CCCCIX.—*Investigation of the Constitution of Glycerides in Natural Fats. A Preliminary Outline of Two New Methods.*

By THOMAS PERCY HILDITCH and COLIN HENRY LEA.

QUANTITATIVE information in regard to the composition of the natural fats is much less available than in the case of most natural organic materials. It is only within the past few years that trustworthy data have been forthcoming as to the quantitative composition of the fatty acids present in combination in natural fats, and even at the present time the fats for which such figures are available form but a small proportion of the total number of those in common use. Information as to the manner in which these fatty acids are combined with glycerol in the natural fats is still more lacking, and it is indeed no exaggeration to say that at present we are without anything approaching quantitative knowledge of the structure of the glycerides in any of the natural fats.

The qualitative detection of individual solid glycerides by subjecting natural fats to exhaustive crystallisation from selected solvents has received much attention by several investigators, for example, Klimont (*Monatsh.*, 1902, **23**, 51; 1903, **24**, 408; 1904, **24**, 929; 1905, **26**, 563; 1912, **33**, 441; etc.), Bömer (*Z. Unters. Nahr. Genussm.*, 1907, **14**, 90; 1909, **17**, 353; 1913, **25**, 321; 1920, **40**, 97; 1924, **47**, 61; etc.) and Amberger (*ibid.*, 1913, **26**, 65; 1918, **35**, 313; 1923, **46**, 276, 291; 1924, **48**, 371; etc.); as Klimont has emphasised, it is difficult (if not impossible) to obtain complete separation of any one component by this procedure.

The present communication is a description of the results so far obtained in some attempts to attack this complex problem by the application of new principles. We are not yet in a position to give a complete analysis of all the glycerides composing any natural fat, but there is reason to believe that further progress along the lines of investigation which we have developed will lead to more complete results in this direction. Many of the products which result from our treatment of the fats are novel in type, and new methods of separation must perforce be devised as the work develops; it is therefore probable that complete execution of the scheme we have in mind will occupy considerable time. Our procedure, however, now permits us to ascertain for the first time the proportion of completely saturated triglycerides present in a fat, and also to suggest certain general features of the structure of natural fats which have already become apparent.

General Description of the Methods employed.—The primary objective has been to alter the chemical characteristics of the glycerides in such a way that, without disturbance of the combined glyceryl radical, new glycerides may be formed which are separable more readily than the original natural mixture.

Two methods have been applied for this purpose, both of them processes of controlled oxidation which have been applied hitherto to the simple esters of the higher unsaturated fatty acids:

1. E. F. Armstrong and one of us (*J. Soc. Chem. Ind.*, 1925, **44**, 43T) showed that the methyl or ethyl ester of a higher unsaturated fatty acid, when dissolved in acetone and submitted to the action of powdered potassium permanganate, broke down almost quantitatively into a monobasic fatty acid and the hydrogen ester of a dicarboxylic acid:

$$CH_3\cdot[CH_2]_7\cdot CH\!:\!CH\cdot[CH_2]_7\cdot CO_2Me \longrightarrow CH_3\cdot[CH_2]_7\cdot CO_2H + HO_2C\cdot[CH_2]_7\cdot CO_2Me.$$

We have now found that the same decomposition can be effected in the case of neutral glycerides without appreciable rupture of the glyceryl ester-linking. In cases where the unsaturated glycerides are derived from either oleic, linoleic or linolenic acid, the products of oxidation are, in addition to nonoic, hexoic or propionic acid, complex glycerides united to at least one azelaic acid residue (the remaining carboxyl group of which is free). If the possible combinations of glycerol with saturated fatty acid ($S\cdot CO_2H$) and oleic acid ($U\cdot CO_2H$) be considered, it will be seen that the following products may arise:—

	Original glyceride.	Product of oxidation.
Fully saturated	$C_3H_5(O\cdot CO\cdot S)_3$	$C_3H_5(O\cdot CO\cdot S)_3$
Mono-oleo-derivative ...	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)_2}{\diagdown(O\cdot CO\cdot U)}$	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)_2}{\diagdown(O\cdot CO\cdot[CH_2]_7\cdot CO_2H)}$
Dioleo-derivative	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)}{\diagdown(O\cdot CO\cdot U)_2}$	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)}{\diagdown(O\cdot CO\cdot[CH_2]_7\cdot CO_2H)_2}$
Triolein	$C_3H_5(O\cdot CO\cdot U)_3$	$C_3H_5(O\cdot CO\cdot[CH_2]_7\cdot CO_2H)_3$

The acid azelaic glycerides (of which it is necessary in due course to synthesise individual representative members, in order to study and compare their characteristics) form as a rule a somewhat complicated mixture, difficult at present to separate satisfactorily. Only general discussion of these products is therefore offered at the moment, and their detailed examination is reserved for a future communication : methods for quantitative separation of the total neutral products (*i.e.*, completely saturated glycerides) from the acidic products of oxidation (emanating from any glycerides containing unsaturated fatty acids) are, however, given.

2. It was found by one of us (J., 1926, 1828) that hydrogen peroxide in concentrated acetic acid solution effected the practically complete conversion of oleic acid or of methyl oleate into a corresponding dihydroxystearic derivative. The dihydroxystearic compounds are in general distinguished from the simply fatty acids by their higher melting points and sparing solubility in most solvents, and it was thought that similar treatment of the natural fats might convert them into mixtures more amenable to fractional crystallisation than the original materials. The products to be expected may be given as follows :—

Glyceride.	Product.
Fully saturated	$C_3H_5(O\cdot CO\cdot S)_3$
Mono-oleo-derivative	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)_2}{\diagdown(O\cdot CO\cdot[CH_2]_7\cdot CH(OH)\cdot CH(OH)\cdot[CH_2]_7\cdot CH_3)}$
Dioleo-derivative	$C_3H_5\genfrac{}{}{0pt}{}{\diagup(O\cdot CO\cdot S)}{\diagdown(O\cdot CO\cdot[CH_2]_7\cdot CH(OH)\cdot CH(OH)\cdot[CH_2]_7\cdot CH_3)_2}$
Triolein	$C_3H_5(O\cdot CO\cdot[CH_2]_7\cdot CH(OH)\cdot CH(OH)\cdot[CH_2]_7\cdot CH_3)_3$

This method has not, up to the present, yielded results quite so promising as the more disruptive oxidation, partly in consequence of complications in the mode of addition of the hydroxyl radicals to the ethylenic linkage which escaped notice in the earlier work on the action of hydrogen peroxide upon oleic acid or its simple esters (compare Experimental, p. 3114). Nevertheless, the unsaturated glycerides can be converted completely into dihydroxylated derivatives by this procedure and its employment in conjunction with the other method has assisted us in formulating the conclusions now put forward.

Discussion of the Results obtained.—We shall refer mainly in this paper to the data which we have collected on the constitution of the glycerides of mutton tallow, cacao butter, and cotton-seed oil. We hope at a later stage to report in fuller detail upon the constitution of individual fats (in addition to the above, fats such as lard, palm-kernel oil, palm oil, Chinese vegetable tallow, Illipé fat, and other vegetable and animal fats are included in the scheme of investigation by these methods in these laboratories), but we desire here to draw comparisons between different types of fat rather than to deal with individual cases.

The composition of the fatty acids combined in mutton tallow, cacao butter and cotton-seed oil is given by Armstrong and Allan (*J. Soc. Chem. Ind.*, 1924, **43**, 216T) as :

	Mutton tallow.	Cacao butter.	Cotton-seed oil.
Myristic	1·5	—	—
Palmitic	21·0	23·2	23·4
Stearic	30·0	33·6	—
Oleic	43·0	41·8	23·0
Linoleic	5·0	1·4	53·6

We may add that the fatty acids of the cacao butter used in the present experiments gave, on separation and fractional distillation of the methyl esters of the saturated and the unsaturated portions, figures in good general agreement with the above : Palmitic 24·3, stearic 34·5, oleic 39·2, linoleic 2·0.

By applying the permanganate–acetone process of oxidation to these fats, we have obtained the following figures for the percentage of completely saturated glycerides present in the original fat; the results of two separate oxidations are given in each case :

	Completely saturated glycerides, %.	Mean equivalent of fatty acids.
Mutton tallow	24·7	268·2
	26·2	266·5
Cacao butter	Below 5 *	275·4
	4·8	—
Cotton-seed oil	1·6 *	—
	1·5	—

* These figures represent maximum, rather than absolute, percentages present.

The non-volatile acidic products of oxidation, as already mentioned, are low-melting materials the complete separation of which may require considerable time; and we therefore limit discussion for the time being to the general relations disclosed between the distribution of saturated and unsaturated fatty acids in the glycerides of vegetable and animal fats.

In mutton tallow, which contains 26% of completely saturated glycerides, the fatty acids of which have a mean equivalent of 266·5, we see that, to a first approximation, the proportions of fatty acids in the unsaturated glycerides are, by difference, about 35% of saturated and 65% of unsaturated acids.

On the other hand, the relative composition of the unsaturated glycerides of cacao butter, allowing for 5% of fully saturated glycerides (p. 3109), is about 56·5% of saturated fatty acids and 43·5% of unsaturated fatty acids.

The mean composition of the unsaturated glycerides lies in each case between that of a mono-oleic and that of a dioleic glyceride, but clearly approximates more closely to the former in cacao butter and to the latter in tallow.

So far as cacao butter is concerned, indeed, there is evidence that the predominating components are mono-oleic derivatives, and that oleopalmitostearin is an important constituent. In one of the oxidations carried out as described on p. 3112, the separation of a considerable quantity of an insoluble soap-like mass was observed at the interface between the ethereal solution and the aqueous sodium carbonate. This material was separated by filtration, washed with ether, and then acidified in the usual manner; a hard fatty substance was then obtained which possessed a saponification equivalent of 191 and an equivalent by direct titration of 703 (oleopalmitostearin requires respectively 191·5 and 766); the amount isolated in this manner (which is from the nature of the case not likely to be the whole of that present) was 60% of the total acidic glycerides produced.

The alternative method of oxidation of cacao butter in acetic acid solution with hydrogen peroxide leads to much the same conclusion. Large amounts of apparently homogeneous material, either in the form of dihydroxylated glyceride or as the monoacetyl derivative of the latter (compare p. 3114), were obtained; the products approximated in composition to glycerides containing palmitic, stearic, and dihydroxystearic acids in proportions corresponding closely with those of the palmitic, stearic, and oleic acids present in the original fat.

Whilst dioleo-derivatives are of course present to a certain extent in cacao butter, we are definitely led from our experience with both methods of oxidation to conclude that mono-oleo-derivatives predominate, and there is considerable evidence that oleopalmitostearin is an important component of the fat. This conclusion is not incompatible with Klimont's results (*loc. cit.*), but is not in accordance with those of Amberger and Bauch (*Z. Unters. Nahr. Genussm.*, 1924, **48**, 371).*

It has been customary in some quarters to conclude that those vegetable fats (of which cacao butter is a notable example) which combine the especially useful qualities of non-greasy, brittle texture and low melting point owe these properties to the presence of certain selectively produced glycerides, and the impression is widely held that certain plants tend to elaborate specific types of glyceride. Our investigations, so far, have led to a somewhat different conclusion, namely, that the fatty acids in vegetable glycerides are distributed impartially rather than selectively, and that there is a distinct tendency towards even distribution of unsaturated acids throughout the whole fat, so that the occurrence of simple triglycerides, and even of mixed saturated glycerides, is reduced to a minimum. If this be the case, the physical properties of a mature vegetable fat depend primarily upon the mixture of fatty acids from which it emanates.

Hence, in the case of cacao butter, in which the proportions of palmitic, stearic and oleic acids approach equality relatively more closely than in most fats, it is not unnatural that the final mixture resembles an individual glyceride in properties more nearly than a fat in which the variation in kind and proportion of the component fatty acids is more pronounced.

Again, permanganate–acetone oxidation of cotton-seed oil has proved that entirely saturated glycerides are present only in very small proportion, although 25% of the combined fatty acids is palmitic acid; this acid is thus practically wholly in combination as mixed glycerides with the unsaturated fatty acids, which in this oil are present in the proportion of about three molecules per molecule of palmitic acid.

Also, work on palm-kernel oil, at present in an early stage in these laboratories, has already indicated that the oleic acid present is combined almost entirely with saturated fatty acids in mono- or di-oleic glycerides.

The only animal fat yet studied by these methods, mutton tallow, has yielded data which point to an entirely different state of affairs, the amount of fully saturated glycerides present being far larger than that in the vegetable fats. We prefer, naturally, to defer further discussion of the animal fats until we have collected a wider range of analytical data.

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* These authors state that cacao butter contains 55% of palmitodiolein, 25% of oleodistearin, and 20% of oleopalmitostearin; the calculated iodine value of the original fat is thus 45·7, whereas the recorded figure is 38·6.

EXPERIMENTAL.

I. *Oxidation of Glycerides with Potassium Permanganate in Acetone Solution.*—The fats employed were of best edible quality and were submitted to oxidation in the neutral condition, *i.e.*, after removal of free fatty acids by washing with dilute aqueous alkali.

The procedure employed in working up the products of oxidation differed somewhat in detail with the various fats oxidised, but a typical experiment with mutton tallow may be described.

Mutton tallow. The tallow (100 g.) was dissolved in anhydrous acetone (1000 c.c.), and powdered potassium permanganate (250 g.) added in small quantities with frequent shaking; the heat of reaction was sufficient to keep the acetone in vigorous ebullition (reflux condenser). The mixture was refluxed for 4 hours and kept overnight. (The admixture of sufficient anhydrous magnesium sulphate with the permanganate to neutralise potassium hydroxide liberated during oxidation is a further precaution which we have employed in some cases in order to restrain any tendency to glyceride saponification.) The acetone was distilled off and the residue after admixture with water was treated with sodium hydrogen sulphite and 30% sulphuric acid (the slightest possible excess of free sulphuric acid being maintained) at a moderate temperature until decolorised. The product was extracted several times with somewhat large quantities of ether. In the particular experiment described, the first ethereal extract deposited on standing a white, crystalline solid, which was recrystallised from acetone (A). The combined ethereal extracts were washed free from mineral acid, evaporated to dryness, and combined with the residue from the acetone mother-liquor of (A). This product was dissolved in a little hot alcohol and titrated against *N*-alcoholic potash; the neutralised solution was now poured into aqueous sodium carbonate and extracted repeatedly with ether to remove neutral matter.

The acidic glyceride products of oxidation are not removed efficiently by simple agitation of the ethereal solution with aqueous sodium carbonate, and as an alternative to the potash neutralisation process outlined above, the following method has been used, particularly when the proportion of fully saturated glyceride in the original fat is fairly large (as in mutton tallow and palm-kernel oil).

The crude oxidation product is boiled with a slight excess of sodium carbonate until a clear layer of neutral fat separates on the surface. The aqueous layer, still retaining a considerable amount of emulsified neutral fat, is syphoned off, and the separated neutral product washed by boiling with water until free from alkali. The united alkali extract and washings are now repeatedly extracted with ether as above.

By these methods a separation is ultimately achieved yielding on the one hand the acidic products of oxidation substantially free from neutral glycerides and on the other the neutral product containing only traces of acid substances. Thus in the experiment cited above, the following results were obtained :

A. White solid separated from ether and acetone—completely neutral, 12·5 g.

B. " Neutral " residue from ether, still containing a little soap, 24 g.

C. Acidic glycerides recovered from alkali solutions : partly freed from monobasic (nonoic) acids produced in the oxidation by heating at 95—100°/2 mm. until the weight became constant. Not submitted to further oxidation, 66·4 g.

B possessed an iodine number of 7·5 (original tallow, iodine number 41·2) and was reoxidised with 4 times its weight of powdered permanganate in acetone solution; the products were worked up by a combination of the methods described above, yielding 15·3 g. of neutral products (D) and 5·1 g. of acidic products (E).

Thus there were obtained in all 27·8 g. of crude neutral glyceride (A and D) and 71·5 g. of acidic glyceride (C and E).

The crude neutral glyceride (27·8 g.) was finally purified by boiling with dilute sodium carbonate solution and then repeatedly with water, the aqueous and alkaline liquors being then united as before and exhaustively extracted with ether. There was thus obtained a total proportion of 26·4% of fully saturated glycerides in the original tallow.

The fully saturated glyceride—iodine number 0·8—gave on saponification a mixture of fatty acids the mean equivalent of which was 266·5, corresponding to 59·8% of palmitic and 40·2% of stearic acid.

In another independent oxidation of mutton tallow, the fully saturated glycerides were 24·7% of the total fat—the fatty acids having a mean equivalent of 268·2.

Cacao butter. Two similar oxidations of cacao butter were undertaken with the following results :

		Expt. I.	Expt. II.	Calc.*
First oxidation	neutral product	22·1	24·7	
	iodine number	6·5	8·9	
Re-oxidation of neutral product	fully-saturated glycerides	4·8	4·8	
	iodine number	1·6	1·1	
	mean equivalent of combined fatty acids	275·4		
Combined acidic products (free from monobasic acids)		79	79·5	81·9
Mean saponification equivalent		174·0	174·4	174·7

* On the basis of 5% and 1·5% of saturated glycerides respectively.

Cotton-seed oil. The final general results obtained so far are :

	Expt. I.	Expt. II.	Calc.*
Fully saturated glycerides	1·6	1·5	
Combined acidic products (free from monobasic acids)	(80) †	68·3	73·4
Mean saponification equivalent		123·1	123·2
Combined acidic products (solids insoluble in cold ethyl acetate)	53·6		
Mean equivalent	137·6		

* On the basis of 5% and 1·5% of saturated glycerides respectively.

† Not freed from nonoic acid.

II. *Oxidation of Glycerides with Concentrated Hydrogen Peroxide and Acetic Acid.*—Neutral unsaturated fats are oxidised with much the same ease as the corresponding fatty acids or methyl esters when agitated at 40—80° with excess of concentrated hydrogen peroxide in moderately concentrated acetic acid solution. The products contain, however, not only glycerides of dihydroxystearic acid, but also a considerable proportion of the latter in the monoacetylated form. The presence of combined acetic acid in these compounds was apparent at an early stage of the investigations and, whilst at first it seemed possible that displacement of one or other higher fatty acid radical by that of acetic acid had occurred, we were able to satisfy ourselves that this took place, if at all, only to an insignificant extent, and that the quantitative data at our disposal definitely pointed to acetylation of one of the hydroxyl groups introduced.

The conditions employed in earlier work by one of us (Hilditch, J., 1926, 1828) on the oxidation of methyl oleate or oleic acid by this reagent probably led to the presence of any monoacetyl derivatives of dihydroxystearic acid or its methyl ester being overlooked, and we hope shortly to return to the study of this action in its simpler applications in the light of the present results.

In the meantime the presence of a considerable degree of acetylation in the products from hydrogen peroxide oxidation of the neutral glycerides has unfortunately complicated matters seriously, since it is obviously very difficult (although apparently not impossible) to remove the acetyl residues without at the same time hydrolysing the glycerides themselves. It is possible to reduce the amount of acetylation to some extent by conducting the process in more dilute acetic acid and at as low a temperature as possible (40—50°), but the oxidation then becomes very slow and is difficult to carry to completion even after several days. We consider at present that the more promising procedure is to operate in concentrated acetic acid solution at a fairly high temperature; the oxidation then proceeds rapidly to completion. A description will be given of preliminary results obtained in this way with cacao butter and mutton tallow.

Cacao butter. Cacao butter (100 g.) was heated with glacial acetic acid (400 c.c.) in a wide-necked conical flask provided with a good mechanical stirrer and immersed in a water-bath maintained at 75—80°. 35% Hydrogen peroxide solution (50 c.c.) was added, further additions of 25 c.c. of this solution being made after 2 hours and after 5 hours from the commencement. After a total period of 9 hours at 75—80° with constant stirring, the product was cooled and poured into water. A large quantity of hard, tough solid separated and was removed, and the filtrate was made alkaline with excess of sodium carbonate solution and then extracted with ether several times. The solid cake was dissolved in the ethereal solution, and the whole washed with water.

The residual fat from the washed ethereal extract was boiled in water with a slight excess of dilute sodium carbonate solution, settled, and separated from the aqueous layer by siphoning off the latter, and then washed similarly and repeatedly with boiling water until neutral. The aqueous and alkaline washings were united and re-extracted with ether. There were thus obtained 101·6 g. of neutral product (95·8 g. from the aqueous extraction, and 5·8 g. recovered by ether from the aqueous layers).

All aqueous and alkaline liquors were methodically acidified and the higher fatty acids (4·3 g.) recovered.

The neutral product (101·6 g.) had an iodine number of 1·6 and a saponification equivalent of 251·5 (calc. from the known composition of the total fatty acids, 302·9 as dihydroxystearic derivative, or 225·3 if the latter are in the monoacetylated form); it contained 3·0% of combined acetic acid (as $CH_3{\cdot}CO$).

From the mean equivalent (251·5) of this product, it may be calculated that (*a*) assuming displacement of fatty acid by acetic acid in the glyceride itself, the fatty acids displaced should have amounted to 22·4 g. of the total product, with production of 86·8 g. of neutral product; (*b*) assuming acetylation of the dihydroxystearic radical produced, there should have been no production of free fatty acids and a yield of 108·5 g. of neutral product.

The experimental data are thus strongly in favour of the latter hypothesis, the small amount of free fatty acid observed being accountable for by slight hydrolysis under the conditions of the reaction.

The neutral product was resolved by repeated systematic fractional crystallisation from acetone into the following fractions:

		% of whole.	Sap. eq.
A.	Least soluble; apparently homogeneous, hard, white, crystalline solid, m. p. 68—69°	22·5	293·4
B.	Sparingly soluble; hard and crystalline; gelatinises with acetone; m. p. 47°	6	256·6
C.	Intermediate fraction; soft, granular mass, m. p. 38—42° (indef.)	4	243·3
D.	From combined mother-liquors (except the first); soft, oily paste	42·5	241·1
E.	Most soluble portion from first crystallisation; oily	25	231·7

Dihydroxystearic acid (m. p. 95°) was present as glyceride in all these fractions, but a rigid method for its separate determination when mixed with palmitic and stearic acids is not yet available.

The mixed acids (mean equivalent 284·5) from fraction A, however, yielded by crystallisation from light petroleum 77% of the amount of this acid which would be required for the product derived from an original mono-oleic glyceride. This fraction apparently still contained traces of acetylated products.

Fractions D and E contained considerable quantities of acetylated products [$CH_3{\cdot}CO$ found: 3·45% (D) and 4·35% (E)].

These results indicate that the hydrogen peroxide treatment of unsaturated glycerides leads to a complicated mixture of products and that the method in its present stage of development is not adapted for quantitative work; nevertheless the above group of fractions affords confirmatory evidence of the general and to a large extent even distribution of oleic acid throughout the glycerides present in cacao butter.

Mutton tallow. Mutton tallow (100 g.) was oxidised in acetic acid with 35% hydrogen peroxide solution under exactly the same conditions as cacao butter. After 9 hours' treatment at 75—80° the mixture was cooled, and the neutral portion of the product obtained free from acetic acid and traces of fatty acid.

The neutral product (100·7 g.) had an iodine number of 1·6 and a saponification equivalent of 245·3; it contained 4·65% of acetic acid (calculated as $CH_3{\cdot}CO$). The yield of neutral product, together with its content of combined acetic acid, points, as in the previous case, to acetylation of one of the hydroxyl groups rather than to displacement of fatty acid residues by acetic acid in the glycerides present.

The product was similar in general appearance to that from cacao butter and was separable by crystallisation from acetone into a hard, white, sparingly soluble material and more soluble constituents. Quantitative resolution of the hydroxylated tallow by this means is at present in progress and the results will form part of a subsequent communication.

We wish to acknowledge the assistance of Mr. G. Collin (who is also engaged in the investigation of palm-kernel oil by these methods) in connexion with one of the oxidations of cotton-seed oil referred to in this paper.

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