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).

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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.

et al. (4). Weigh the fat into an Evelyn cuvette. Add 10 ml of solvent (80 volumes ethyl alcohol; 20 volumes benzene) to dissolve the fat. Add 1 drop of ammonium thiocyanate solution and read the absorbancy of the fat blank. Add 1 drop of ferrous chloride solution and measure the absorbancy of the red color with the 515 mμ filter.

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Preparation of 9-*trans*, 12-*trans* - Octadecadienoic Acid[1]

DURING CURRENT INVESTIGATIONS of the metabolism of *trans* fatty acids, it became necessary to prepare large quantities of 9-*trans*, 12-*trans*-octadecadienoic acid, the all-*trans* isomer of linoleic acid. This material is most conveniently made by *cis-trans* isomerizing natural linoleic acid and then purifying by recrystallization.

Jackson, et al. (1) and Kass and Burr (2) have reported methods for preparing 9-*trans*,12-*trans*-octadecadienoic acid using a Se catalyst. However, Se *cis-trans* isomerization has two major disadvantages. First of all, complete removal of toxic Se is essential for biological investigations. The above workers used vacuum distillation to accomplish Se removal after isomerization, but they did not report any analysis of residual Se in their final product. Teeter, et al. (3) have reported that Se was not completely removed by standard vacuum distillation nor by several other chemical treatments, but was only removable by repeated molecular distillations. Secondly, Se isomerization of linoleic acid is known to produce *trans* monoenes as a by-product (4,5). If these *trans* monoenes are present in sufficient quantity (as when recrystallization filtrates are re-isomerized), they cannot be removed by recrystallization.

Kass and Burr (2) have used a HNO_2 catalyst to prepare 9-*trans*,12-*trans*-octadecadienoic acid from linoleic acid, but reported extensive nitrogenous by-product formation and a very poor yield. We have found that by-product formation can be minimized by shortening reaction time, and that those nitrogenous by-products formed can be completely and easily removed on a column of silicic acid. Furthermore, Litchfield, et al. (5) have demonstrated that HNO_2 isomerization of linoleate does not produce the *trans* monoene by-products that Se does. This allows recrystallization filtrates to be re-isomerized to improve yields. Therefore, HNO_2 appeared to be the better isomerization catalyst for our purposes.

A procedure for making 9-*trans*,12-*trans*-octadecadienoic acid has been developed using readily available safflower oil as a starting material. A linoleic acid concentrate was prepared from safflower fatty acids by urea adduct formation. After HNO_2 isomerization, nitrogenous reaction by-products were removed on a column of silicic acid. Recrystallization from acetone yielded 4.5 g of 9-*trans*,12-*trans*-octadecadienoic acid per 100 g of safflower oil.

Preparation of Linoleic Acid Concentrate. A solution of 215 g of NaOH in 430 ml of distilled water was prepared and cooled to room temperature. This solution was slowly added with stirring to 1 kg of alkali-refined safflower oil and one liter of 95% ethanol in a 6 liter reaction flask. The mixture was stirred until homogeneous and then let stand for one hour with occasional stirring. One liter of distilled water and enough 3 M H_2SO_4 (about 850 ml) were added to bring the solution to a pH of 3. The solution temperature was not allowed to rise above 50C during acid addition. The fatty acid layer was allowed to separate, and the lower aqueous layer was siphoned off and discarded. The fatty acid was then washed with distilled water (NaCl was added to the first washings to hinder emulsion formation) until the wash water was neutral. The fatty acid layer was then dried under vacuum.

825 g of urea was dissolved in 2.55 liters of methanol by heating in a six liter reaction flask. 940 g of safflower fatty acids was heated to 80C under nitrogen and added to the methanol solution under nitrogen. If the solution became cloudy, it was heated until clear. The solution was cooled overnight under nitrogen to room temperature. The urea adduct crystals were then filtered and discarded. Another 825 g of urea was dissolved in the filtrate by heating under nitrogen, and the solution was cooled overnight and filtered as before. 4 liters of distilled water and 500 ml of petroleum ether (30–60C boiling range) were added to the filtrate with mild stirring, and the solvent layer was allowed to separate. The solvent layer was removed, washed twice with distilled water, once with 100 ml of 1% HCl, and then with distilled water until the wash water was neutral. The solvent and any traces of water were removed from the fatty acids under vacuum. The yield was about 340 g of fatty acid showing a purity of 98–99% linoleic acid by gas-liquid chromatography (GLC) analysis. (Linoleic acid purity at this point was probably less than GLC results indicated, since non-volatile safflower unsaponifiables were probably concentrated by the urea crystallizations.) Yield and purity depended somewhat on the room temperature used for the urea crystallizations. Lower room temperatures gave a lower yield and a higher purity. If GLC analysis indicated less than 98% linoleic acid at this point, another urea adduct crystallization was performed. If the final acetone recrystallization filtrates were to be re-isomerized to increase yields, 99+% linoleic acid was required.

Isomerization and Purification Procedure. 340 g of linoleic acid concentrate and 135 ml of 6 M HNO_3 were placed in a 2 liter reaction flask fitted with a dropping funnel, a stirring shaft, an outlet to a bubble trap, and a connection to a source of nitrogen. The flask was purged with nitrogen. 205 ml of freshly prepared 2 M $NaNO_2$ was added over a period

[1] Presented at the AOCS meeting in Atlanta, 1963.