differences were found to be similar in both grains. Germ fats were the most unsaturated, contained the least free fatty acids and the least unsaponifiable matter. Starch fats were 70 to 90% free fatty acids and contained large amounts of palmitic acid. Gluten and fiber fats contained up to 32% unsaponifiables and about 20% free fatty acids.

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The Preparation of Stearolic Acid and Methyl Dideutero-Oleate, and Certain of Their Derivatives[1,2]

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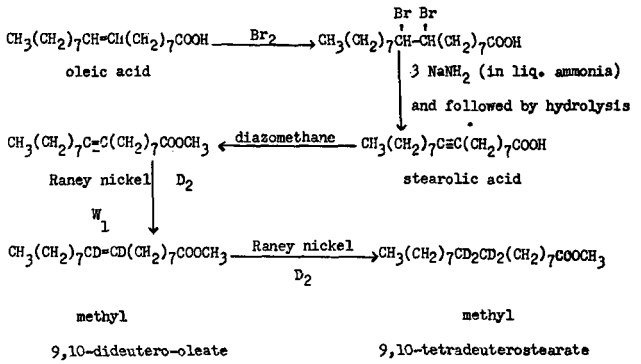
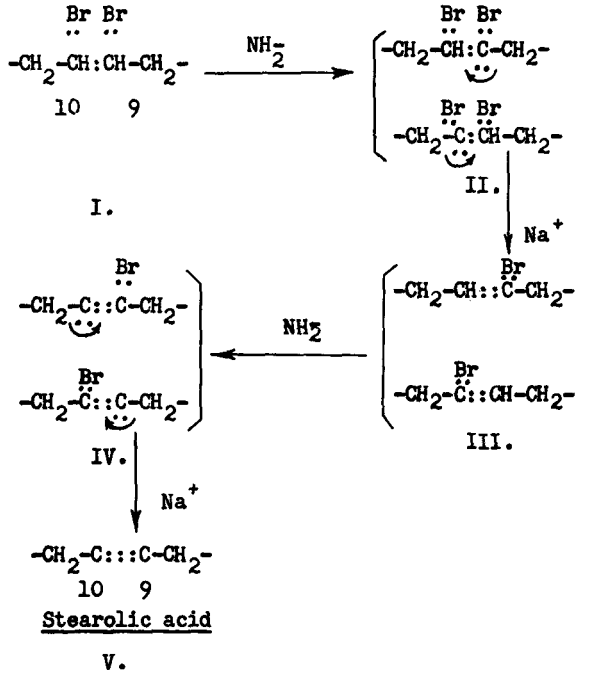
IN connection with proposed studies on the mechanism of the autoxidation of methyl oleate it was desired to prepare methyl dideutero-oleate (methyl 9,10-dideutero-cis-9-octadecenoate). This compound was synthesized by the selective reduction of methyl stearolate with deuterium, using W_1 Raney nickel (1). By fractional crystallization at low temperature (2,3) the 9,10-dideutero-oleate was separated from the unreacted stearolate and the complete reduction product, methyl tetradeuterostearate.

Earlier methods (5-8) and the more recent one published in "Organic Syntheses" (4), in which alcoholic potassium hydroxide was used for the dehydrohalogenation of 9,10-dibromostearic acid and its esters, gave relatively low yields of stearolic acid. However by reaction of sodamide on dibromostearic acid in liquid ammonia, 58-68% yields were readily obtained. Further, dehydrobromination of methyl dibromostearate by this procedure gave the amide of stearolic acid which was hydrolyzed to stearolic acid in relatively better yield and purity.

The over-all scheme of methyl dideutero-oleate synthesis is as follows:



Examination of the ozonization and peracid decomposition products of stearolic acid, thus prepared, showed no evidence of simultaneous formation of allenic and 8- and 10-octadecynoic acids (9). This fact, together with the observed nature of the freezing point curve and the homogeneity of the fractions of methyl stearolate, obtained by fractional distillation, are indicative of its purity. The mechanism of the dehydrobromination may, therefore, be outlined as follows:



The dehydrobromination appears to be completed in two stages, I-III and III-V, each involving 1,2-elimination of a mole of hydrogen bromide, and leaves no room for shifting of triple bond to give isomers.

9,10-Diketostearic acid was obtained in 90% yield by neutral permanganate oxidation of stearolic acid. [Yields of less than 27% have been previously reported (10-11)]. We have also prepared diketostearic acid directly from the oleic acid in 40% yield

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[2] Supported in part by grants-in-aid from the Graduate School and the Institute of Nutrition and Food Technology.
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[cf. much poorer yields by previous methods (12-13)]. In addition, certain other derivatives of stearolic acid were prepared in order to establish its purity.

Experimental

Preparation of Oleic Acid. Oleic acid was prepared from olive oil by two modifications of low temperature crystallization procedures. The first was essentially the method of Brown and Shinowara (2). The saturated acids were mostly removed by cooling a 5% solution of olive oil acids in acetone to —24°C. The oleic acid was then crystallized four times from acetone at —60° and finally freed from a small additional amount of saturated acids by cooling the filtrate to —35°. The product gave the following constants: I.N. (Iodine number) 88.4 (purity about 98%); n^{20}, 1.4597; neutralization equiv., 282.5.

In the second procedure a 5% solution of the acids was left for two days at —20° and the crystals were removed at this temperature; the filtrate was taken down —30° very slowly and left long enough (two or more hours) to remove additional amounts of saturated acids. The filtrate was cooled to —60° and the oleic acid crystals thus obtained were washed once with solvent at the same temperature; I.N., 91.5 (95-97% pure); neut. equiv., 281.5. This 95-97% oleic acid was used in the preparation of stearolic acid in the large scale experiments. The presence of small amounts of linoleic acid did not seem to affect the purity of the resultant stearolic acid.

Preparation of Dibromostearic Acid. Oleic acid was brominated in dry ether solution with the temperature maintained below 5°. A slight excess of bromine was added dropwise to the stirred solution. The excess bromine was discharged by addition of a few drops of oleic acid.

Preparation of Stearolic Acid by Dehydrobromination of Dibromostearic Acid With Sodamide in Liquid Ammonia. A 2-liter, three-necked flask was fitted with a mechanical stirrer, a gas inlet tube, and a block tin condenser, which was cooled with an acetone-dry-ice mixture and connected to drying towers to exclude moisture. One liter of liquid ammonia was introduced into the flask. Ferric chloride (C.P., anhydrous), 0.3-0.4 g., was added with stirring. After 20 seconds metallic sodium (1-2 g.) was dropped into the brown solution, i.e., enough to convert the iron salt into metallic iron (14). After the evolution of hydrogen gas had stopped, the remainder of the sodium (9 g.) was added in small portions within 15-20 minutes. When the violent evolution of hydrogen ceased, grey crystals of sodamine settled out.

The dibromostearic acid solution from 28 g. (0.1 mole) oleic acid (98%) was introduced in a continuous stream from a separatory funnel. After refluxing for six hours with mechanical stirring, an equivalent of solid ammonium chloride (15 g.) was added to neutralize excess sodamide. After complete evaporation of ammonia 300-400 ml. of water was added and the mixture was washed into a beaker and mildly acidified with 6N. H_2SO_4 acid solution. The solid reaction products were extracted with two 300-ml. portions of ether. The ether solution was washed with water and then dried over anhydrous sodium sulfate. After removal of ether the residual acids were crystallized from a minimum amount of petroleum ether at 0-3°; yield, 19 g. of white crystalline needles (68% of theory). Yields of stearolic acid reported in Organic Syntheses (4) range between 33-42%.

Generally, for each mole of oleic acid, 4-5 moles of sodium are required, and for each mole of sodium, 0.3-0.5 g. of ferric chloride and 1 liter of liquid ammonia.

In large scale reactions starting with 250 g. oleic acid (97%), acidulation of the sodium salt from the reaction with HCl was carried out with vigorous stirring and cautious heating in order to melt the crude acids and to avoid entrained soap. On cooling to

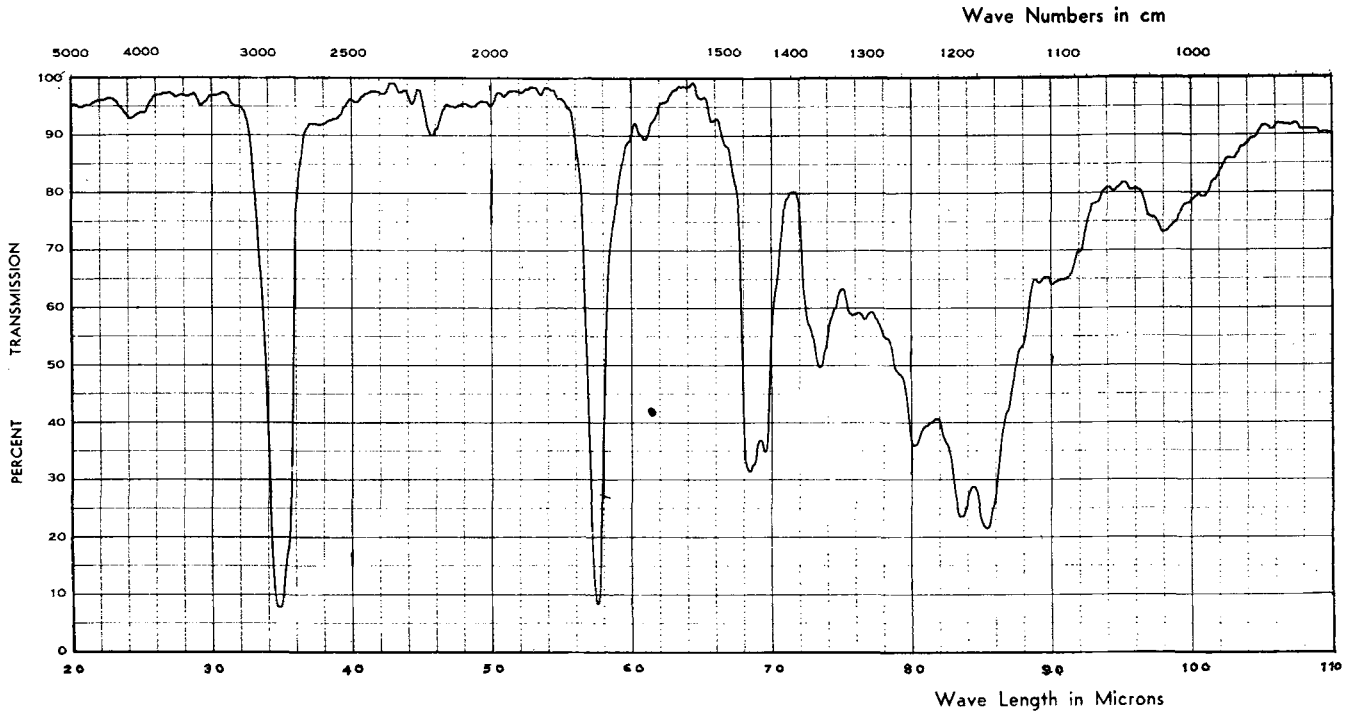

FIG. 1. Infra-red absorption curve, methyl 9,10-dideutero-oleate.

0-2°, the solid layer of acids could be separated and washed with cold water and then freed from adhering water and dried under vacuum. The acids were then dissolved in sufficient petroleum ether (2 l. for 100 g. of acid) and further dried with anhydrous sodium sulfate. Stearolic acid was crystallized 2-3 times from petroleum ether at 2-3°; yield, 145-156 g. (58-62%); m.p. 46-46.5°.

The pure stearolic acid may also be obtained by vacuum distillation of the crude material; b.p. 189-190° at 1.8 mm.; m.p. 46-46.5°. The mixed melting point with stearolic acid made by the method of Organic Syntheses (4) showed no depression. With pure elaidic acid (m.p. 44-44.5°) the melting point was depressed to 40-40.5°. Other data include: I.N., 89.5, (Wijs ½ hr.); $n^{54.5}$, 1.4510; $n^{61.5}$, 1.4484; carbon, 77.58% (theory 77.1); hydrogen, 11.64% (theory 11.43); n.e., 279.6 (theory 280). The UV absorption curve showed slight maxima at 2740 and 2950 Å, similar to the results described by Kass (15). The hydrogen uptake was quantitative.

Dehydrobromination of Methyl Dibromostearate With Formation of Stearolamide

Methyl dibromostearate from 54 g. of methyl oleate was treated with sodamide from 20 g. of sodium (4 moles per mole of methyl oleate) in 1½ liters of liquid ammonia under reflux with stirring. After removing excess sodamide and liquid ammonia, 200 ml. of water was added and then the solution was acidified with HCl. The solid product was filtered off, dissolved in 95% alcohol with heating and twice crystallized at 0-3°; yield, 39 g. white crystalline stearolamide; m.p., 82-83°; N, 5.15% (theory for $C_{18}H_{33}ON$, 5.3%).

Hydrolysis of Stearolamide to Stearolic Acid. Ten g. of the amide was refluxed with an excess of alcoholic potash for 5 hrs., the alcohol removed, the soap dissolved in water and acidified with HCl. The stearolic acid was filtered, washed, dried, and crystallized from petroleum ether; yield, 8.4 g. (84% of theory), m.p., 46-46.5°. The yield based on the amount of methyl oleate used, was 64.8%.

Ketostearic Acid. Stearolic acid was treated with conc. H_2SO_4 according to the method of Robinson (16) to obtain a mixture of 9-keto- and 10-ketostearic acids; yield, 31%, m.p. 72-73°.

Dibromo-oleic Acid and Dibromo-oleyl Alcohol. Stearolic acid did not absorb bromine by the ordinary methods (17) in ether or acetic acid solution at room temperature [cf. Overbeck (18)] but by bromination with pyridine-dibromo-sulfate solution, made by the method of Yasuda (19) and containing 0.0172 g. of bromine per ml., two atoms of bromine were added. After being extracted with petroleum ether, a heavy viscous product resulted upon removal of solvent. Yield, 96.4%; $n^{29.5}$, 1.4998; Br, 38.1% (theory for $C_{18}H_{32}O_2Br_2$, 36.6%).

Attempted replacement of bromine of the dibromo-oleic acid with hydrogen, carried out with $LiAlH_4$ (20) (4 moles of $LiAlH_4$ per mole of acid), was unsuccessful but dibromo-oleyl alcohol was obtained indicating the failure of $LiAlH_4$ in this replacement of olefinic bromine; yield, 89%; $n^{29.5}$. 1.4970; Br, 38.6% (theory for $C_{18}H_{34}OBr_2$, 37.5%).

Stearolyl Alcohol (9-Octadecynol-1). Stearolic acid was allowed to react with $LiAlH_4$ [25% excess (20)] in ether solution under an atmosphere of dry nitrogen. After the usual addition of water and acidification the stearolyl alcohol was extracted with ether and the ether solution washed with water, dried, and the solvent then removed. The product was dissolved in petroleum ether and crystallized at 0°, giving water white leaflets. The yields from two runs were 87 and 91%. Analytical data were: m.p., 33-34°; $n^{54.5}$, 1.4520; $n^{61.5}$, 1.4489; mol. wt. from acetyl no. 266.5 (theory 266); acetyl no., 181.5 (29); C, 81.93% (found), 81.2 (theory); H, 13.19% (found), 12.79 (theory). Quantitative microhydrogenation showed an uptake of 1.97 moles.

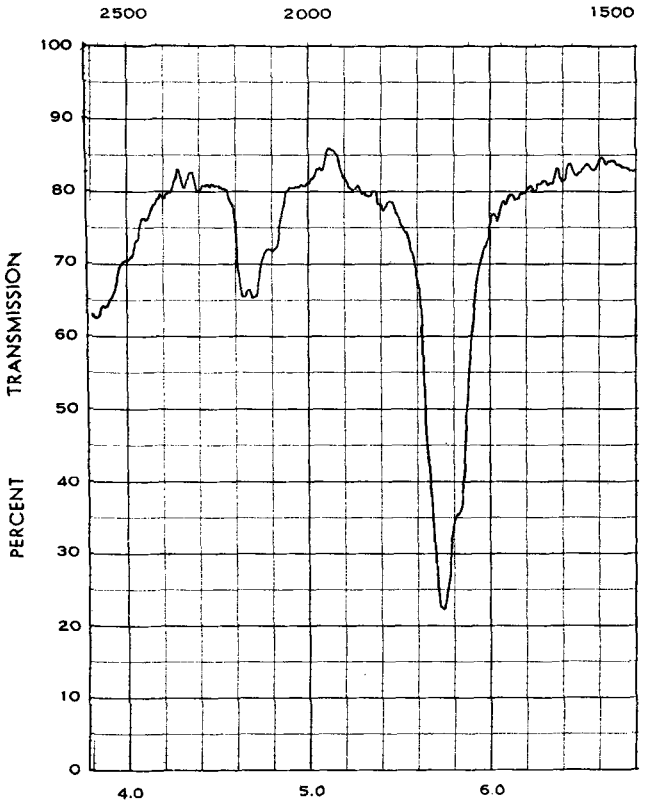

FIG. 2. Infra-red absorption curve, methyl 9,10-tetradeuterostearate and 9,10-tetradeuterostearic acid.

Stearolamide and stearolyl and dibromo-oleyl alcohols have not been previously described.

Ethyl Stearolate was made by the usual azeotropic method (21). The yield was 96%; b.p. 180-181° at 2.5-2.8 mm.; n^{20} 1.4555; $n^{29.5}$ 1.4515; sap. equiv., 308.4; I.N., 82.05; hydrogen uptake, 1.97 moles.

Methyl Stearolate. The acid was esterified by reaction with diazomethane (22) in ether solution. The neutral ester was obtained by passing the solution through a column of alumina followed by distillation under vacuum. Six fractions were obtained by distilling 18 g. of the ester through a Todd Scientific microscale column at 0.3 mm. These fractions gave essentially the same refractive index as the original ester, indicating the homogeneity of the product: n^{20}, 1.4562; n^{26}, 1.4545; $n^{52.5}$, 1.4435; $n^{62.5}$, 1.4394; d^{26}, 0.8933; M.W. 293 (theory, 294); b.p. 174-175° (2.6-3 mm.); sap. equiv., 294.9; I.N. 86.2. Microhydrogenation showed a theoretical uptake of hydrogen.

9,10-Diketostearic Acid From Stearolic Acid. 2.8 g. of stearolic acid, dissolved in an equivalent of NaOH in two liters of water with mild warming was cooled to +10° and mechanically stirred while permanganate solution (3 g. in 125 ml. of water) was added in one

portion and stirred for 20-25 minutes. The solution was decolorized with SO_2 gas and then acidified with HCl. The yellowish precipitate was filtered, washed, and dried. The product was then dissolved in hot 80% alcohol and crystallized at 2-3°. Yield, 2.8 g. (90%); yellowish leaflets, m.p., 84.5-85.5°; mixed melting point with an authentic sample showed no depression; C, 69.53%; hydrogen, 10.15% (calculated for $C_{18}H_{34}O_4$, 69.24 and 10.25% respectively). The UV absorption curve showed maxima at 2650 and 4250 Å [cf. Burr (23)].

The following procedure was used to prepare diketostearic acid without actually isolating stearolic acid as an intermediate. The dibromostearic acid from 14 g. of 97% oleic acid was dehydrobrominated as before, except that a large excess (2 liters) of liquid ammonia was used. The reaction products containing crude sodium stearolate were transferred to a 4-liter beaker with 2 liters of water. After neutralization (phenolphthalein) with HCl, the stearolate was oxidized as described above; yield 6.2 g. (about 40% based on original oleic acid).

Oxidative Degradation of Stearolic Acid. Ozonization (24) of 2 g. of stearolic acid in 50 ml. acetic acid for 4 hours (25) gave, after hydrolysis and crystallization, 1.04 g. of azelaic acid (80% of theory); m.p., 105-106°; there was no melting point depression with an authentic sample of azelaic acid.

Oxidation with 3-4% peracetic acid in acetic acid or with performic acid in formic acid (26-27) in excess and at room temperature resulted in complete destruction of the stearolic acid. Only 66-72% of the theoretically possible azelaic acid was recovered.

Selective Reduction of Stearolic Acid and Methyl Stearolate. In preliminary hydrogenation trials leading up to the synthesis of methyl dideutero-oleate, oleic acid, free of more unsaturated substances, was prepared by hydrogenating in Joshel's apparatus (25) 5 g. of stearolic acid in 100 ml. of dioxane in the presence of W_1 Raney nickel (8-10% of wt. of substrate) at ordinary temperature and pressure; hydrogenation was continued until 1.03-1.05 moles of hydrogen were absorbed. After removal of the catalyst by filtration and the solvent by evaporation *in vacuo,* the product from 3 or 4 runs was dissolved in dry alcohol-free ether [4] to make a 10% solution. This was slowly cooled to —35° and kept at this temperature for more than 2 hours with occasional stirring after first appearance of the crystals. After inverted suction filtration the mother liquor containing oleic acid was again crystallized at —35° to remove additional small amounts of crystalline material which was mostly oleic acid. The final filtrate was dried with anhydrous sodium sulfate and the solvent removed and the residue distilled at 1.8-2.0 mm.; b.p., 183-184°; yield, 60-70% of theory; I.N., 89.4 (theory, 90); n^{20}, 1.4600.

Methyl oleate, free of more unsaturated impurities, was similarly prepared, except that crystallization was carried out at —41 to —43°; b.p. 171-172° at 1.9-2 mm.; yield, 67-71% of theory; I.N., 85.1 (theory, 85.75); n^{20}, 1.4521. Microhydrogenation for both products showed a theoretical absorption of hydrogen.

Synthesis of Methyl 9,10-Dideutero-oleate. The catalytic reduction of methyl stearolate with deuterium [5] and purification of methyl 9,10-dideutero-oleate were carried out by the procedures mentioned above for methyl oleate; yield, 65-75% of theory; b.p. 173-174° at 1.8-2.0 mm.; I.N., 84.7 (theory, 85.23); n^{20}, 1.45186; d^{26}, = 0.8938; molecular refractivity, 89.8; sap. equiv., 299.3 (theory, 298); quantitative uptake of one mole of deuterium in presence of Raney nickel, W_1; D, 5.05% (theory for $C_{18}H_{32}D_2O_2$, 5.55%).

The infra-red curve (Fig. 1) showed a maximum at 4.48 microns characteristic of the C-D bond; no absorption at 10.36 microns proved the absence of the trans form.

Preparation of Methyl 9,10-Tetradeuterostearate. The further reduction of methyl dideutero-oleate to methyl 9,10-tetradeuterostearate was successful; yield, 98-99% of theory; m.p., 38-38.5°.

Preparation of 9,10-Tetradeuterostearic Acid. The ester was hydrolyzed by saponification to give the acid; m.p., 69-69.2°. Infra-red analysis of the tetradeutero ester and acid in tetrahydrofurane as solvent (Fig. 2) showed maxima at 4.65 and 4.7 microns, characteristic of the $C\text{-}D_2$ bond. The deutero compounds, methyl dideutero-oleate, tetradeuterostearate, and tetradeuterostearic acid, have not been previously described.

Summary

1. Improved methods of synthesizing stearolic acid and 9,10-diketostearic acid in high yields are described. Dehydrobromination of dibromostearic acid by sodamide in ammonia is shown to be a very smooth and practical reaction.

2. Dibromo-oleic acid, methyl stearolate, and ethyl stearolate have been prepared and characterized.

3. Stearolamide, dibromo-oleyl alcohol, and stearolyl alcohol (9-octadecynol-1) have been synthesized and their properties described.

4. Selective hydrogenation and low temperature crystallization have been utilized to prepare oleic acid from stearolic acid and methyl oleate and methyl 9,10-dideutero-oleate, free of other higher unsaturated substances, from methyl stearolate.

5. Complete reduction of dideutero-oleate yielded 9,10-tetradeuterostearate which, on hydrolysis, gave tetradeuterostearic acid.

6. Infra-red absorption curves for 9,10-dideutero-oleate, tetradeuterostearate, and tetradeuterostearic acid are also reported.

Acknowledgment

The authors wish to thank Prof. Christopher Wilson for the infra-red spectra and Prof. Herrick Johnston for the deuterium analysis by mass-spectroscopy.

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[4] For ordinary purposes these restrictions of solvent (free of labile hydrogen) and apparatus are not necessary.

[5] Deuterium gas was obtained as 99.5% pure from Stuart Oxygen Company by the permission of A.E.C., and all possible measures were taken to avoid exchange of deuterium with hydrogen in the system.

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Molecularly Distilled Monoglycerides. III. Nutritional Studies on Monoglycerides Derived From Cottonseed Oil[1,2]

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TRIGLYCERIDES, from a quantitative viewpoint, are the most important lipids of foods and of tissues and have been of prime interest to the lipid biochemist for a long time. However certain intermediate products of triglyceride metabolism, mono-, and di-glycerides, have received attention in recent years. Monoglycerides, in particular, have been studied with respect to their beneficial role in fat absorption from the intestinal tract (1) and to their presence normally in pancreas and other tissues (2). Furthermore Tidwell (3) has shown that monoglycerides of olive oil fatty acids were more efficiently absorbed than olive oil. Also the nutritive properties of monostearin and monolinolein were investigated by Braun and Shrewsbury (4), who indicated that these two monoglycerides were "practically equal" to lard in promoting the growth of young rats.

These experiments were designed to investigate the problem of whether relatively large amounts of monoglycerides of a natural mixture of fatty acids would be utilized *in vivo*, as efficiently as natural fats composed of triglycerides of the same mixture of fatty acids. Monoglycerides of the fatty acids of cottonseed oil have been compared with cottonseed oil itself at 15% and at 25% levels in the diet of three generations of rats, for adequacy in supporting growth, reproduction, and lactation. Results indicate that for all these functions cottonseed oil monoglycerides are equal to the fat from which they were prepared.

Experimental

Diets. The detailed composition of the diets is given in Table I. The additional fat was added at the expense of a portion of both cerelose and starch. The cottonseed oil was deodorized, refined salad oil (Wesson oil), and the cottonseed oil monoglycerides were a commercial product (Myverol Type 18-85 Monoglyceride) made by molecular distillation (5). Each diet was prepared to contain 400 mg. of vitamin E/kg. The cottonseed oil contained 0.75 mg./gm. of vitamin E and the cottonseed oil monoglyceride, 0.22 mg./gm.; the extra amount needed was furnished as distilled d,α-tocopherol concentrate. Fresh diets were prepared weekly, and the prepared diets were stored in the refrigerator until used. No increase in peroxide value (about 2) of the diet was observed on storage for one week. All diets were fed *ad libitum*.

TABLE I

Composition of Diets

Ingredient	Content (%)
Casein, crude	16.0
Casein, vitaminized	2.0
Salt mixture, U.S.P. No. 2	4.0
Yeast, dried brewers	7.8
Liver extract, Wilson's 1-10	0.2
Lipid, cottonseed oil or monoglycerides	15.0 or 25.0
Corn starch	36.7 or 30.0
Dextrose (Cerelose)	18.3 or 15.0

Vitamins: per 10 grams of diet—

A—400 I.U.	B_1—100 μg.	Ca-pantothenate—250 μg.
D— 40 I.U.	B_2—100 μg.	Niacin—200 μg.
E— 4 mg.	B_6—100 μg.	Choline—10 mg.
K—500 μg.		Inositol—5 mg.

Treatment of Animals. Eighty albino rats, forty males and forty females, were selected at time of weaning from our stock colony. The animals (10 per group) were placed immediately on the four diets previously described; 15% and 25% cottonseed oil, and 15% and 25% cottonseed oil monoglycerides. The growth of these animals was observed for an 8-week period, at which time reproduction and digestibility experiments were started. Six females and two males were selected from each dietary group for breeding purposes. Throughout the breeding and lactation period the females were maintained on the same diets previously fed. The males were interchanged between breeding cages and subsisted for short periods on diets other than those to which they were originally assigned. The young of the original ani-

TABLE II

Growth Response of Male Rats (8-Week Period)

Level of Fat	Generation	Mean Gain ± S.E. (gms.)	
		Cottonseed Oil	Monoglycerides
15%	1	219.3± 9.5	213.9±6.5
	2	251.1± 9.4	220.5±5.9
	3	225.7± 2.7	235.9±6.7
25%	1	217.3± 9.5	228.0±8.5
	2	226.9± 4.2	219.0±7.7
	3	226.5±10.5	219.8±5.3
Over-all Mean		227.8± 4.0	222.9±2.8

Diff. of Means = 4.9; S.E. of Diff. = 5.0; t = 0.98; p = 0.3-0.4.

Each group contained approximately 10 animals. The over-all mean gain for cottonseed oil is based on 61 animals and for monoglycerides on 59 animals.

[1] Presented at San Francisco fall meeting, American Oil Chemists' Society, Sept. 26-28, 1950.
[2] Communication No. 174.