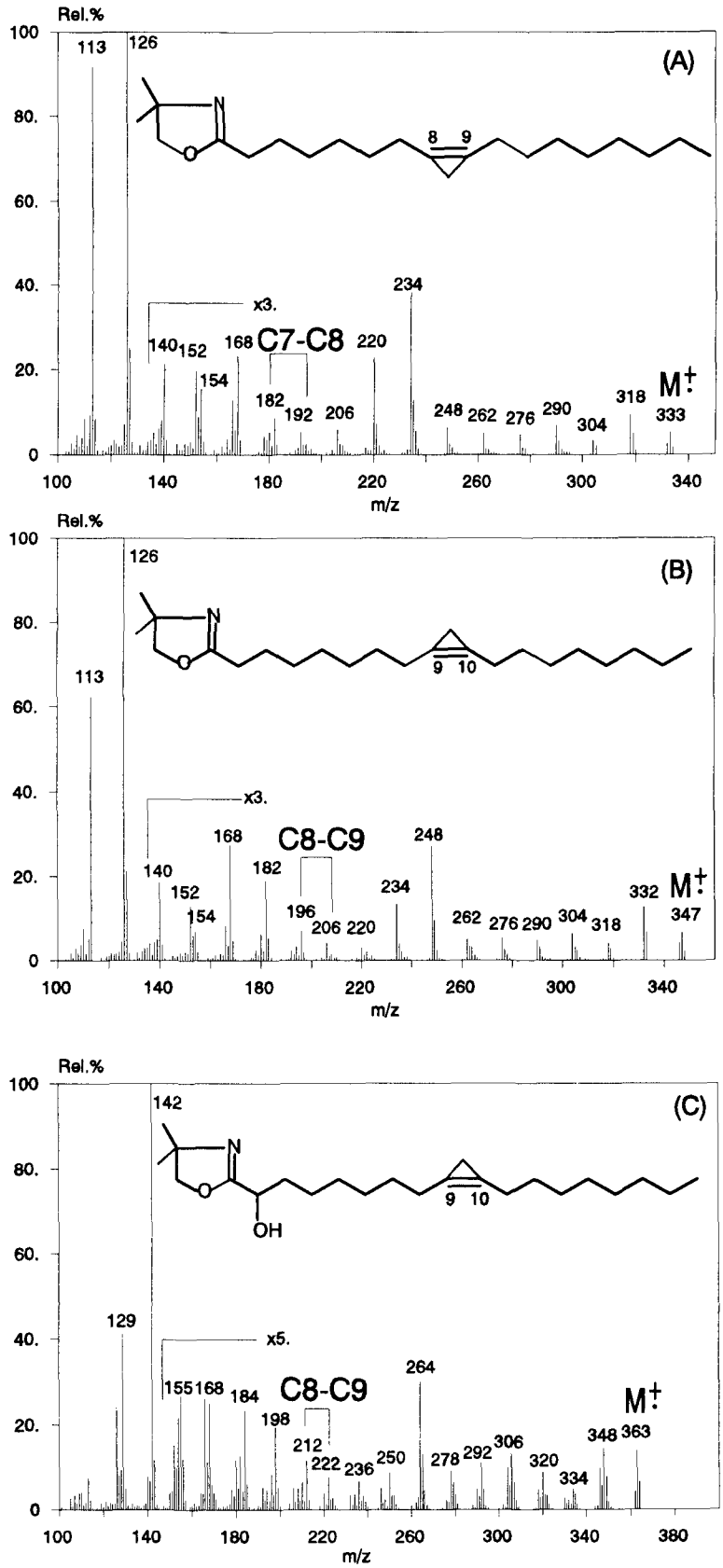


FIG. 2. Mass spectra of the oxazoline derivatives of (A) malvalic, (B) sterculic, and (C) α-OH-sterculic acid.

sterculic acid). This fact enables differentiation between mass spectra of the oxazoline derivatives of acetylenic acids (38), which also show a mass separation of 10 u at their acetylenic linkage, but their intensity pattern of the neighbor ions remains different. Thus, the oxazoline derivatives of CPeFA can be used for ring location and identification of these compounds by the following empirical rule—a mass separation of 10 u between the ions containing n-1 and n carbon atoms and a relatively abundant, higher homologous ion containing n+3 carbon imply the presence of a cyclopropene ring between carbon n and n+1 of the original FA [analogous to the findings from Zhang *et al.* (29) for cyclopropane FA]. In combination with the results from the CI mass spectra of the FAME, this method is very suitable for structure elucidation of CPeFA by GC/MS, as well as for new compounds.

The spectrum of the reduction product of the oxazoline derivative of α-OH-dihydrosterculic acid exhibited an analogous fragmentation pattern as those of α-OH-sterculic acid (Fig. 2C). Thus, the ions *m/z* 129 and 142, derived from the α-OH-group (explained above), were found to be the most intense peaks and, according to the cyclopropane ring, a mass separation of 12 u between *m/z* 212 and 224 and an intense beta cleavage ion *m/z* 266 (65%) were observed.

NMR-spectroscopy. Chemical shifts of the ^{13}C-NMR- and ^{1}H-NMR spectra of α-OH-sterculic acid ME, isolated by TLC, are shown in Table 3. The ^{13}C shifts were assigned by comparison with the data from α-OH-stearate ME (39) and with the data from the synthetic 6,7-positional isomer of sterculic acid (40). The proton shifts were assigned by comparison with the published data of sterculic acid ME (41) and the two characteristic shifts (exo-methylene *H* and -C*H*OH) published by Morris and Hall (16) for α-OH-sterculic acid ME.

TABLE 3

NMR Spectral Data of 2-OH-Sterculic Acid Methyl Ester

Carbon	δ (ppm) ^{13}C-NMR	δ (ppm) ^{1}H-NMR
1	175.93	—
2	70.49	4.18 (1H, m)
3	34.44	1.75 (2H, m)
4	24.77	1.3 (16H, m)
5	29.15-29.47	1.3 (16H, m)
6	27.33*	1.3 (16H, m)
7	29.15-29.47*	1.5 (4H, m)
8	26.02	2.37 (4H, t)
9	109.23	—
10	109.52	—
11	26.1	2.37 (4H, t)
12	29.15-29.47*	1.5 (4H, m)
13	27.45*	1.3 (16H, m)
14	29.15-29.47	1.3 (16H, m)
15	29.15-29.47	1.3 (16H, m)
16	31.94	1.3 (16H, m)
17	22.74	1.3 (16H, m)
18	14.18	0.88 (3H, t)
19 (exo methylene)	7.43	0.75 (2H, s)
OCH_3	52.54	3.78 (3H, s)
OH		2.75 (1H, s)

*Doubtful assignments.

The ^{13}C-NMR spectrum of the total FAME of *P. aquatica* showed some interesting features—the signal for the exo-methylene carbon atoms (7.4 ppm) and the six resolved signals for the two unsaturated carbons in each cyclopropene ring in the range between 109.16 and 109.55 ppm (Fig. 3) showed clearly both the presence and

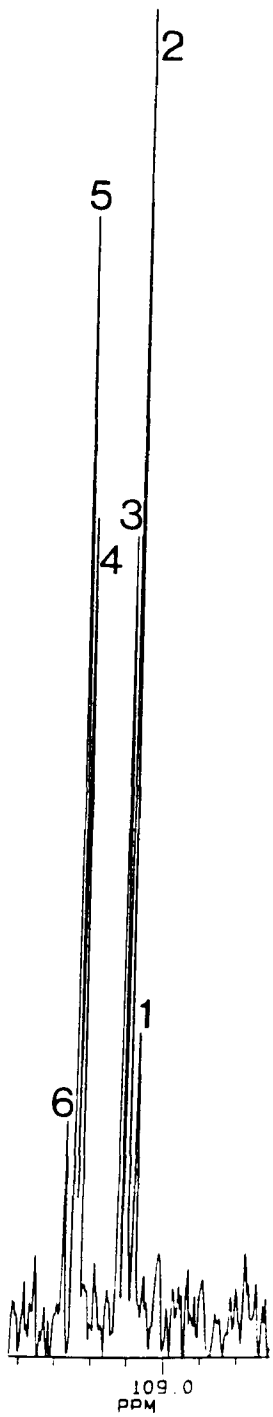


FIG. 3. ^{13}C-NMR spectrum of the mixed FAMES; 109-110 ppm region (ppm scale expansion factor = 23). The signal numbers, chemical shifts (in ppm) and assignments are: 1 (109.16) = C-8 of malvalate; 2 (109.21) = C-9 of 2-OH-sterculate; 3 (109.24); 4 (109.47) = C-9, C-10 of sterculate; 5 (109.5) = C-10 of 2-OH-sterculate; and 6 (109.55) = C-9 of malvalate.

the number (3) of cyclopropenoid compounds. The assignment of the latter signals was done in consideration of the known percentage (GC) of malvalic acid, sterculic acid and α-OH-sterculic acid. To ensure this assignment, isolated α-OH-sterculic acid was added to the mixture (5%) and a peak enhancement was observed for the signals at 109.21 and 109.5 ppm. In addition, the α-OH group could be detected easily in the mixture.

As far as these findings are concerned, it would be interesting to investigate whether quantitative ^{13}C-NMR spectroscopy could be useful for analysis of the composition of natural cyclopropenoid acids in seed oils.

ACKNOWLEDGMENTS

Thanks to Prof. Dr. J.G.S. Maia, Belém (Brazil) for suppling the seeds, and to Mr. C. Schmid for NMR spectroscopy.

REFERENCES

1. Badami, R.C., and K.B. Patil, *Progr. Lipid Res. 19*:119 (1981).
2. Christie, W.W, in *Topics of Lipid Chemistry*, edited by F.D. Gunstone, Logos Press, London 1970, p. 1-47.
3. Raju, P.K., and R. Reiser, *J. Biol. Chem. 242*:379 (1967).
4. Johnson, A.R., A.C. Fogerty, J.A. Peterson, F.S. Shenstone and A.M. Bersten, *Lipids 4*:1 (1969).
5. Pande, S.V., and J.F. Meade, *J. Biol. Chem. 245*:1856 (1970).
6. Lee, D.J., J.H. Wales, L. Ayres and R.O. Sinnhuber, *Cancer Res. 28*:2312 (1968).
7. Struthers, B.J., J.H. Waba, D.J. Lee and R.O. Sinnhuber, *Exp. Mol. Pathol. 23*:164 (1975).
8. Scarpelli, D.G., *Science 185*:958 (1974).
9. Durbetaki, A.J., *Anal. Chem. 28*, 2000 (1956).
10. Halphen, G.J., *Pharmacol. 6*:390 (1897).
11. Bailey, A.V., G.T. Boudreaux and E.L. Shau, *J. Am. Oil Chem. Soc. 42*:637 (1965).
12. Pawlowski, N.E., J.E. Nixon and R.O. Sinnhuber, *Ibid. 49*:387 (1972).
13. Pawlowski, N.E., Eisele, T.A., D.J. Lee, J.E. Nixon and R.O. Sinnhuber, *Chem. Phys. Lipids 13*:164 (1974).
14. Morris, L.J., and S.W. Hall, *Chem. Ind. (London)*, 32 (1967).
15. Jevans, A.W., and C.Y. Hopkins, *Tetrahedron Lett.*, 2167 (1968).
16. Hooper, N.K., and J.H. Law, *J. Lipid. Res. 9*:270 (1968).
17. Raju, P.K., and R. Reiser, *Lipids 1*:10 (1966).
18. Eisele, T.A., L.M. Libbey, N.E. Pawlowski, J.E. Nixon and R.O. Sinnhuber, *Chem. Phys. Lipids 12*:316 (1974).
19. Christie, W.W., in *Gas Chromatography and Lipids*, The Oily Press, Ayr, Scotland, 1989, p. 117.
20. Christie, W.W., in *High-Performance Liquid Chromatography and Lipids*, Pergamon Press, Oxford 1987, p. 161.
21. Recourt, J.H., G. Jurriens and M. Schmitz, *J. Chromatog. 30*:35 (1967).
22. Bohannon, M.B., and R. Kleiman, *Lipids 13*:270 (1978).
23. Cattaneo, P., E.G. Gros, M.S. Vigo and J.R. Avanza, *An. Asoc. Quim. Argent. 73*:225 (1985).
24. Hara, A. and N.S. Radin, *Anal. Biochemistry 90*:420 (1976).
25. Zhang, J.Y., Q.T. Yu, B.N. Liu and Z.H. Huang, *Biomed. Environ. Mass Spectrom. 15*:33 (1988).
26. Christie, W.W., in *Gas Chromatography and Lipids*, The Oily Press, Ayr, Scotland, 1989, p. 77.
27. Schneider, E.L., S.P. Loke and D.T. Hopkins, *J. Am. Oil Chem. Soc. 45*:585 (1968).
28. Lago, R.C.A., D.A Pereira, F.A.R. Siqueira, R.R. Szpiz and J.P. de Oliveira, *Acta Amazonica 16/17*:369 (1986/1987).
29. Zhang, J.Y., Q.T. Yu and Z.H. Huang, *Shitzuryo Buneski (Japan) 35*:23 (1987).
30. Odham, G., and E. Stenhagen, in *Biochemical Applications of Mass Spectrometry*, edited by G.R. Waller, Wiley-Interscience, New York, 1972, Chapter 8, p. 211.
31. Zhang, J.Y., Q.T. Yu, Y.M. Yang and Z.H. Huang, *Chem. Scr. 28*:357 (1988).
32. Munson, M.S.B., and F.H. Field, *J. Am. Chem. Soc. 88*:4337 (1966).
33. Tsang, C.W., and A.G. Harrison, *J. Chem. Soc. Perkin Trans. II*, 1718 (1975).
34. Arsenhault, G.P., J.J. Dolhun and K. Biemann, *Chem. Commun.*, 1542 (1970).
35. Murata, T., S. Takahashi and T. Takeda, *Anal. Chem. 47*:573 (1975).
36. Suzuki, M., T. Ariga and M. Sekine, *Ibid. 53*:985 (1981).
37. Rezanka, T., *LC-GC INTL 3*:46 (1990).
38. 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).
39. Tulloch, A.P., *Org. Magn. Res. 11*:109 (1978).
40. Pollard, M., in *Analysis of Oils and Fats*, edited by R.J. Hamilton and J.B. Rossel, Elsevier Applied Science, London, 1986, p. 412.
41. Williams, J.L., and D.S. Sgoutas, *Chem. Phys. Lipids 9*:295 (1972).

[Received June 12, 1991; accepted September 24, 1991]