The Mass Spectra of the 4,4-Dimethyloxazoline Derivatives of the Methoxymethyl Olefins of Malvalic and Sterculic Acids

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ABSTRACT: A new approach to the gas chromatography–mass spectrometry analysis of cyclopropenoid fatty acids is described. The method is based on the "on-site"-modification of the cyclopropenoid ring with $AgNO_3$-saturated methanol to the methoxymethyl olefin and a subsequent "remote-site"-derivatization of the carboxyl group to its 4,4-dimethyloxazoline. The mass spectra of the isomeric reaction products obtained from malvalic and sterculic acid were found to be useful for locating the original cyclopropenoid ring in the fatty acid chain.
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KEY WORDS: Cyclopropenoid fatty acids, 4,4-dimethyloxazoline derivatives, GC–MS, methoxymethyl olefin derivatives.

Cyclopropenoid fatty acids (FAs) occur in seed oils of many plant families (1) and are known to produce numerous physiological effects (2–4) including carcinogenic and co-carcinogenic activities (5–7) in animals. Therefore, the identification of those compounds is important. A number of different analytical techniques have been published in recent years (8–14). One of the most popular methods involves gas chromatography (GC) of the fatty acid methyl esters (FAME) of the methoxymethyl olefins and α,β-unsaturated keto-derivatives that are formed by reaction of the cyclopropenoid ring with $AgNO_3$-saturated methanol (11). These compounds produce more complex spectra than the unmodified cyclopropenoid FAME and can be carefully interpreted in terms of the position of the original ring (15). While searching for new possibilities for the characterization of cyclopropenoid FA, we examined the 4,4-dimethyloxazoline derivatives of the methoxymethyl olefins of malvalic acid (8,9-methylene-heptadeca-8-enoic acid) (MA) and sterculic acid (9,10-methylene-octadeca-9-enoic acid) (SA) by GC–mass spectrometry (MS) analysis.

MATERIALS AND METHODS

For the present study, the FA mixture of the seed oil of *Pachira aquatica* was used as the source for MA and SA. Extraction, hydrolysis, and separation of the free FA of the oil and the identification procedures of the FA mixture have been described previously (13).

To obtain the methoxymethyl olefins of MA and SA, 10 mg of the FA mixture was treated at 40°C for two hours with 5 mL of absolute methanol saturated with silver nitrate. The reaction mixture was diluted with 5 mL of water and extracted twice with 5 mL hexane (16). The organic layer was dried over Na_2SO_4 and evaporated under vacuum at 30°C. The products obtained were then converted to 4,4-dimethyloxazoline derivatives as described by Zhang *et al.* (17). For comparison purposes, a part of the unmodified FA mixture was converted to 4,4-dimethyloxazoline derivatives.

Separation of the oxazolines was achieved on a DB 23 (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25 mm i.d., 0.2 μm) using a temperature program (150–245°C, 2°C/min). GC–MS analysis was done with the NERMAG AUTOMASS 120 (Paris, France), and the mass spectra were obtained with 70 eV ionization energy.

RESULTS AND DISCUSSION

Comparative GC–MS analysis of the oxazoline derivatives of the unmodified FA mixture and of the FA mixture reacted with the $AgNO_3$-saturated methanol solution showed that the peaks of the oxazoline derivative of MA and SA present in the first mixture disappeared completely in the chromatogram of the second mixture. Instead of those peaks, two poorly resolved pairs of new peaks were observed in the chromatogram (Fig. 1). GC–MS analysis demonstrated that these compounds represent the isomeric methoxymethyl olefins of MA (the CH_2OCH_3-group can be at C-8 or C-9) and SA (the CH_2OCH_3-group can be at C-9 or C-10). As expected (8), the peaks for the α,β-keto compounds appeared in low abundance and were not considered further.

The mass spectra of each of the isomeric methoxymethyl olefins of MA and SA (Fig. 1) showed principally the same fragments, and only slight differences in the ion intensities were found. Thus, it was not possible to determine the position of the side-chain by MS. Due to the similarity of the

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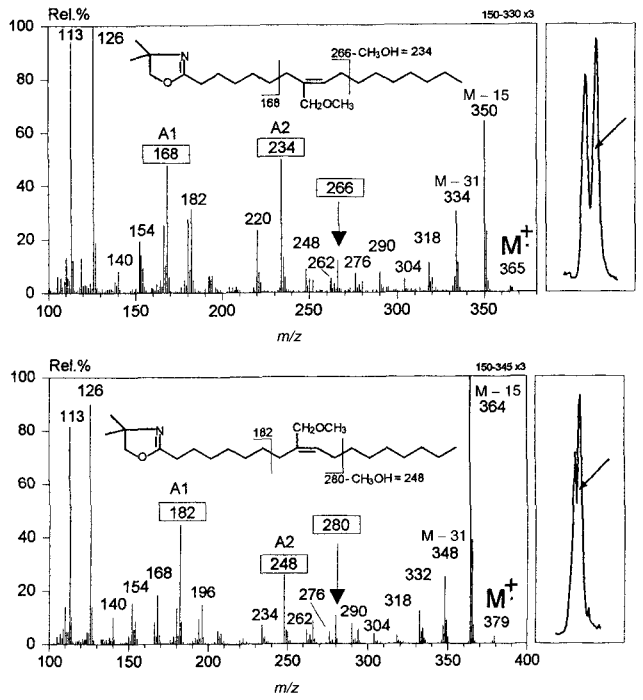

FIG. 1. Mass spectra (70 eV) of the 4,4-dimethyloxazolines of the methoxymethyl olefin derivatives of malvalic acid and sterculic acid with their corresponding total ion curve (TIC) peaks (spectra were taken from the TIC peaks marked with an arrow). The position of the CH_2OCH_3-groups are not proved by the spectra and can be reverse.

spectra of the isomers, only one of each isomer will be described here.

In the low mass range appear prominent fragment ions at *m/z* 113 and 126, which are produced by McLafferty rearrangement and cyclization–displacement reactions, respectively (17). The even-mass homologous series 126 + 14n, deriving from cleavage at each methylene group, show local maxima at *m/z* 168 (A1) (MA) and *m/z* 182 (A1) (SA), that are probably due to a preferred cut at the allylic position of the double bond. These 14n-series are then interrupted at the position of the double bond [at *m/z* 182 (MA) and at *m/z* 196 (SA)] and are substituted by a new regular series at *m/z* 220 + 14n (MA) and 234 + 14n (SA). The latter series could be interpreted by cleavage at each methylene group at the distal side of the double bond with subsequent elimination of methanol. Due to the theoretically preferred allylic cleavage, the ions at *m/z* 234 (A2) (266-32) (MA) and at *m/z* 248 (280-32) (A2) (SA) are found to be the most intense fragments in this part of the spectra. The corresponding fragments produced by the cleavage at the distal side of the double bond without the loss of methanol [*m/z* 252/266 (MA) and at *m/z* 266/280 (SA)] are also present. In the high mass range, all spectra show weak molecular ions [*m/z* 365 (MA derivative) and 379 (SA derivative)], which are accompanied by intense $[M - 15]^+$ fragments. In the spectra of the isomeric reaction products of SA, this fragment is found to be the base peak. It is probable that this ion is mainly caused by the loss of a CH_3-radical from the methoxy group of the side chain. In the spectra of the oxazoline derivatives of usual FA (17) or methyl branched FA (18), the $[M - CH_3]^+$ fragment is less intensive. A further abundant signal in the high mass range is observed at $[M - 31]^+$, which can be attributed to the loss of the methoxy group.

In comparison with the mass spectra of the FAME derivatives of the methoxymethyl olefins of MA and SA, the oxazoline derivatives are simpler and easy to interpret. The position of the original cyclopropenoid ring is clearly indicated by the intense allylic cleavage product peaks A1 and A2 (A2 = A1 + 66 mu). Moreover, the spectra are easy to distinguish from the spectra of usual oxazoline derivatives of unsaturated FA, which do not show a $[M - 31]^+$ peak or such an intensive $[M - 15]^+$ signal.

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REFERENCES

1. Badami, R.C., and K.B. Patil, *Progr. Lipid Res. 19*:119 (1981).
2. Raju, P.K., and R. Reiser, *J. Biol. Chem. 242*:379 (1967).
3. Johnson, A.R., A.C. Fogerty, J.A. Peterson, F.S. Shenstone and A.M. Bersten, *Lipids 4*:1 (1969).
4. Pande, S.V., and J.F. Meade, *J. Biol. Chem. 245*:1856 (1970).
5. Lee., D.J., J.H. Wales, L. Ayres and R.O. Sinnhuber, *Cancer Res. 28*:2312 (1968).
6. Struthers, B.J., J.H. Waba, D.J. Lee, and R.O. Sinnhuber, *Exp. Mol. Pathol. 23*:164 (1975).
7. Scarpelli, D.G., *Science 185*:958 (1974).
8. Christie, W.W., in *Topics in Lipid Chemistry*, edited by F.D. Gunstone, Logos Press London, 1970, Volume 1, pp. 1–49.
9. Pawlowski, N.E., J.E. Nixon and R.O. Sinnhuber, *Ibid. 49*:387 (1972).
10. Pawlowski, N.E., T.A. Eisele, D.J. Lee, J.E. Nixon and R.O. Sinnhuber, *Chem. Phys. Lipids 13*:164 (1974).
11. Eisele, T.A., L.M. Libbey, N.E. Pawlowski, J.E. Nixon and R.O. Sinnhuber, *Ibid. 12*:316 (1974).
12. Christie, W.W., in *High-Performance Liquid Chromatography and Lipids*, Pergamon Press, Oxford, 1987, p. 161.
13. Spitzer, V., *J. Am. Oil Chem. Soc. 68*:963 (1991).
14. Spitzer, V., F. Marx and K. Pfeilsticker, *Fat Sci. Technol. 96*:335 (1994).
15. Christie, W.W., in *Gas Chromatography and Lipids*, The Oily Press, Ayr, 1989, p. 178.
16. Schneider, E.L., S.P. Loke, and D.T. Hopkins, *J. Am. Oil Chem. Soc. 45*:585 (1968).
17. Zhang, J.Y., Q.T. Yu, B.N. Liu and Z.H. Huang, *Biomed. Environ. Mass Spectrom. 15*:33 (1988).
18. Yu, Q.T., B.N. Liu, J.Y. Zhang and Z.H. Huang, *Lipids 23*:804 (1988).

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