Gas-Liquid Chromatography of Hydroxy Fatty Esters: Comparison of Trifluoroacetyl and Trimethylsilyl Derivatives[1]

BERNARD FREEDMAN,
Western Regional Research Laboratory,[2] Albany, California

Abstract

Transformations of hydroxy to keto fatty esters have been monitored successfully by converting the hydroxy compounds to their trifluoroacetyl (TFA) or trimethylsilyl (TMS) derivatives followed by gas-liquid chromatography (GLC). The TFA derivatives have shorter retention times, are better separated from the keto esters, and permit more complete resolution of saturated and unsaturated hydroxy fatty esters than the TMS derivatives. Improved methods for derivative preparation which are rapid and convenient are described. Methyl dimorphecolate (methyl 9-hydroxy-*trans,trans*,10,12-octadecadienoate), which is thermally unstable and cannot be chromatographed as the trifluoroacetate or free hydroxy compound, was chromatographed satisfactorily as the trimethylsilyl ether.

Introduction

THE PURPOSE of this investigation was to develop a GLC method for monitoring conversions of hydroxy fatty esters to the corresponding keto fatty esters. Hydroxy fatty esters are difficult to analyze by GLC because they have long retention times, they tail, they interact with polar substrates, and some are thermally unstable. In addition, the separation of saturated and unsaturated hydroxy fatty esters, e.g. methyl 12-hydroxystearate and methyl ricinoleate, is generally unsatisfactory on packed columns.

A number of investigators have tried to improve the GLC analysis of hydroxy fatty esters by use of derivatives such as acetates (1–4), methyl ethers (5), trimethylsilyl ethers (6,7), and trifluoroacetates (8,9). TFA and TMS derivatives appear to be more suitable for this purpose than acetates or ethers because they have shorter retention times and are more conveniently and rapidly prepared (6,9,10). However, a direct comparison of TFA and TMS derivatives has not been made. In this paper these derivatives are compared with regard to their retention times on various packed columns, how their method of preparation influences quantitation, their usefulness in separating methyl 12-hydroxystearate from methyl ricinoleate, and their suitability for monitoring conversions of hydroxy to keto fatty esters.

Experimental

GLC Apparatus

A laboratory-constructed, programmable instrument equipped with dual columns and a 4-filament thermal conductivity detector was used in conjunction with a 1-mv recorder. All analyses were conducted isothermally using on-column injection. Columns and operating conditions are described in "Results and Discussion."

Thin-Layer Chromatography (TLC)

Silica Gel G chromatostrips were used as previously reported (17). The developing solvent was 70% Skellysolve F—30% diethyl ether (v/v). Spots were detected after spraying with a 25% H_2SO_4 solution and charring.

Materials

The preparation of the methyl esters of ricinoleic (11), 12-hydroxystearic (12), 12-ketostearic (13), dimorphecolic (14), 9-hydroxystearic (15) and 9-ketostearic (15) acids has been described. Except for methyl dimorphecolate, these esters contained no impurities detected by GLC. Ultraviolet spectral analysis indicated a methyl dimorphecolate content of 93% (15) in the sample used. Methyl stearate was purchased from the Hormel Institute; trimethylchlorosilane and hexamethyldisilazane from Perco Supplies, San Gabriel, California. Trifluoroacetic anhydride was obtained from Eastman Organic Chemicals. Reagent-grade pyridine was dried over barium oxide before use.

Derivative Preparation

TFA Derivatives. To 5-10 mg of hydroxy ester in a ½-dram vial was added 100 μl chloroform and 50 μl trifluoroacetic anhydride (TFAA). The solution was shaken for 30 sec and allowed to stand for 5 min at room temperature. Trifluororacetic acid, excess TFAA, and solvent were removed by evaporating the solution under a stream of nitrogen. The residue was diluted to a 10% solution with chloroform, prior to GLC analysis.

TMS Derivatives. Two workup procedures were used. In Procedure 1 the unmodified reaction mixture was directly analyzed by GLC. In Procedure 2, excess starting material and ammonium chloride were removed from the reaction mixture prior to GLC analysis. Procedure 1. To a 1-dram vial (polyethylene stopper) containing 5–10 mg of hydroxy ester was added 0.5 ml pyridine, 100 μl hexamethyldisilazane, and 40 μl trimethylchlorosilane. The contents were shaken for 60 sec, and the mixture was analyzed or further purified as follows: Procedure 2. The mixture was filtered through a fine, sintered glass funnel, and volatiles were removed from the filtrate by evaporation at aspirator pressure. After the residue was diluted with chloroform, the solution was analyzed.

Results and Discussion

Derivative Preparation

TFA Derivatives. The procedure described in the experimental section for converting a hydroxy compound to its trifluoroacetate is somewhat simpler than other reported methods (8,9). Excess trifluoroacetic anhydride was removed by evaporation under a stream of nitrogen instead of destroying it with added water or butanol (8). These last two reagents give rise to peaks which can interfere with the analy-

[1] Presented at the AOCS Meeting in Philadelphia, October 1966.
[2] W. Utiliz. Res. Dev. Div., ARS, USDA.

sis. By use of a much lower ratio of TFAA to hydroxy compound than that reported (9), trifluoroacetic acid was easily removed. The residue was redissolved in $CHCl_3$ rather than TFAA (9), because $CHCl_3$ is less toxic, less volatile and more readily available.

Methyl 12-hydroxystearate was converted completely to its trifluoroacetate as shown by GLC and TLC. The reaction of methyl ricinoleate with TFAA, however, was accompanied by some decomposition (TLC). The reaction produced a yellow color which became darker on standing. Peak areas were significantly lower for the methyl ricinoleate derivative than for the methyl 12-hydroxystearate derivative. The difference in peak areas between these two derivatives is discussed later.

TMS Derivatives. Methyl 12-hydroxystearate reacted completely to form the TMS derivative in 1 min as shown by GLC and TLC. When Procedure 1 was applied to methyl ricinoleate, TLC indicated by-products were present. Peak areas (GLC) were considerably lower for the methyl ricinoleate derivative than for the methyl 12-hydroxystearate derivative. Removal of extraneous compounds after TMS ether formation by filtration and evaporation (Procedure 2) is simpler and more convenient than another published method (10) which involves water extraction.

The necessity for removing extraneous compounds after derivative formation was investigated. Some workers (16) stated ammonium chloride did not interfere with GLC analyses. Others (10) found the extraneous compounds undesirable. To determine whether differences in workup (Procedures 1 or 2) would affect peak areas, methyl 12-hydroxystearate and methyl ricinoleate as their TMS ethers were analyzed separately. Methyl stearate was used as an internal standard.

When the TMS ethers of methyl ricinoleate and methyl 12-hydroxystearate were prepared by Procedure 1 (extraneous compounds not removed) peak areas were greater by only 0.6 and 3.0%, respectively, than when these derivatives were prepared by Procedure 2. A Carbowax column (Table I, No. 4) was used for these studies. The method of preparation of the TMS derivative thus affected peak area only slightly.

To determine if the resolution of a mixture of methyl 12-hydroxystearate and methyl ricinoleate, as their TMS ethers, was influenced by the method of preparation, mixtures of the ethers prepared by Procedures 1 and 2 were analyzed. On polar columns (No. 3–5), the derivatives prepared by either procedure were not separated (see Table I). On Apiezon L columns (No. 1 and 2) the mixture prepared by Procedure 2 was somewhat resolved, but the mixture prepared by Procedure 1 failed to emerge. Pyridine and/or ammonium chloride apparently prevent elution of the TMS ethers under these conditions.

Both TFA and TMS derivatives of methyl 12-hydroxystearate and methyl ricinoleate which had stood for several days at room temperature showed no significant decrease in peak area compared to freshly prepared samples.

Separation of Methyl 12-Hydroxystearate, Methyl Ricinoleate, and Methyl 12-Ketostearate

Comparison of Packed Columns. To separtae these three components various packed columns were examined. On polar columns the keto ester was easily separated from the hydroxy esters (Table I, No. 3–5), but the hydroxy esters were not resolved satisfactorily. On the 20% Apiezon L column (No. 1), as shown in Figure 1A and Table I, methyl 12-hydroxystearate was partially separated from the other esters, but these were not separated from each other. In the absence of the keto esters, the hydroxy esters were only partially resolved on this column.

The hydroxy compounds were then converted to their TMS derivatives in the presence of the keto ester. As previously noted, the TMS ethers were not resolved on polar columns. On the 20% Apiezon column, the three components were partially separated (Fig. 1B and Table I). However, the proximity of the keto ester and TMS ether peaks reduced the usefulness of this separation. Even less resolution of these derivatives was observed on the 2% Apiezon column (No. 2).

A mixture of the keto ester and the trifluoroacetates of the hydroxy compounds showed no separation of the TFA derivatives on the polar columns. On the

TABLE I

Retention Times of Some Oxygenated Fatty Etsers on Packed Columns [a]

Column		Temp, C	Helium flow, ml/min	Hydroxy compounds		TMS derivatives of		TFA derivatives of		Methyl 12-keto-stearate
No.	Length and packing			Me Ri[b]	Me 12-HO[c]	Me Ri[b]	Me 12-HO[c]	Me Ri[b]	Me 12-HO[c]	
1	10 ft—20% Apiezon L on 60/80 mesh Gas-Chrom P	250	30	25.5	27.8	20.3	22.1	10.5	11.6	25.5 23.6 22.9
2	10 ft—2% Apiezon L on 70/80 mesh AW-DMCS[d] Chromosorb G	245	30	......	.....	13.5	14.6	7.1	8.3	......
3	8 ft—15% Degs[e] on 70/80 mesh Gas-Chrom P	220	50	6.4	6.6	2.0	2.0	2.1	2.1	5.3 4.9 5.2
4	3 ft—5% Carbowax 20 M on 60/80 mesh HMDS[f] Chromosorb W	210	50	5.0	5.0	1.8	1.8	1.4	1.4	3.6[g]
5	5 ft—10% FFAP[h] on 70/80 mesh AW-DMCS Chromosorb W	220	50	15.8	15.8	4.3	4.3	3.7	3.7	12.1[g]

[a] Columns were made of ⅛ in. O.D. stainless-steel or aluminum. Retention times are in minutes.
[b] Methyl ricinoleate.
[c] Methyl 12-hydroxystearate.
[d] Acid-washed dimethyldichlorosilane.
[e] Diethylene glycol succinate.
[f] Hexamethyldisilazane.
[g] Retention time was not changed significantly by other compounds present during analysis.
[h] FFAP (a trade name) was purchased from Varian Aerograph.

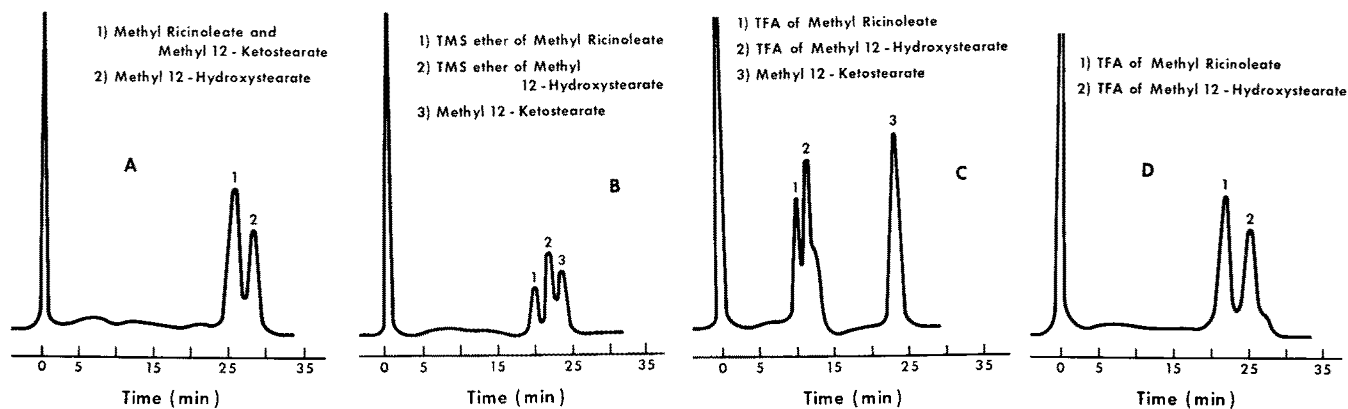

FIG. 1. Separation of methyl ricinoleate, methyl 12-hydroxystearate, methyl 12-ketostearate, and TMS and TFA derivatives. Column: 10 ft ⅛ in. aluminum; 20% Apiezon L on 60/80 mesh Gas-Chrom P; 1A, 1B, 1C, temperature 250C, helium flow 30 ml/min; 1D, temperature 220C, helium flow 50 ml/min.

20% Apiezon L column, both the trifluoroacetates and the keto ester were fairly well resolved (Fig. 1C and Table I). The TFA derivatives were almost completely separated at lower temperature and greater gas flow (Fig. 1D). Methyl ricinoleate and methyl 12-hydroxystearate were thus better separated in the presence of methyl 12-ketostearate as their TFA than as their TMS derivatives.

Thermal Decomposition of TFA Derivatives. Examination of Figures 1C and 1D reveals a shoulder on the peak corersponding to the trifluoroacetate of methyl 12-hydroxystearate (peak 2). This shoulder is more pronounced at 250C (Fig. 1C) than at 220C (Fig. 1D). At 260C the peak area of the shoulder increased markedly. The same trend was observed when the TFA derivatives of methyl 12-hydroxystearate or methyl ricinoleate were analyzed separately under these conditions. Because the peak areas of the shoulders were temperature dependent, the compounds corresponding to these shoulders appeared to be decomposition products formed on the 20% Apiezon column. Such decomposition products did not form on the polar columns at temperatures of 220C or below.

The TMS derivatives, in contrast to the TFA derivatives, showed only a slight tendency to decompose at 250C on the 20% Apiezon column. This difference may result from greater thermal stability of the TMS ether relative to the trifluoroacetate. The decomposition products from the mixture of the TFA derivatives of methyl 12-hydroxystearate and methyl ricinoleate appeared to be C18 monoenes and dienes. Their retention times were similar to those of methyl oleate and methyl linoleate. When analyses were performed as in Figure 1D, decomposition was minimized, and a satisfactory qualitative analysis was possible.

Quantitation. Factors affecting quantitation of the TFA and TMS derivatives on polar columns were examined briefly. Methyl stearate was used as an internal standard. Per cent composition of the derivative and methyl stearate mixture as determined by peak area (triangulation) was compared to the per cent composition determined by weight per cent. The best agreement between observed and calculated values was obtained with a well-conditioned Carbowax column (No. 4).

Separate analyses of the TFA and TMS derivatives of methyl 12-hydroxystearate on this column showed the observed values were 97.3% (average of 5 trials) and 95.3% (average of 2 trials), respectively, of the calculated values. By use of correction factors these results could be made quantitative. In contrast, the observed value for the TMS ether of methyl ricinoleate on this column was only 79.0% of the calculated value. Analysis of the TFA derivative of methyl ricinoleate was also far from quantitative. Differences between the values obtained for the derivatives of methyl 12-hydroxystearate and the derivatives of methyl ricinoleate may result from greater adsorption of the latter compounds on the column, their partial decomposition after formation and during analysis, and differences in detector response. On other polar columns, e.g. FFAP (Varian Aerograph), polyethylene glycol succinate, and a newly prepared Carbowax column, observed values for both the TFA and TMS derivatives of methyl 12-hydroxystearate were significantly lower than those obtained on the well-conditioned Carbowax column. These results suggest that column conditioning and type as well as the structure of the TFA or TMS derivatives influence quantitation.

Analysis of Methyl Dimorphecolate

Methyl dimorphecolate has been shown to dehydrate during GLC analysis to conjugated trienes (4). Acetylation provided no protection (4). GLC analysis of the TFA or TMS derivatives of methyl dimorphecolate was investigated to determine if these derivatives dehydrated less readily during analysis than the parent compound. Chromatography of the trifluoroacetate of methyl dimorphecolate was not successful. Either this derivative did not form, or it decomposed on the column; it did not elute from the column. In view of the tendency of methyl dimorphecolate to be dehydrated by acidic catalysts (18,19), this result was not surprising. The conditions used for this analysis were the same as those described below for the TMS derivative.

Morris and co-workers (4) found that methyl dimorphecolate decomposed not on the GLC columns but in the flash heater. In the present investigation, an attempt was made to eliminate decomposition by injecting the ester directly on column. Extensive dehydration still occurred. As shown in Figure 2A, the broad peak which eluted at 2.6 min was probably a mixture of conjugated trienes. For comparison, Figure 2B shows the chromatogram of the methyl esters of tung oil which are composed mainly of mixed conjugated trienes. Under these conditions methyl dimorphecolate would have a retention time of ca. 5 min. Dehydration of methyl dimorphecolate was as extensive at 200C as at 220C.

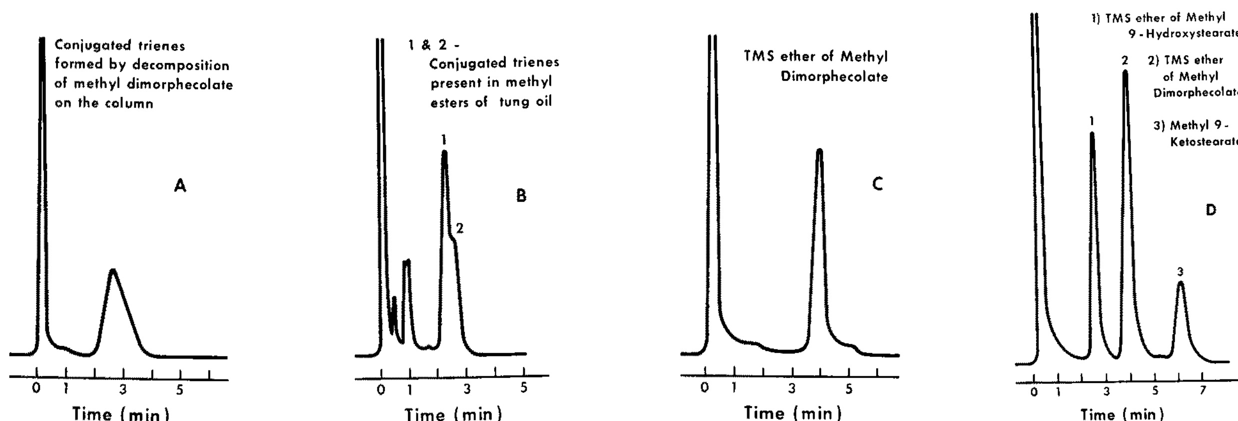

FIG. 2. Analysis of methyl dimorphecolate and derivatives. Column 3 ft ⅛ in. stainless-steel; 5% Carbowax 20 M on 60/80 mesh hexamethyldisilazane-treated Chromosorb W; helium flow 50 ml/min. Temperature: 2A and 2B, 220C; 2C and 2D, 200C.

The TMS ether of methyl dimorphecolate (pyridine, etc., not removed) was successfully chromatographed (Fig. 2C). The presence of only a slight amount of triene (4.5–5.3 min under the conditions of Fig. 2C) shows that little of the TMS derivative decomposed. The same stability was observed at 220C as at 200C. Methyl dimorphecolate and methyl 9-hydroxystearate, as their TMS ethers, were well separated from each other and from methyl 9-ketostearate (Fig. 2D). The use of trimethylsilylation thus provided a convenient method for following the conversion of methyl dimorphecolate to methyl 9-ketostearate.

REFERENCES

1. O'Brien, J. S., and G. Rouser, Anal. Biochem. *7*, 288–296 (1964).
2. Kishimoto, Y., and N. S. Radin, J. Lipid Res. *4*, 130–138 (1963).
3. Tulloch, A. P., JAOCS *41*, 833–836 (1964).
4. Morris, L. J., R. T. Holman and K. Fontell, J. Lipid Res. *1*, 412–420 (1960).
5. Kishimoto, Y., and N. S. Radin, *Ibid. 1*, 72–78 (1959).
6. Wood, R. D., P. K. Raju and R. Reiser, JAOCS *42*, 81–85 (1965).
7. Radin, N. S., *Ibid. 42*, 569–580 (1965).
8. Morrissette, R. A., and W. E. Link, J. Gas Chrom. *3*, 67–68 (1965).
9. Wood, R. D., E. L. Bever and F. Snyder. Presented at AOCS meeting, Los Angeles, April, 1966.
10. Wood, R. D., P. K. Raju and R. Reiser, JAOCS *42*, 161–165 (1965).
11. Swern, D., and E. F. Jordan, Jr., Biochem. Prep. *2*, 104–105 (1952).
12. Freedman, B., and T. H. Applewhite, JAOCS *43*, 125–127 (1966).
13. Freedman, B., and T. H. Applewhite, *Ibid. 43*, 342–344 (1966).
14. Diamond, M. J., R. E. Knowles, R. G. Binder and L. A. Goldblatt, *Ibid. 41*, 430–433 (1964).
15. Freedman, B., R. G. Binder and T. H. Applewhite, *Ibid. 43*, 458–460 (1966).
16. Sweeley, C. C., R. Bentley, M. Makita and W. W. Wells, J. Am. Chem. Soc. *85*, 2497–2507 (1963).
17. Freedman, B., J. S. Nelson, R. G. Binder and T. H. Applewhite, JAOCS *42*, 340–344 (1965).
18. Smith, C. R., Jr., T. L. Wilson, E. H. Melvin and I. A. Wolff, J. Am. Chem. Soc. *82*, 1417–1421 (1960).
19. Hopkins, C. Y., and M. J. Chisholm, Can. J. Chem. *43*, 3160–3164 (1965).

[Received September 19, 1966]