reported in the literature but are likely to be correct because they are not based on acetyl or hydroxyl values or the solubility of the acid in ethyl acetate or ether.

The content of saturated acids, 3–3.5%, was confirmed in one sample by the isolation of the saturated acid from the nonhydroxy ester fraction.

The estimation of α,β-glycolic compounds (3, 34) by oxidation with periodic acid is a standard procedure and does not need any elaboration. However, incidental to the present study, it has been shown that the true periodic acid content of a solution is best determined by titration with ferrous ammonium sulfate because the usual iodometric method determines the total oxidative capacity. The iodometric method is however more convenient for assessing the consumption of periodic acid. The recommended method for dihydroxystearic acid gives results agreeing well with those calculated and thus confirms the composition arrived at in this paper. It should also prove useful in estimating dihydroxy compounds in fats and other materials.

Summary

Methyl esters of castor oil were prepared by saponifying the oil with potassium hydroxide in methanol, splitting the potassium soaps *in situ* with an excess of hydrochloric acid, and esterifying at room temperature. The esters had hydroxyl values comparable with those of the parent oils. The methyl esters were quantitatively resolved into hydroxy and nonhydroxy esters after reacting with succinic anhydride in toluene. The composition of castor oil was calculated from a) amount of nonhydroxy esters, b) methyl linoleate content of methyl esters determined spectrophotometrically, c) iodine value of the methyl esters determined by the Wijs method at 15–20°C., and d) iodine value of the nonhydroxy esters determined by the Woburn method. This composition was confirmed by the estimation of saturated acids in one sample and dihydroxystearic acid in all. Castor oil was readily hydrogenated with Raney nickel in alcohol at room temperature (30–33°C.) without any hydrogenolysis of the hydroxyl groups. Methyl dihydroxystearate content of the methyl esters of this hydrogenated oil was determined by reaction with 80–100% excess periodic acid at 15–20°C.

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A Semi-Micro Procedure for the Separation and Degradation of Long-Chain Fatty Acids[1,2]

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STUDIES of the biochemistry of fatty acids have been greatly facilitated by the use of carbon-14 compounds, and such studies have in turn resulted in the development and refinement of semi-micro procedures for the separation and degradation of long-chain fatty acids. In spite of this, there has not yet appeared in the literature a complete semi-micro procedure for the isolation, separation, and stepwise degradation of radioactive fatty acids from plants or animals. Such a procedure is presented in this paper. For the most part, the procedure can be carried out with equipment usually available in the laboratory, and the few pieces of special apparatus which are required can be assembled with little diffi-

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culty or expense. The method of separation of fatty acids is based on the work of Brown (2, 9), and the degradation procedure is a combination and modification of those described by Dauben *et al.* (3, 5), and Mikeska, Smith, and Lieber (8).

Experimental

I. *Extraction and Separation of Component Fatty Acids.* The radioactive fatty acids used in the following procedure were obtained by culturing developing flax fruits *in vitro* in a solution containing sodium acetate specifically labeled with carbon-14 (6). The approximate composition of fatty acids in flax seed oil is 10% palmitic and stearic, 29% oleic, 19% linoleic, and 42% linolenic. To adapt the procedure to a fatty acid mixture obtained from a different plant or animal source, it would probably be only necessary to modify the extraction procedure and to take into consideration the composition and temperature of crystallization of the component fatty acids.

The seeds are dried for 3 hrs. at 70 to 80°C., and the total lipid is extracted with petroleum ether (B.P. 30–60°C.) in a Soxhlet apparatus. The extract is transferred to a flask, and the solvent is evaporated by standing over-night. The lipids are saponified by refluxing for 6 hrs. with 12% alcoholic KOH. The mixture is diluted with two volumes of water, and the unsaponified residue is removed by extracting with diethyl ether. The aqueous solution is acidified with HCl, and the free fatty acids are extracted several times with diethyl ether. The ether solution is dried over anhydrous $MgSO_4$.

The crude fatty acids obtained upon evaporation of the ether are dissolved in anhydrous acetone (1 g. fatty acid/5 ml. acetone). The concentration of fatty acids in acetone depends upon the kind and relative amounts of fatty acids present (7), but the concentration given has proved satisfactory for our purpose.

The acetone solution of fatty acids is placed in a low temperature fractionation chamber. A suitable chamber may be constructed so that the fatty acid mixture in a test tube is surrounded by a low temperature fractionation bath consisting of a 1:1 mixture of chloroform and carbon tetrachloride. The temperature of the bath is regulated by varying the voltage supplied to an aquarium warmer, which is immersed in the bath and by placing small pieces of dry ice directly in the bath. With practice any temperature between —5° and —79°C. may be maintained with a ±2° variation. The temperature of the fatty acid mixture is first maintained at —50°C. for 2 to 3 hrs. The solution is filtered as rapidly as possible through sharkskin filter paper in a funnel surrounded with finely chopped dry ice. The fatty acid fraction which is obtained from the filtrate upon evaporation of the solvent is largely linolenic acid. The solid remaining on the filter paper from this filtration is redissolved in acetone (1 g. fatty acid/ 5 ml. acetone), placed in the sample holder, and maintained at —30°C. for 2 to 3 hrs. The solution is filtered as before, and a fraction which is largely linoleic acid is obtained from the filtrate upon evaporation of the solvent. The solid from this filtration is dissolved in diethyl ether (1 g. fatty acid/10 ml. ether) and fractionated as before at —30°C. for 2 to 3 hrs. The solution is filtered, and the oleic acid fraction is obtained from the filtrate upon evaporation of the solvent. The solid from this filtration is a mixture of stearic and palmitic acid.

If the amount of starting material is sufficiently large, or if a high degree of fatty acid purity is required, the low temperature fractionation may be repeated for further purification. In trial fractionations of non-radioactive fatty acid mixtures, the purity of the fatty acid fractions may be determined by means of iodine values, but in most work with radioactive mixtures this is usually not feasible because of insufficient material.

II. *Hydrogenation of the Unsaturated Fatty Acids.* After separation of the unsaturated fatty acids they should be immediately hydrogenated to prevent isomerization and oxidation. The fatty acid fraction is placed in the side arm of a reaction flask. For each gram of fatty acid to be reduced, 10 ml. of absolute ethanol and 35 mg. of platinum oxide catalyst (1) are placed in the main chamber of the reaction flask. A heating mantle is secured to the flask, and the solution is warmed to 70°C. with shaking. Pure, dry hydrogen gas at low pressure is bubbled through the ethanol solution, and after the appearance of platinum black in the ethanol solution, the fatty acid is emptied into the main chamber from the side arm. Shaking is discontinued, and the reaction flask is tilted several times in order to rinse the side arm. Hydrogen at low pressure is then passed through the solution with shaking for 15 min. Hydrogen is then passed through the reaction mixture at a pressure of 1 atmosphere for an additional 10 min. The solution is cooled and filtered, and the solvent is removed from the filtrate by evaporation. The saturated fatty acid (stearic acid) is obtained in almost 100% yield.

III. *Degradation of the Saturated Fatty Acid.* In this procedure the carboxyl carbon of the fatty acid is removed and isolated as the carboxyl carbon of benzoic acid. A mixture of 910 mg. of stearic acid, 1.5 ml. of freshly distilled thionyl chloride, and 10 µl. of pure pyridine is placed in a 3-neck flask, equipped with a stirrer and reflux condenser. The mixture is heated to 40°C. and stirred for 30 min. Excess thionyl chloride is removed at reduced pressure (30 mm. Hg for 45 min.). The flask is cooled to 0°C., and 3.5 ml. of anhydrous benzene are added. Approximately 1 g. of anhydrous $AlCl_3$ is added in one portion, and the mixture is stirred for 16 hrs. at room temperature. The mixture is cooled to 0°C., and small portions of cold, dilute HCl are added until hydrolysis of the aluminum chloride-complex is complete. The precipitate is removed by filtration and washed with hexane. The filtrate and hexane washings are transferred to a separatory funnel and extracted several times with hexane. The hexane layer is dried over anhydrous $MgSO_4$ and then saturated with dry ammonia gas. The unreacted stearic acid precipitates as the ammonium salt. The stearophenone is crystallized from hexane in a dry ice bath, filtered, and twice recrystallized from hexane.

The stearophenone (990 mg.) is transferred to a 3-neck flask, equipped with a stirrer, reflux condenser, and dropping funnel. Ten ml. of freshly distilled dioxane and 0.6 ml. of concentrated HCl are added, and the mixture is heated to 50°C. A solution of 0.6 ml. freshly distilled (less than 1 hr. old) isoamyl nitrite in 5 ml. of freshly distilled dioxane is placed in the dropping funnel. The contents of the dropping funnel are added to the flask during a

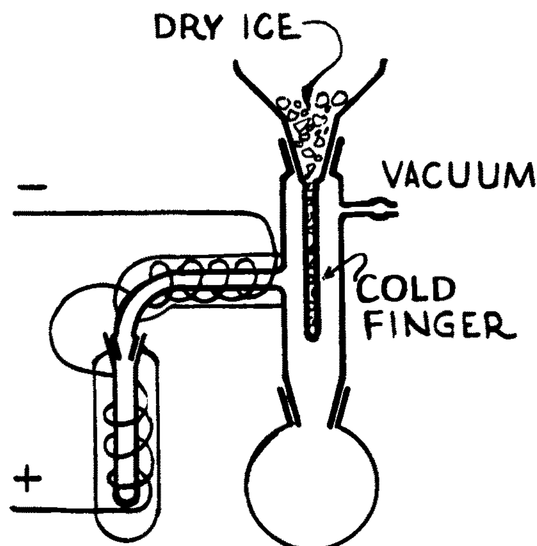

FIG. 1. Micro-sublimation apparatus. The impure benzoic acid (in ether) is placed in the left chamber. After evaporation of the ether, the benzoic acid is sublimed onto the cold finger. It is then shaken down into the round-bottom flask.

45-min. period. This reaction with isoamyl nitrite should be carried out in semi-darkness. After an additional 15-min. reaction period, the solution is made basic with 12 ml. of 3N NaOH. The solution is cooled to 0°C., and 2.0 g. p-toluenesulfonyl chloride are added in small portions over a 20-min. period. The solution is warmed to 50°C. and stirred for 2 hrs. The mixture is diluted with 300 ml. of water, transferred to a separatory funnel, and extracted with several portions of pentane.

The aqueous layer is warmed and then cooled slowly. The large particles of sodium heptadecanoate which precipitate are removed by filtration to yield 15–30 mg. The aqueous filtrate is acidified and extracted with several portions of diethyl ether (peroxide-free), and the extracts are transferred to a sublimation apparatus (Figure 1). After evaporation of the diethyl ether, the benzoic acid is sublimed at 78° to 80°C. and 200 mm. Hg pressure. The benzoic acid may be counted directly for beta radioactivity, or it may be converted to barium carbonate (4, 5). If the acid is counted directly, 8 to 10 mg. are uniformly spread over the surface of a copper planchet, air-dried for 36 to 48 hrs., and the specific activity determined.

The pentane solution is evaporated to dryness, and the remaining heptadecanonitrile is hydrolyzed by refluxing with 10 ml. of 15% KOH in n-propanol for 24 hrs. Hexane is slowly added to the solution until the precipitation of sodium heptadecanoate is complete. The solid is filtered out and recrystallized several times from hexane in a dry ice bath. This product is combined with that obtained previously from the aqueous layer. The combined salt is acidified and extracted with pentane. The free fatty acid is recrystallized twice from pentane in a dry ice bath.

This completes the degradation of the first carbon. The degradation procedure is repeated, using the heptadecanoic acid, and degradations may be continued until an insufficient amount of acid is obtained. Yields of at least 70–75% fatty acid and benzoic acid may be expected. Consequently a minimum starting quantity of 1 g. of fatty acid is required in order to degrade three carbon atoms. The procedure permits the removal of at least six carbon atoms from stearic acid.

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Selective Acidolysis, a Method for the Segregation of Drying and Semi-Drying Oils[1]

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IN RECENT YEARS the glyceride structure of oils and fats has attracted considerable attention. From a consideration of the properties and uses of many oils it becomes obvious that products with improved technical applications can be obtained by the elimination or extraction of certain constituents (1). Thus drying oils contain a number of different fatty acid groups of varying degrees of unsaturation, and their value largely depends upon the proportion in which acids having two or more unsaturated groups are present. By removing the more saturated constituents, the performance of the oil as a film-former can be improved. This might be done either by fractionating the mixture of fatty acids obtained by hydrolysis and reesterifying, or by segregating the glycerides themselves into fractions of different degrees of unsaturation. Both of these methods have been investigated and have resulted in a better understanding of the number and types of glycerides present in drying and semi-drying oils as well as of valuable technical products.

In effecting separations of the oil glycerides, the efficiency of any process is limited by the mixed glyceride structure of the oil. Thus sardine oil, which contains about 25–27% saturated fatty acids, contains only about 0.5% of fully saturated glycerides. The remaining saturated acids are distributed on glyceride molecules also containing unsaturated acids. However with sardine oil a partial separation of the

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