TABLE V

Radioactivity of Degradation Products of Arachidic and Behenic Acids Derived from Eicosapentaenoic and Docosahexaenoic Acids

Sample	d.p.s./mmole
Arachidic acid (diluted)	871
Benzoic acid (C-1 of arachidic)	57
Benzoic acid (C-2 of arachidic)	18
Benzoic acid (C-3 of arachidic)[a]	557
Margaric acid	235
Behenic acid	758
Benzoic acid (C-1 of behenic)	167
Benzoic acid (C-2 of behenic)	86
Benzoic acid (C-3 of behenic)	46
Benzoic acid (C-4 of behenic)	13
Benzoic acid (C-5 of behenic)	310
Margaric acid	145

[a] Calculated from the difference between the radioactivities of C_{17} and C_{18} acids purified by reversed-phase chromatography.

those shown to occur in the conversion of linoleic to arachidonic acid (11,12,13), and of oleic to 5,8,11-eicosatrienoic acid (23). In this case, the pathway is probably: 9,12,15-octadecatrienoic (linolenic) acid ⟶ 6,9,12,15-octadecatetraenoic acid ⟶ 8,11,14,17-eicosatetraenoic acid ⟶ 5,8,11,14,17-eicosapentaenoic acid ⟶ 7,10,13,16,19-docosapentaenoic acid ⟶ 4,-7,10,13,16,19-docosahexaenoic acid.

The shorter chain more saturated intermediates in this process were not studied in this case, but the structures proposed seem likely not only because of the analogy with the linoleic and oleic conversions but also because some of them have been identified in various fish oils. For example, Klenk and Brockerhoff characterized 6,9,12,15-octadecatetraenoic (2) and 7,10,13,-16,19-docosapentaenoic (1,3) acids in herring and cod liver oils, and Stoffel and Ahrens (7) have identified these and also 8,11,14,17-eicosatetraenoic acid in menhaden body oil. Klenk and Mohrhauer (17), in a very interesting study, have demonstrated the occurrence of steps 2, 3, and 5 in the rat. There is thus ample evidence to suggest that the same pathway occurs in the fish and that the fatty acids of the fish lipids (in analogy with those of mammals) are the result of predictable series of stepwise alterations of a known small number of dietary or biosynthesized acids.

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REFERENCES

1. Klenk, E., 1st Internat. Conf. on Biochem. Problems of Lipids, Brussels, 33 (1953).
2. Klenk, E., and H. Z. Brockerhoff, Physiol. Chem., *307*, 272 (1957).
3. Klenk, E., and H. Z. Brockerhoff, *Ibid.*, *310*, 153 (1958).
4. Silk, M. H., and Hahn, H. H., Biochem J., *57*, 582 (1954).
5. Whitcutt, J. M., and D. A. Sutton, *Ibid.*, *63*, 469 (1956).
6. Stoffel, W., and E. H. Ahrens, Jr., J. Am. Chem. Soc., *80*, 6604 (1958).
7. Stoffel, W., and E. H. Ahrens, Jr., J. Lipid Res., *1*, 139 (1960).
8. Toyama, Y., Y. Iwata, and K. Fjuimura, Fette-Seifen Anstrichmittel, *61*, 846 (1959).
9. Mead, J. F., M. Kayama, and R. Reiser, JAOCS, *37*, 483 (1960).
10. Mead, J. F., G. Steinberg, and D. R. Howton, J. Biol. Chem., *205*, 683 (1953).
11. Steinberg, G., W. H. Slaton, Jr., D. R. Howton, and J. F. Mead, *Ibid.*, *220*, 257 (1956).
12. Mead, J. F., and D. R. Howton, *Ibid.*, *229*, 575 (1957).
13. Howton, D. R., and J. F. Mead, *Ibid.*, *235*, 3385 (1960).
14. Kayama, M., R. Reiser, D. W. Hood, and B. Stevenson, Unpublished.
15. Steinberg, G., W. H. Slaton, Jr., Howton, D. R., and J. F. Mead, J. Biol. Chem. *224*, 841 (1957).
16. Nevenzel, J. C., and D. R. Howton, J. Org. Chem., *23*, 933 (1958).
17. Klenk, E., and H. Mohrhauer, Z. Physiol. Chem., *320*, 218 (1960).
18. Stein, R. A., and M. Kayama, J. Org. Chem., *26*, 5231 (1961).
19. Howard, G. A., and A. J. P. Martin, Biochem. J., *46*, 532 (1950).
20. Mead, J. F., J. Biol. Chem., *227*, 1025 (1957).
21. Fillerup, D. L., and J. F. Mead, Proc. Soc. Exptl. Biol. Med., *83*, 574 (1953).
22. Dauben, W. G., E. Hoerger, and J. W. Petersen, J. Am. Chem. Soc., *75*, 2347 (1953).
23. Fulco, A. J., and J. F. Mead, J. Biol. Chem., *234*, 1411 (1959).

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The Preparation and Properties of Some Synthetic Glycerides. II. Control of the Migration of Acyl Groups[1,2]

J. E. JACKSON, State University College, Geneseo, New York, and W. O. LUNDBERG, The Hormel Institute, University of Minnesota, Austin, Minnesota

Abstract

In a study of the use of tritylglycerols as starting materials for synthesis of mono- or diglycerides and mixed triglycerides, it was found that acyl migration can be minimized or prevented by detritylation in petroleum ether, avoiding contact with polar solvents or contaminants. Under conditions favoring acyl migration, tetrabromostearoyl radicals were found to migrate less readily than stearoyl radicals.

Introduction

FORMATION OF THE trityl (triphenylmethyl) ether is a convenient method for temporarily blocking one or both of the primary hydroxyls of glycerol. However, the use of tritylglycerols as starting materials for synthesis of mono- or diglycerides has been limited by the facile migration of the secondary acyl radicals during detritylation with dry hydrogen chloride (1).

As a result, it has been necessary to devise relatively complex procedures for the synthesis of 2-monoglycerides or 1,2-diglycerides. In one class of procedures hydroxyl groups are made available in analogs of glycerol by suitable transformations after the desired acylation is complete (2). In another, the hydroxyl-blocking trityl groups are removed by hydrogenolysis under mild conditions (3). In reactions leading to mixtures of 1,2- and 1,3-diglycerides, the two have been separated by fractional crystallization (4).

The need for a more direct procedure for the synthesis of 1,2-diglycerides led to a reinvestigation of the comparatively simple method which uses monotritylglycerol as starting material. It was found that cleavage of the trityl ether with hydrogen chloride does not cause migration of acyl radicals if the tritylglyceride is sufficiently pure and the detritylation is effected in petroleum ether without subsequent exposure of the product to acidic diethyl ether. Thus, by varying the solvent in which detritylation takes place, the same procedure may be used for preparing either symmetrical or unsymmetrical glycerides.

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[2] Publication No. 4265, Agricultural Experiment Station, University of Minnesota.

Experimental

Tritylated Intermediates. 1-Monotrityldistearin was prepared by mixing, at room temperature, 1-monotritylglycerol, mp 109.7–110.1°C (5), dissolved in dry pyridine and 5% excess of stearoyl chloride, bp 143C at 0.2 mm of mercury (6), dissolved in chloroform. After standing 30 min at room temperature, the mixture was refluxed 4 hr under a condenser vented through a drying tube, cooled and poured into water. The product was extracted with diethyl ether and washed successively with copious amounts of dilute sulfuric acid, dilute sodium bicarbonate and water. After drying and removing the ether, the slushy syrup which was obtained was taken up in benzene, boiled briefly with carbon black and filtered. The benzene was removed and 3 vol of acetone was added. A gelatinous precipitate which formed was removed by filtering and 2 vol of absolute ethyl alcohol added to the acetone solution. A granular precipitate melting at 46–48C was obtained after 24 hr at 5C. This precipitate was recrystallized from 12 vol of acetone, then from 5 vol of acetone containing 5% of petroleum ether and finally from 5 vol of a mixture of 2 parts absolute ethyl alcohol and 1 part acetone. The final product was a white crystalline solid, melting at 48–49C and remelting at 33.5–34C. Less rigorously purified 1-monotrityldistearin also melted at 48–49C but was slightly yellow and did not yield 1,2-distearin when detritylated.

1,3-Ditritylstearin was prepared by similarly acylating ditritylglycerol, mp 170.5–172C, (5). After decolorization with carbon black in benzene and repeated crystallization from acetone and 50–50 mixtures of acetone and absolute ethyl alcohol, the 1,3-ditritylstearin melted at 82–83C.

With minor variations these same procedures were used in preparing the tetrabromostearins. In preparing 1-monotrityldítetrabromostearin, after tetrabromostearoyl chloride, mp 61.7–62.2C, was added, the reaction mixture was allowed to stand for 2 days in a stoppered flask in the dark, then refluxed 2 hr. Emulsification was severe during the washing step. Final crystallizations were from mixtures of diethyl ether and petroleum ether. As precipitated, the 1-monotrityldítetrabromostearin melted over a range from 48.5–99C. When recovered from petroleum ether solution by evaporation of the solvent, the melting point was 72–78C. When similarly recovered from a mixture of two parts petroleum ether and one part diethyl ether, the mp was 48.4–48.8C. In the latter case, as the temperature of the melting point bath was raised, the sample resolidified and again completely melted at 95C. 1,3-Ditrityltetrabromostearin was prepared by letting the reaction mixture stand 20 days at room temperature in a tightly stoppered flask. No emulsification occurred during the washing step. However, the product could not be crystallized.

Detritylation. In order to obtain 1,2-distearin or 2-monostearin, dry hydrogen chloride was bubbled slowly through a petroleum ether solution of the corresponding trityl compound. Neither temperature nor concentration appeared to be critical. Cleavage of the ether to form the glyceride and triphenylchloromethane was effected satisfactorily at room temperature. From 3 to 6 vol of petroleum ether were used as solvent. Typically, a copious precipitate began to form in 10 to 15 min and the treatment with hydrogen chloride was continued until no further precipitation occurred. The reaction flask was stoppered and left to stand for approximately 1 hr at room temperature. Essentially pure products in nearly quantitative yield were obtained by filtering and washing the precipitate on the filter paper with copious amounts of petroleum ether.

Using diethyl ether as solvent in the same procedure, 1,3-distearin was obtained from monotrityldistearin and 1-monostearin from ditritylstearin. These products, or mixtures of them with 1,2-distearin or 2-monostearin, also were obtained when using petroleum ether as solvent if the tritylglycerides were inadequately purified or were intentionally contaminated with water, fatty acid or triphenylcarbinol.

Like monotrityldistearin, monotrityldítetrabromostearin was converted to the 1,3-diglyceride when treated in diethyl ether with dry hydrogen chloride. After one recrystallization from petroleum ether, the 1,3-ditetrabromostearin melted at 90–93C. When detritylation was effected in a mixture of two parts of petroleum ether and one part diethyl ether, the product melted at 103–104.2C and remelted at 80.5–81.5C. The latter compound could be obtained only from very pure monotrityldítetrabromostearin, whereas the low melting compound could be obtained by detritylating contaminated monotrityldítetrabromostearin in either solvent system. Hence, by analogy with the distearins, the ditetrabromostearin with the high melting point was designated 1,2-ditetrabromostearin.

The monotetrabromostearins were anomalous in that, depending on the duration of exposure to hydrogen chloride, either the 1- or the 2-compound could be obtained. When the detritylation reaction (in diethyl ether) was followed by prompt isolation of the product, the compound obtained melted at 84.5–86C and was designated 2-monotetrabromostearin. When the reaction mixture was allowed to stand 48 hr in a tightly stoppered flask at 5C after treatment with hydrogen chloride, the product melted at 106–110C and appeared to be identical to the 1-monotetrabromostearin described by Black and Overly (7).

Stability. 1,2-Distearin could be converted quantitatively to 1,3-distearin by treating it with dry hydrogen chloride in diethyl ether. No change occurred in petroleum ether, nor could the 1,3-distearin be converted reversibly to 1,2-distearin in either solvent. Attempts to transform the monostearins in diethyl ether led to mixtures.

Unlike the distearins, the ditetrabromostearins were stable, and neither could be converted to the other although the solvent in which detritylation was effected determined which positional isomer was obtained. However, the treatment with dry hydrogen chloride in diethyl ether converted 2-monotetrabromostearin, melting at 86C, to 1-monotetrabromostearin melting at 110C.

Triglycerides. The mono- and diglycerides described above were characterized further by converting them in mixed triglycerides by acylation with tetrabromostearoyl chloride or stearoyl chloride. These triglycerides, as well as the diglycerides containing the tetrabromostearoyl radical, were debrominated by the method described previously (5) to obtain unsaturated compounds which could be characterized by their iodine values.

Table I shows the melting points and Wijs iodine values of the glycerides derived from 1-monotrityldistearin and 1,3-ditritylstearin. Table II summarizes these data for the glycerides derived from 1-monotrityldítetrabromostearin and 1,3-ditrityltetrabromostearin.

TABLE I

Glycerides Derived from 1-Monotrityldistearin and 1,3-Ditritylstearin

Glyceride	mp C	Remelt C	Iodine value		Yield %
			Found	Theory	
1,2-Distearin	69.2–69.4	59.7–60.2			85.5
1,3-Distearin	78.0–78.3	77.6–77.8			62.5
1-Tetrabromostearodistearin	50.5–51.5	48.5–49.5			72.8
2-Tetrabromostearodistearin	53.5–54.5				60.0
1-Linoleodistearin	39.5–40.8		57.9	57.3	79.6
2-Linoleodistearin	36.3–37.0		56.3	57.3	77.8
1-Monostearin	79.7–80.0	73.4–74			55.2
2-Monostearin	73.5–74	67 –68			48.8

TABLE II

Glycerides Derived from 1-Monotrityldibromostearin and 1,3-Ditrityltetrabromostearin

Glyceride	mp C	Remelt C	Iodine value		Yield %
			Found	Theory	
1,2-Ditetrabromostearin	103.0–104.2	80.5–81.5			72
1,3-Ditetrabromostearin	90.0– 93.0				
1,2-Dilinolein	Liquid at RT		162.1	165	89.5
1,3-Dilinolein	Liquid at RT		163.0	165	96.0
1-Stearoditetrabromostearin	43.5– 44.0				63.5
2-Stearoditetrabromostearin	40.0– 41.0				78.8
1-Stearodilinolein	Liquid at RT		114.2	115.2	90.0
2-Stearodilinolein	Liquid at RT		112.7	115.2	95.0
1-Monotetrabromostearin	106.0–110.0				
2-Monotetrabromostearin	84.5– 86.0				

Results and Discussion

The experimental results indicate that unsymmetrical diglycerides and the 2-monoglycerides may be arranged in the following order of tendency toward acyl migration during their formation from tritylated intermediates: 2-monostearin > 1,2-distearin > 1,2-ditetrabromostearin > 2-monotetrabromostearin.

Once these compounds have been formed, however, their tendency to rearrange on further exposure to hydrogen chloride in diethyl ether is: 2-monostearin > 1,2-distearin > 2-monotetrabromostearin > 1,2-ditetrabromostearin.

At least under the conditions used here, there was no apparent tendency for acyl radicals to migrate in the reverse direction from a primary position to a secondary position in the glyceride. Neither was there a tendency toward migration of acyl radicals in the absence of polar solvents or polar contaminants.

In their melting points and relative stability the tetrabromostearoyl glycerides resemble the aromatic glycerides. King and coworkers (8) found that the aromatic glycerides are much less likely to undergo migration of acyl groups than are the aliphatic glycerides and pointed out that the symmetrical aromatic glycerides melt lower than the unsymmetrical isomers, reversing the order for the saturated aliphatic glycerides.

Characteristics of both classes of compounds seem to be combined in the tetrabromostearodistearins and derived unsaturated triglycerides. The melting point of 2-tetrabromostearodistearin was found to be higher than that of the unsymmetrical compound. After debromination, however, the order of the mp was reversed, the symmetrical isomer then melting at a lower temperature than the unsymmetrical isomer. The mp of both pairs of compounds were close together (within 3C) and their appearance quite different, as noted by Carter and Malkin (9).

REFERENCES

1. Lohuizen, O. E., and P. E. Verkade, Rec. Trav. Chim., *79*, 133 (1960). Hartman, L., Chem. Rev., *58*, 845 (1958).
2. Burry, P. J., and B. M. Craig, Can. J. Chem., *33*, 716 (1955). Schlenk, H., Beverly G. Lamp, and B. W. DeHaas, J. Am. Chem. Soc., *74*, 2550 (1952). Bergman, M., Z. Physiol. Chem., *137*, 27 (1924).
3. Verkade, P. E., Rec. Trav. Chim., *72*, 393 (1943). Daubert, B. F., J. Am. Chem. Soc., *62*, 1713 (1940).
4. Radlove, S. B., R. V. Madrigal, and R. Slutkin, JAOCS, *37*, 570 (1960). Crossley, A., I. P. Freeman, B. J. F. Hudson, and J. H. Pierce, J. Chem. Soc., *1959*, 760; Grasas y Aceites, *10*, 245 (1959).
5. Jackson, J. E., and W. O. Lundberg, JAOCS, *40*, 276 (1963).
6. Craig, B. M., W. O. Lundberg and W. F. Geddes, *Ibid.*, *29*, 169 (1952).
7. Black, H. C., and C. A. Overly, J. Am. Chem. Soc., *61*, 3051 (1939); U.S. Patent 2,408,905 (October 8, 1946).
8. Jackson, D. T., and C. G. King, J. Am. Chem. Soc., *55*, 678 (1933).
9. Carter, M. G. R., and T. Malkin, J. Chem. Soc., *1939*, 1518.

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• *Letters to the Editor*

Modified Procedure for the Determination of Peroxide Value of Fats by the Ferric Thiocyanate Method[1]

DETERMINATION OF PEROXIDE VALUE is one of the most frequently performed tests in studies on edible fats. In the course of stability studies within the authors' laboratories, published procedures were modified in the interests of greater reliability, speed, and simplicity. A simplified ferric thiocyanate procedure for peroxides in fats is described here for the possible benefit which it may provide to others engaged in food research or quality control.

The solvent mixture in the Hills and Thiel (1) method for fats consists of 70 parts of benzene and 30 parts of methyl alcohol. In order to develop the red ferric thiocyanate color the reaction mixture is heated for 2 min at 50C. In the methods of Sumner (2) and of Koch et al. (3) solutions in ethyl alcohol and water are used and heat is not required for developing the final red color. However, the latter methods are applicable only to fatty acids or to esters of fatty acids. By proper selection of solvents it was possible to develop a procedure for fats in which the color develops at room temperature. A mixture of 80 parts of ethyl alcohol and 20 parts of benzene served that purpose.

Elimination of the heating step permitted further simplification of the method. The entire test can be performed in a cuvette, thereby eliminating a separate reaction vessel. An Evelyn Colorimeter tube or other large cuvette is most convenient for the purpose. Further, a replicate weighing of the fat for a blank determination is unnecessary because the fat blank can be measured just prior to the addition of ferrous chloride solution to the cuvette. The red color which develops has good stability. An increase in intensity which develops very slowly is fully compensated by the reagent blank which changes at the same rate. When applied to cottonseed oil and to butterfat, the modified procedure gave the same peroxide values as did the Hills and Thiel method, and with considerable saving in time.

Procedure

Except for the modification indicated above, the procedure and standardization are described by Stine

[1] Paper number 2174.