Gas Liquid Chromatography of the Hydroxy-, Acetoxy- and Oxo-Stearic Acid Methyl Esters[1]

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Abstract

Carbon numbers have been determined for all the 17 isomeric methyl hydroxy- and acetoxystearates and for 15 of the 16 isomeric methyl oxostearates using silicone SE-30, silicone QF-1, and ethylene glycol succinate (EGS) as liquid phases. The carbon numbers of the isomers increase with increasing distance of the point of substitution from the carboxyl end of the fatty acid chain (the increases for the 4- and 5-hydroxy esters are unexpectedly large). The change in carbon number from one isomer to the next is greatest when the substituents are attached near either end of the chain. The best separation of isomers with the substituent near the carboxyl end is obtained for oxo esters using the QF-1 column, and that of isomers substituted near the methyl end is obtained for acetoxy esters using the EGS column. Isomers with substituents at carbons 9–13 are not distinguishable on any of the columns used, but the other isomers are partly or completely separated. The quantitative aspects of the separations have not been investigated.

Introduction

DURING THE LAST TEN YEARS there has been an increasing number of reports of the isolation of hydroxy fatty acids from natural sources, particularly from bacteria and fungi. Unsaturated hydroxy acids with 18 carbon atoms occur in plant seed oils, the hydroxyl group of these usually being at positions 8,9,12 or 18. Mixtures of hydroxy acids are also found among the products of autoxidation.

Most of the major physical methods have been used in attempts to identify the isomeric monohydroxy fatty acids of the same chain length. X-ray crystallography has been applied to the hydroxy- and oxostearic acids (2) and the isomers in which the hydroxyl group is near the ends of the chain can be distinguished but those in which it is at positions 5–14 cannot; however, all the oxo acids can be identified by this method. It also has been reported that the oxo esters can be distinguished by IR spectroscopy (10), but this method has not been successful with the hydroxy esters. Mass spectrometry can apparently be used to determine the structure of any one of the methyl esters of the hydroxy- or oxostearic acids (25).

Usually physical methods require a fairly pure sample to identify an isomer and cannot be used to measure the composition of mixtures or be applied directly to natural oils which contain only small amt of oxygenated acids. Thus it appeared that GLC might have some advantages over the other methods. GLC had been used previously with a few hydroxy esters and it was shown that they could be distinguished from non-oxygenated esters by their quite different emergence times on non-polar and polar columns (31, 20,11). Morris et al. (21) appear to have been the first to show that isomeric hydroxystearates could be separated by GLC when they separated 2- and 12-hydroxystearates using a polyester column. Kitagawa et al. (17) also examined a few methyl hydroxy-, acetoxy- and oxostearates using a silicone column. However, it appeared that no systematic investigation of all the isomeric hydroxy- and oxostearates had been made.

In early work, using silicone columns, it was found that the 17- and 18-hydroxy esters were readily separated (32). Later it was observed, during the characterization of a 3-acetoxystearate (33), that while the carbon numbers of 3- and 8-acetoxystearates were fairly close to each other those of 8- and 17-acetoxystearates were much further apart. The investigation was then extended to the other isomers and it was found that the separation of the acetoxystearates improved as more polar columns were used. An ethylene glycol phthalate on C-22 firebrick column (6) was first used, but the work of Craig (7) indicated that ethylene glycol succinate on Chromosorb W was a more polar combination and this column was used in the present investigation. The fluorinated silicone QF-1 was used particularly to achieve a better separation of the oxo esters, since compounds with an oxo function have greater retention times on this column than those with hydroxy or ester functions (34). The silicone XE-60 and the polyamide resin Versamid 900 (22) were also tried as liquid phases but were not found to be as useful.

When this work had been completed, O'Brien and Rouser (23) reported the retention times of all the isomeric methyl hydroxypalmitates using Apiezon and polyester columns and of the methyl acetoxypalmitates using only the polyester column. They found that the retention times differed appreciably from each other if the substituent was near the ends of the chain, but were very similar to each other if it was at positions 6–12.

Materials and Methods

Preparation of Compounds Used. Bergström et al. (2) described the synthesis of all the methyl oxo- and hydroxystearates, but since many of the intermediates used in their syntheses were not readily available, new syntheses were devised for some of the isomers using more common starting materials. 2-Hydroxystearic acid was obtained by the method used for 2-hydroxypalmitic acid (30) and converted to the methyl ester with diazomethane. Methyl 3-D-hydroxystearate (33), (−) methyl 8-hydroxystearte, methyl 12-hydroxystearate, methyl 17-L-hydroxystearate, and methyl 18-hydroxystearate had all been obtained in previous work (32). Methyl 3-oxostearate was prepared as described by Ställberg-Stenhagen (28), and the methyl esters of 2-,8-,12- and 17-oxostearates were prepared by chromic acid oxidation of the corresponding hydroxy esters (2,11).

Diethyl *n*-dodecylmalonate and 3-carbomethoxybutyryl chloride were used to prepare 5-oxostearate, and pentadecanoyl chloride and diethyl 2-ethoxycarbonylsuccinate were used to prepare 4-oxostearate; both syntheses were by the general method of Bowman (3). Hydrogenation over W-6 Raney nickel at 25C and atmospheric pressure (2) yielded methyl 5-hy-

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TABLE I

Carbon Numbers of Methyl Hydroxy-, Acetoxy- and Oxostearates Using Different Liquids Phases

Substituent position	Methyl hydroxystearate			Methyl acetoxystearate			Methyl oxostearate		
	SE-30	QF-1	EGS	SE-30	QF-1	EGS	SE-30	QF-1	EGS
2	19.25	20.10	23.55	20.30	21.95	23.50	18.95	21.00	22.55
3	19.50	20.80	24.45	20.35	22.35	23.95	.....	.....	.....
4	19.85[a]	24.55[b]	27.15[c]	20.40	22.45	24.40	19.40	21.95	24.70
5	20.10	25.10	28.00	20.45	22.60	24.45	19.50	22.40	24.85
6	19.85	21.70	26.15	20.50	22.85	24.50	19.65	22.80	25.20
7	19.90	21.75	26.20	20.50	22.95	24.60	19.65	22.90	25.25
8	19.95	21.75	26.20	20.50	23.00	24.60	19.65	23.00	25.30
9	20.00	21.80	26.20	20.50	23.00	24.65	19.70	23.05	25.30
10	20.00	21.80	26.25	20.50	23.05	24.65	19.70	23.05	25.35
11	20.00	21.80	26.25	20.55	23.05	24.70	19.70	23.10	25.35
12	20.00	21.80	26.25	20.55	23.10	24.70	19.75	23.15	25.40
13	20.05	21.80	26.30	20.60	23.15	24.80	19.80	23.15	25.40
14	20.05	21.80	26.30	20.65	23.20	24.90	19.85	23.15	25.50
15	20.05	21.80	26.35	20.75	23.30	25.10	19.90	23.15	25.60
16	20.10	21.90	26.50	20.95	23.60	25.50	19.95	23.36	25.90
17	20.10	22.10	26.95	21.15	23.90	25.95	20.00	23.75	26.40
18	21.00	23.05	.....	21.90	24.90	27.40	.....	.....	.....

The carbon number of γ-stearolactone was a) 19.80; b) 24.55; c) 27.15.

droxystearate; however, under the same conditions the 4-oxo ester gave γ-stearolactone with mp 49C [lit (15) gives 52–53C]. The NMR spectrum (in carbon tetrachloride), which had no peak at 6.4 ppm relative to tetramethylsilane = 10.00 (13), and the IR spectrum (0.7% in carbon tetrachloride), which had carbonyl absorption at 1783 cm^{-1} characteristic of a 5-membered lactone ring, confirmed that the product was the lactone and not the methyl ester. Hydrolysis of the lactone with aqueous sodium hydroxide followed by acidfication with 2 N hydrochloric acid gave 4-hydroxystearic acid which was converted to methyl 4-hydroxystearate (mp 56.5–57.5C) by treatment with diazomethane. 6-Oxostearic acid was prepared as described by Hünig and Lendle (14) and 7-oxostearic acid as described by Hünig et al. (15).

9-,10-,11-,13-,14-,15- and 16-Oxostearic acids were prepared by the following general method:

$$CH_3-(CH_2)_y-CO-(CH_2)_{4\text{ or }5}-CO_2H + HO_2C-(CH_2)_x-CO_2CH_3 \rightarrow CH_3(CH_2)_y-CO-(CH_2)_{x+4\text{ or }5}-CO_2CH_3$$

The appropriate 6- or 7-oxo monocarboxylic acids were prepared by the methods of Hünig and coworkers (14,15) and anodically coupled with the appropriate dicarboxylic acid half methyl esters using the conditions described by Greaves et al. (12). By using both 6- and 7-oxo acids of the same chain length some half esters could be used for two preparations. When the coupling reaction was complete, the reaction mixture was acidified with acetic acid, methanol added to give an approx 10% solution of product and the insoluble dioxo hydrocarbon by-product was filtered off. In the preparation of the 9-,10-,11- and 16-oxo acids the solvent was removed, the product distilled and the fraction with bp 150–180C/0.15 mm collected. The ester was then hydrolyzed and the oxo acid crystallized from ethanol. In the preparation of the 13-,14- and 15-oxo acids, the crude product was saponified and acidic material isolated, the desired oxo acid was taken up in hexane, filtered from insoluble long chain dicarboxylic acid and crystallized from ethanol. The yields of oxo acid obtained in the coupling reaction were 25–40%.

The oxostearic acids were prepared from the intermediates given below. Where the intermediate 6- or 7-oxo acid has been previously synthesized by a route other than that of Hünig et al. the melting point obtained in the present work is compared with the literature value. 9-Oxostearic acid was prepared from 6-oxopentadecanoic acid [mp 75–76C, lit (16) gives 75.3C] and methyl hydrogen glutarate; 10-oxostearic acid from 6-oxomyristic acid (4) and methyl hydrogen adipate; 11-oxostearic acid from 7-oxomyristic acid (29) and methyl hydrogen adipate; 13-oxostearic acid from 6-oxoundecanoic acid [mp 53–54C, lit (9) gives 53–54C] and methyl hydrogen azelate; 14-oxostearic acid from 7-oxoundecanoic acid [mp 48.5–49.5C, lit (8) gives 51.5–52C] and methyl hydrogen azelate; 15-oxostearic acid from 7-oxodecanoic acid [mp 40.5–41.5C, lit (19) gives 42–43C] and methyl hydrogen sebacate; and 16-oxostearic acid from 6-oxocaprylic acid (14) and ethyl hydrogen dodecanedioate. The methyl oxo esters were prepared by treatment with diazomethane. The methyl esters of 6-,7-,9-,10-,11-,13-,14-,15- and 16-hydroxystearic acids were prepared from the oxo esters by hydrogenation over Raney nickel using the conditions described by Skogh (26). The oxo- and hydroxystearic acids and esters had melting points which were almost the same as those reported by Bergström et al. (2).

Methyl 4- and 5-acetoxystearates were prepared by treating the hydroxy esters with excess acetic anhydride and pyridine (1:1) at zero C for 18 hr and then removing the reagents at 0C and 0.1 mm (27). The acetates of all the other hydroxy esters were prepared by refluxing with acetic anhydride for one hr and then evaporating the excess at 70C and 10 mm.

Gas Liquid Chromatography. The GLC units were of conventional design using thermal conductivity cells for detection. It was found to be important to use solid supports which did not have a basic reaction for preparation of the columns. Chromosorb W, which had not been acid washed, was sufficiently alkaline to cause complete decomposition of all the hydroxystearates and also of 3-acetoxystearate which is very sensitive to alkali (33). The columns used were a 30 in. x ¼ in. copper column packed with silicone SE-30 on 60–80 mesh acid washed celite (1:6 w/w) operated at 220C and a flow rate of 40 ml helium/min; a 6 ft x 3/16 in. copper column packed with silicone QF-1 on 60–70 mesh Anachrom AB (1:6 w/w) operated at 202C and a flow rate of 40 ml helium/min; and a 12 ft x 3/16 in. copper column packed with ethylene glycol succinate on 60–80 mesh acid washed Chromosorb W (1:4 w/w) operated at 224C and a flow rate of 60 ml helium/min. The injectors were maintained at a temp 40C above that of the column. The recorder chart speeds were 1 cm/30 sec for the SE-30 column, 1-in./5 min for the QF-1 column and 1 in./6 min for the polyester column. Since sample size has a considerable effect on the emergence of peak maximum (1,24) the retention times were measured from the start of the air or solvent peak to the point of intersection of the base line with a tangent drawn to the forward slope of the peak. Carbon numbers were determined as described by Woodford and van

Gent (35) using a standard mixture of the methyl esters of normal C_{16}, C_{18}, C_{20} and C_{22} acids.

Results and Discussion

The carbon numbers obtained are shown in Table I. It is considered that the figures accurately represent the carbon number of each isomer relative to its immediate neighbors. However, if the isomers are run randomly at intervals of a week or more, when small changes in operating conditions have occurred, the carbon numbers are only reproducible within ±0.1 of a carbon number for the SE-30 and QF-1 columns. For the polyester column, which has a higher "bleeding" rate, it was found that as the column aged the retention times of the oxygenated esters decreased to a greater extent than did those of the normal esters used for the determination of carbon numbers. Magidman et al. (18) also found that as polyester columns aged the retention times of unsaturated esters changed at a different rate than those of saturated esters. Craig (7) observed a similar effect when he measured the relative emergence times of saturated and unsaturated esters on a number of polyester columns which had a wide variation in the ratio of liquid phase to solid support. The figures which were obtained using the polyester column are not absolute values but refer only to a column of the particular age used in these experiments (2–3 weeks at operating temp). A column which had been in use for six weeks yielded figures which were all 0.3–0.4 of a carbon number lower than those in the table, but relative to each other they had not changed appreciably.

Methyl Hydroxystearates. As might be expected from the ease of formation of intramolecular hydrogen bonds (21,17) the 2- and 3-isomers had much lower carbon numbers than the rest on all three columns. The 4- and 5-isomers had carbon numbers which, in the case of the SE-30 column, were slightly greater than those expected and which in the case of the other two columns were markedly greater than those of any of the other isomers. γ-Stearolactone gave peaks with the same carbon numbers as those given by methyl 4-hydroxystearate. It would not be surprising if the 4- and 5-hydroxy esters were converted to lactones at the temp of the injector but it is still difficult to explain why a lactone with an appreciably lower mol wt would have such high carbon numbers. The carbon numbers of the rest of the isomers increased gradually as the substituent approached the end of the chain. However, only the 2-,3- and 17- or 18-isomers differed from the others by more than 0.4 of a carbon atom. The curve obtained for some hydroxy esters is shown in Figure 1A. The 18-isomer did not give a peak on the poly-ester column perhaps because the primary hydroxy group reacts with the liquid phase particularly readily.

Methyl Acetoxystearates. Using the SE-30 column acetylation was found to have a considerable effect on the carbon numbers of the 2- and 3-isomers since hydrogen bonding was no longer possible. All the isomers now formed a series of continuously increasing carbon number; there was only 0.25 of a carbon number difference between the 2- and 12-acetates but the 13- to 18-isomers showed an increasingly greater difference between each other. The total change in carbon number from the 2- to the 18-isomer was 1.6 units. Similar results were obtained using the QF-1 column except that there was a greater change between isomers, the overall change from the 2- to the 18-isomer was ca. 2.9 units. A still larger change, of 3.9 units, was obtained using the polyester column; Figure 1B shows the separation obtained with some of the isomers.

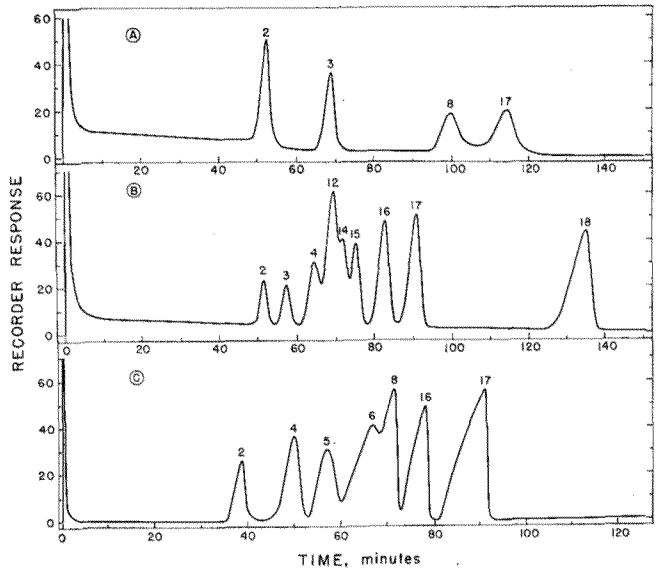

FIG. 1. A) GLC separation of methyl 2-,3-,8- and 17-hydroxystearates using the EGS column. B) GLC separation of methyl 2-,3-,4-,12-,14-,15-,16-,17- and 18-acetoxystearates using the EGS column. C) GLC separation of methyl 2-,4-,5-,6-,8-,16- and 17-oxostearates using a 12 ft x 3/16 in. silicone QF-1 column.

Methyl Oxostearates. Methyl 3-oxostearate decomposed on all three columns, giving a product with a carbon number of about 16, using the SE-30 column. The IR spectrum of the product, which was collected when batches of methyl 3-oxostearate were put through the SE-30 column, did not show the original ester carbonyl absorption at 1746 cm^{-1} (10), but only ketonic carbonyl absorption at 1710 cm^{-1}, which indicated that the product was probably 2-heptadecanone. The carbon numbers increased wih change in substituent position using the SE-30 column but the overall change was quite small. There was a considerably greater overall change using the QF-1 column, especially for the region of the 2- to 8-isomers, which is illustrated in Figure 1C. A considerable change in carbon number from the 2- to 17-isomers also was found using the polyester column but the change in the region of the 2- to 8-isomers was smaller than that found for the QF-1 column. Methyl 2-oxostearate appeared to decompose to some extent using the polyester column since the peak to which the carbon number of 22.55 was assigned was followed by a smaller broader peak. The major peak also may have been a decomposition peak as its carbon number was unexpectedly low.

Applications to the Identification and Analysis of Oxygenated Stearates. Any oxygenated ester can be distinguished from a non-oxygenated ester which has the same carbon number on the SE-30 column by reanalysis on the QF-1 or the polyester column. In attempting to identify an unknown isomer it is probably best to compare it with a known compound such as 12-oxo, hydroxy- or acetoxystearate, which can easily be derived from hydrogenated castor oil esters, rather than to rely on measurements of carbon number alone. If the unknown ester is a hydroxy ester only the 2-,3-,17- and 18-hydroxy esters can be positively distinguished and separated from 12-hydroxystearate on any of the columns. The 4- and 5-hydroxy esters also are separated from 12-ester on the more polar columns but their behavior is exceptionl and may not be the same under all operating conditions.

For further identification the unknown hydroxy ester should be converted to the acetoxy ester. Using the polyester column and 12-acetoxystearate as standard it can be seen from Figure 1B that 14-acetoxy ester can be partly resolved from 12-ester if the relative amt in the mixture are suitable and that 2-,3-,4-, 15-,16-,17- and 18-acetoxy esters are partly or completely separated. If the unknown hydroxy ester is converted to the oxo ester (2,11) further information can be obtained by analysis on the QF-1 column, particularly about isomers with the substituent at positions 4–8. For this purpose it was found best to use a 12 ft x $\frac{3}{16}$ in. column, as was used to obtain Figure 1C, instead of the 6 ft x $\frac{3}{16}$ column used to obtain the carbon numbers. Figure 1C shows that the 2-,4-, 5-,16- and 17-oxo esters all are separated from each other and that 6-oxo is partly separated from 8-oxo. Using this column the 6- and 12-oxo esters are completely separated, 7- and 12-oxo esters are partly separated and 8-oxo ester forms a pronounced shoulder on the 12-oxo ester peak. Thus by using a combination of the three types of oxygenated ester and the three columns 2-,3-,4-,5-,6-,7-,8-,14-,15-,16-,17- and 18-isomers can be identified using the 12-isomer as a known standard but the 9-,10-,11- and 13-isomers cannot be characterized in this way.

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REFERENCES

1. Bens, E. M., and W. R. McBride, Anal. Chem. *31*, 1379 (1959).
2. Bergström, S., G. Aulin-Erdtman, B. Rolander, E. Stenhagen and S. Östling, Acta Chem. Scand. *6*, 1157 (1952).
3. Bowman, R. E., J. Chem. Soc. 325 (1950).
4. Christie, W. W., F. D. Gunstone and H. G. Prentice, *Ibid.* 5768 (1963).
5. Clutterbuck, P. W., *Ibid.* 2330 (1924).
6. Craig, B. M., Chem. Ind. (London), 1442 (1960).
7. Craig, B. M., in "Gas Chromatography," 3rd Symposium, ed. by N. Brenner, J. E. Callen and M. B. Weiss, Academic Press Inc., New York, 1962, pp. 37–56.
8. Diaper, D. G. M., Can. J. Chem. *33*, 1720 (1955).
9. Fieser, L. F., and J. Szmuszkovicz, J. Am. Chem. Soc. *70*, 3352 (1948).
10. Fischmeister, I., Arkiv Kemi. *18*, 101 (1961).
11. Gorin, P. A. J., J. F. T. Spencer and A. P. Tulloch, Can. J. Chem. *39*, 846 (1961).
12. Greaves, W. S., R. P. Linstead, B. R. Shephard, S. L. S. Thomas and B. C. L. Weedon, J. Chem. Soc. 3326 (1950).
13. Hopkins, C. Y., JAOCS *38*, 664 (1961).
14. Hünig, S., and W. Lendle, Ber. *93*, 913 (1960).
15. Hünig, S., E. Lücke and E. Benzing, Ber. *91*, 129 (1958).
16. Keskin, H., Rev. Fac. Sci. Univ. Istanbul, *17A*, 344 (1952).
17. Kitagawa, I., M. Sugai and F. A. Kummerow, JAOCS *39*, 217 (1962).
18. Magidman, P., S. F. Herb, R. A. Barford and R. W. Riemenschneider, *Ibid.* *39*, 137 (1962).
19. Manyik, R. M., F. C. Frostick, J. J. Sanderson and C. R. Hauser, J. Am. Chem. Soc. *75*, 5030 (1953).
20. Miwa, T. K., K. L. Mikolajczak, F. R. Earle and I. A. Wolff, Anal. Chem. *32*, 1739 (1960).
21. Morris, L. J., R. T. Holman and K. Fontell, J. Lipid Res. *1*, 412 (1960).
22. Morrissette, R. A., and W. E. Link, *JAOCS 41*, 415 (1964).
23. O'Brien, J. S., and G. Rouser, Anal. Biochem. *7*, 288 (1964).
24. von Rudloff, E., Can. J. Chem. *38*, 631 (1960).
25. Ryhage, R., and E. Stenhagen, Arkiv Kemi. *15*, 545 (1960).
26. Skogh, M., Acta Chem. Scand. *6*, 809 (1952).
27. Spencer, E. Y., and G. F. Wright, J. Am. Chem. Soc. *65*, 1281 (1941).
28. Ställberg-Stenhagen, S., Arkiv Kemi. Mineral. Geol. *20A*, No. 19, 1 (1945).
29. Stork, G., A. Brizzolara, H. Landesman, J. Szmuszkovicz and R. Terrell, J. Am. Chem. Soc. *85*, 207 (1963).
30. Sweet, R. S., and F. L. Estes, J. Org. Chem. *21*, 1426 (1956).
31. Tulloch, A. P., B. M. Craig and G. A. Ledingham, Can. J. Microbiol. *5*, 485 (1959).
32. Tulloch, A. P., J. F. T. Spencer and P. A. J. Gorin, Can. J. Chem. *40*, 1326 (1962).
33. Tulloch, A. P., and J. F. T. Spencer, *Ibid.* *42*, 830 (1964).
34. VandenHeuvel, W. J. A., E. O. A. Haahti and E. C. Horning, J. Am. Chem. Soc. *83*, 1513 (1961).
35. Woodford, F. P., and C. M. van Gent, J. Lipid Res. *1*, 188 (1960).

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Analytical Fractionation of Complex Lipid Mixtures: DEAE Cellulose Column Chromatography Combined With Quantitative Thin Layer Chromatography

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Abstract

A quantitative chromatographic procedure for the fractionation of complex lipid mixtures is described. The method utilizes diethylaminoethyl (DEAE) cellulose column chromatography followed by thin layer chromatography (TLC). Spots produced in TLC are charred with sulfuric acid-potassium dichromate and heat and are then measured by quantitative densitometry. Results obtained with beef brain and beef heart mitochondrial lipids are presented, and the close correspondence between column isolation procedures and the new procedure is demonstrated. Methods utilizing only column chromatography, column chromatography and TLC, and one- and two-dimensional TLC without column chromatography are compared.

Introduction

CHROMATOGRAPHIC PROCEDURES are widely used for the determination of lipid class composition. Silicic acid column chromatography has been the most commonly utilized approach based on the initial observations of Borgström (1), Fillerup and Mead (2), Barron and Hanahan (3) and Lea et al. (4). Diethylaminoethyl (DEAE) cellulose column chromatography was introduced by Rouser et al. (5) to eliminate the problem of the elution of acidic lipids with other lipid classes encountered with silicic acid column chromatography. DEAE column chromatography combined with silicic acid column chromatography and with silicic acid-silicate column chromatography was used to obtain separation of most lipid classes of brain (5,6) and other mixtures (6). Recently, Privett and Blank (7) and Blank et al. (8) have greatly extended the possibilities of TLC for quantitative applications. These investigators demonstrated that a spray reagent composed of H_2SO_4 and potassium dichromate can be used to char spots obtained by TLC to a reproducible optical density. Under proper conditions the extent of charring is independent of the degree of unsaturation of lipid classes such as lecithin and sphingomyelin, can be made to give a linear response over a fairly wide concentration range, and is readily determined by transmission densitometry.

The present report describes a new approach to