•*Technical*

Fractionation of Fats, Oils, and Waxes on Thin Layers of Silicic Acid[1]

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THE TECHNIQUE of "thin-layer chromatography," as described by Stahl (7), has recently been used by us for the fractionation of radio-actively labelled lipid derivatives into classes of compounds (4) and for the analysis of acetin fats (1) which were obtained from various fish oils.

The separation and identification of natural lipids by this technique is described here. Procedures for the segregation of fats, oils, and waxes into their constituent lipid classes are presented. The resolution, on a micro scale, of unusual fatty acids in total acid fractions isolated after saponification of lipids from vegetable and animal sources is also described. Methods are outlined for separating the unsaponifiable fractions obtained from natural lipid mixtures.

Materials and Methods

The following natural products were investigated: a) vegetable lipids: jojoba oil *(Simmondsia chinensis)*, castor oil *(Ricinus communis)*, olive oil *(Olea europaea)*, and oiticica oil *(licania rigida)*; b) animal lipids: lanolin *(Adeps lanae* U.S.P.*)*, liver oils of soupfin shark *(Galeorhinus galeus)*, basking shark *(Cetorhinus maximus)*, and ratfish *(Hydrolagus colliei)*, fur seal *(Callorhinus ursina cynocephala)* blubber oil.

Most of the individual compounds used as reference materials were commercial products.

The procedures recommended by Stahl for the preparation of thin layers (250 to 275 μ) of silicic acid on glass plates (20 × 20 cm.), *i.e.*, "open columns" or "chromatoplates," were followed throughout this investigation. Thin layers of alumina were obtained similarly. The equipment for making the plates was purchased from C. Desaga G.m.b.H., Heidelberg, Germany, together with "Silica Gel G," which is manufactured by E. Merck A. G., Darmstadt, Germany.[2]

Amounts of lipids between 5 and 500 γ were separated with mixtures of petroleum ether, diethyl ether, and acetic acid. The chromatograms were run by ascending technique for 40 min. at room temperature. Iodine vapors were used for locating unsaturated substances as brown spots on a yellow background (3). Both unsaturated and saturated lipids were manifested in ultraviolet light with a maximum emission at 270 mμ[3] after spraying the chromatograms with a solution of 0.2% 2′,7′-dichlorofluorescein[4] in 95% ethanol. In general, as little as 3 γ of a lipid may be detected by one indicator or the other. The use of the two indicators increases the probability that no substance remains undetected. Other spray reagents commonly used for identifying lipids on paper chromatograms (2) can in most cases be applied to thin-layer chromatograms.

Results

Adsorption chromatography on silicic acid fractionates lipids into classes of compounds. A slight degree of subfractionation within these classes can be observed in thin-layer chromatography. For example, triolein, trilinolein, and trilinolenin migrate at a slightly lower rate than triglycerides of C-12 to C-18 saturated acids. However these subfractionations do not interfere with the separation of lipids according to classes.

Some typical examples of thin-layer chromatography, on silicic acid, of total fats, oils, and waxes are illustrated in Figure 1. Lanolin (a) and soupfin shark liver oil (e) were resolved with the system of 95 volumes of n-hexane and 5 volumes of diethyl ether. Mixtures of oleyl alcohol, cholesteryl palmitate, and squalene (b) and of tristearin, vitamin A palmitate, and squalene (d) were chromatographed simultaneously with the natural mixtures. The presence of fatty alcohols, steryl esters, and hydrocarbons is probable in lanolin. Esters of higher alcohols with fatty acids, which are to be expected in this wax, appear in the hydrocarbon fraction. The major part of the liquid wax, jojoba oil, migrates under these conditions to the solvent front. Triglycerides, vitamin A esters, steryl esters, and hydrocarbons are demonstrated in soupfin shark liver oil.

Ratfish (e) and basking shark (g) liver oils were fractionated with the system of 90 volumes of petroleum ether (B.P. 30–60°C.), 10 volumes of diethyl ether, and 1 volume of acetic acid. They were compared with standard mixtures of batyl alcohol, vitamin A, and batyl distearin (f), vitamin A, tristearin, and squalene (h). Glyceryl ethers, vitamin A, triglycerides, and glyceryl ether diesters were found to be constituents of ratfish liver oil. Glyceryl ethers, vitamin A, triglycerides, a small amount of glyceryl ether diesters, and a substantial quantity of hydrocarbons are demonstrated in basking shark liver oil.

Constituents of castor (i) and olive oils (k) were resolved with the system of 70 volumes of petroleum ether, 30 volumes of diethyl ether, and 2 volumes of acetic acid. The major spot in the chromatogram of castor oil essentially represents triglycerides contain-

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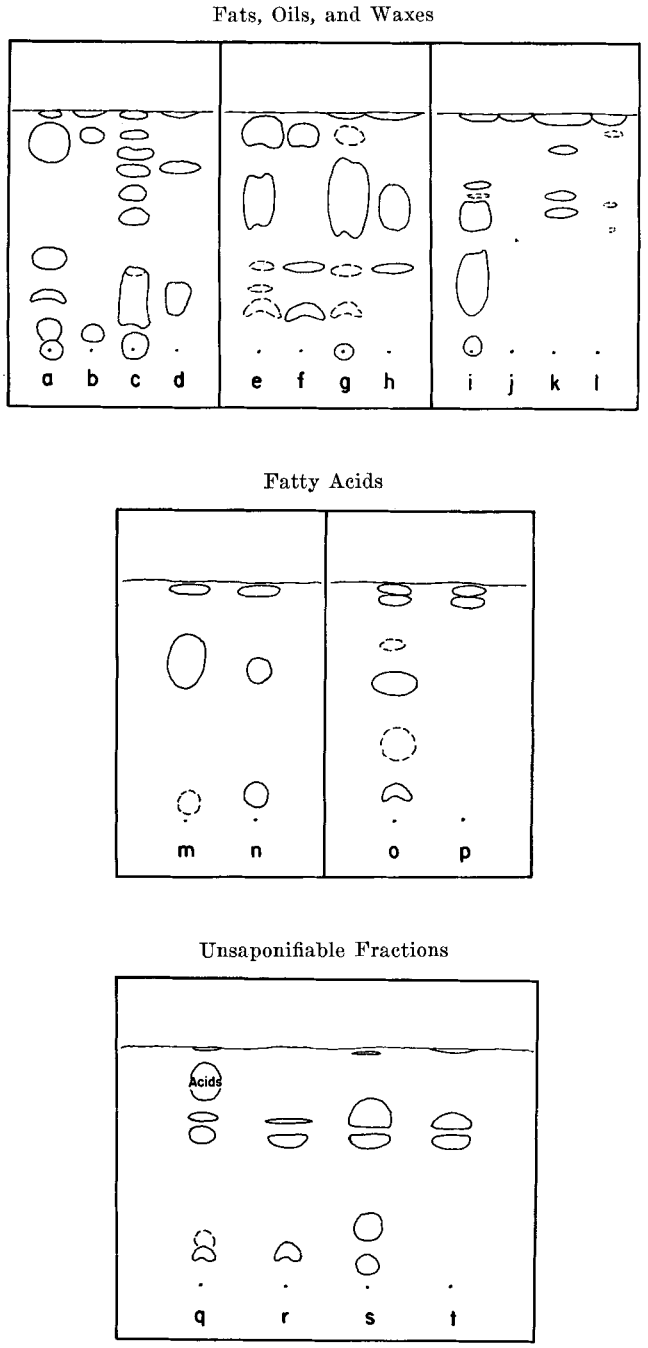
[2] "Silica Gel G" may be obtained from Terra Chemicals Inc., 500 Fifth avenue, New York 36, N.Y., or from C. A. Brinkmann and Company, 115 Cutter Mill road, Great Neck, L. I., N.Y. The latter firm is also U. S. representative of C. Desaga G.m.b.H., Heidelberg, Germany.

[3] "Mineralight," Ultra-Violet Products Inc., San Gabriel, Calif.

[4] Eastman Kodak Company, Rochester 3, N.Y.



Fractionation of Complex Lipid Mixtures

The letters a, e, etc., relate to natural mixtures, 200–300 γ of each, the composition of which is explained in the text. The groups b, d, etc., are mixtures of reference compounds. The order of mentioning the substances found in each vertical column is from the bottom up. The figures represent tracings of actual chromatograms. Dotted lines indicate minor constituents.

a) Lanolin; b) oleyl alcohol, cholesteryl palmitate, and squalene; c) soupfin shark liver oil; d) tristearin, vitamin A palmitate, and squalene; e) ratfish liver oil; f) batyl alcohol, vitamin A, and batyl distearin; g) basking shark liver oil; h) vitamin A, tristearin, and squalene; i) castor oil; j) squalene; k) olive oil; l) tristearin;

m) fatty acids of castor oil; n) dihydroxy stearic acid, ricinoleic acid, and stearic acid; o) fatty acids of oiticica oil; p) α-elaeostearic acid and licanic acid;

q) hydrolyzate of fur seal blubber oil; r) selachyl alcohol, cholesterol, and vitamin A; s) unsaponifiable fraction of lanolin; t) cholesterol, docosanol, and octadecene.

ing three ricinoleic moieties. The spot immediately above it consists mainly of triglycerides possessing two ricinoleic acid groups. It may be assumed that triglycerides containing one ricinoleic acid with two nonhydroxylated acids migrate slightly beyond the latter compound. The spot at the solvent front contains hydrocarbons. Squalene (j), which was chromatographed simultaneously, cannot be separated, by this system, from small amounts of normal triglycerides, the presence of which might be expected. Olive oil and tristearin (l), which were run on the same plate, also appear largely in the solvent front. Impurities of mono- and distearin were present in the tristearin, which was used as a reference compound.

Figure 2 illustrates separations of unusual acids from the common fatty acids found in castor oil and oiticica oil. The mixture of 70 volumes of petroleum ether, 30 volumes of diethyl ether, and 1 volume of acetic acid resolves dihydroxystearic acid, ricinoleic acid, and the nonhydroxylated fatty acids of castor oil (m). Representative samples of these three classes of acids were chromatographed simultaneously (n).

The fatty acids (o) of oiticica oil were chromatographed in the system of 70 volumes of petroleum ether, 30 volumes of diethyl ether, and 2 volumes of acetic acid so that two of the major constituents, α-elaeostearic acid and licanic acid, were segregated. A composite (p) of these two acids was run on the same plate. Iodine as an indicator fails to differentiate these acids. The chromatogram was stained with dichlorofluorescein and viewed under ultraviolet light. Licanic acid emits a yellow-green fluorescence whereas α-elaeostearic acid exhibits a strong purple color. α-Elaeostearic acid was not resolved from the common fatty acids on silica gel. A satisfactory separation was achieved however on thin layers of alumina.

Figure 3 shows separations of the unsaponifiable fractions of fur seal blubber (q) and of lanolin (s). A mixture of 70 volumes of petroleum ether, 30 volumes of diethyl ether, and 1 volume of acetic acid was used. Glyceryl ethers, cholesterol, vitamin A, and hydrocarbons were identified in fur seal blubber unsaponifiables by comparison with reference compounds (r and t). The hydrolysate of the fur seal blubber had been acidified, but the free fatty acids were not removed. This simplified procedure allows for the identification of the major unsaponifiable constituents despite the presence of the acids.

In the unsaponifiable fraction of lanolin two spots were found that are believed to represent aliphatic and/or cyclic diols. In addition, cholesterol, substantial amounts of long-chain alcohols, and small quantities of hydrocarbons were found. Cholesterol, docosanol, and octadecene served as reference materials (t).

Discussion

Thin-layer chromatography permits rapid fractionation of complex lipid mixtures ranging from hydrocarbons to phospholipids, according to classes. Closely related groups of compounds, such as triglycerides, glyceryl ether diesters, wax esters, and vitamin A esters in fish oils, or triglycerides containing one, two, or three oxygenated fatty acids as in castor oil, can be separated within less than one hour. Only ten minutes are required to segregate less complicated mixtures, such as fatty acids and their alkyl

esters. Good separations can be obtained with much less material than is required with standard techniques of column chromatography.

Most indicators used in paper chromatography of lipids may be applied in thin-layer chromatography as well. In addition, corrosive spot-test reagents, such as sulfuric acid, can be used to char nearly all organic compounds. Thin-layer chromatograms usually yield spots which are much smaller and more distinct than those observed in cellulose- or glass-fiber paper chromatography.

It is possible to separate up to 10 mg. of a mixture of lipids on one plate. The various fractions can be eluted for identification or further fractionation if nondestructive indicators are used for their localization. The solvent systems utilized in this microtechnique can also be applied on columns of silicic acid if larger amounts are required.

Thin-layer chromatography has been applied to test a variety of seed oils for the occurrence of epoxy and hydroxy acids, and some striking results were obtained (5, 6).

Summary

Adsorption chromatography on thin layers of silicic acid or alumina provides a new and highly efficient analytical tool for the rapid separation of lipids according to classes of compounds.

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A Study of Octadecenoic Acids by Gas-Liquid Partition Chromatography and Infrared Spectrophotometry

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NUMEROUS AUTHORS (1,2,3,6,9) have reported the use of gas-liquid partition chromatography (G.L.P.C.) for the separation of the methyl esters of fatty acids. Techniques were generally used with conventional columns packed with high boiling greases or with polyesters. Such closely related compounds as methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate have been readily separated and determined by this technique. Lipsky, Lovelock, and Landowne (4) and Lipsky, Landowne, and Lovelock (5) reported the use of capillary columns for the separation of the methyl esters of oleic and elaidic acids.

Infrared analysis for *trans*-octadecenoic acid is a standard method (8). Good quantitative results of the *trans*-isomers are possible by infrared analyses, and such analyses are done routinely by many laboratories.

This laboratory would like to report the separation of geometric isomers of octadecenoic acid methyl esters from commercially hydrogenated oils by means of G.L.P.C., using a capillary column coated with Apiezon L. Comparisons with a conventional column packed with a polyester and with *trans*-isomer values from infrared spectrophotometry are also given.

Experimental

A Barber-Colman Model 10 gas chromatograph with an ionization detection system was used for all G.L.P.C. analyses.

A 6-ft. glass column 0.25 in. in inside diameter, containing 60–100 mesh Chromosorb W coated with 20% succinic acid-diethylene glycol polyester, was used for the regular packed column G.L.P.C. Temperatures employed were: column 180°C., detector cell 233°C., flash heater 234°C. The argon carrier gas pressure was 20 p.s.i. with an outlet flow of 155 ml./min. The ionization voltage applied to the cell electrodes was 900 volts, and a radium D source was used in the cell. All experimental conditions were maintained constant throughout the analysis. The sample was dissolved in n-hexane to make a 1% solution, from which a sample of 2.0 microliters was applied to the column, using a 10-microliter syringe. The recorder sensitivity range was 3×10^{-8} amps.

For capillary column chromatography a 100-ft. stainless steel column 0.010 in. in inside diameter, coated with Apiezon L, was used. Temperatures employed were: column 180°C., detector cell 230°C., flash heater 228°C. The ionization voltage applied to the cell electrodes was 1,250 volts, and a radium D source was used in the cell. The argon carrier gas pressure was 40 p.s.i. with a stream-splitting arrangement used in injecting the sample so that only a small percentage of the sample went through the capillary column. The remainder was vented to the atmosphere, using a scavenging flow arrangement. No sample dilution was used with the capillary column. A 1.0-microliter sample was used in this manner.

For infrared analyses a Perkin-Elmer Model 21 infrared spectrophotometer was used.

Results and Discussion

A sample of commercial vegetable oil (mixed cottonseed and soybean) was hydrogenated to an iodine value of 82 by using hydrogen at 5 p.s.i. pressure at 180–200°C. with 0.17% nickel catalyst. This oil was