ON THE SEPARATION OF GLYCERIDES.

PART I. LINSEED OIL.

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Former studies on constituents of fats and oils have usually dealt with the isolation and identification of fatty acids, if possible quantitatively. This is sufficient to account for the nature of fats and oils if they be composed of simple glycerides, but there are many indication that the principal components of fats and oils are mixed glycerides and in reality not a few of them have been isolated by different chemists. Unless we assume the presence of mixed glycerides we cannot account for the difference of nature which exists between cocoa butter and mutton tallow, both being composed of nearly the same quantity of the same kinds of fatty acid. If fats and oils are composed of mixed glycerides, it is of primary importance to isolate glycerides as such in order to know what fats and oils are.

Taking advantage of the different solubilities of brominated glycerides against different solvents, one of the authors (S.), in co-operation with several co-workers, has up to the present, succeeded in isolating more than 20 glycerides from 6 fats and oils though some of the experiments are too incomplete to publish as yet. There is nothing new about the process, for the same method has been applied previously for the same purpose by several different chemists. But these investigators adopted the process of fractional precipitation while we used different solvents to separate glycerides and we found our process the better.

The outline of our process undertaking for the separation of glycerides of linseed oil may be given in the following scheme.

The brominated glycerides when separated are not crystalline when inspected under a microscope. To insure purity, therefore, each fraction was

reprecipitated from a suitable solvent several times until it gave a constant composition and melting point. After the bromine content and melting point were determined the glycerides were hydrolyzed with hydrochloric acid, and the resultant acids were identified and weighed to ascertain their molecular proportion in the glyceride.

The following glycerides were isolated from linseed oil.

	m. p.
1. Dilinoleo-linolenin bromide	78°C.
2. Linoleo-dilinolenin bromide (a)	117-118°C.
3. Linoleo-dilinolenin bromide	158°C.
4. Dilinoleo-olein bromide	oil

The figures correspond to those of the above mentioned scheme.
2 and 3 are isomeric to each other.

Experimental

Bromination 200g. of linseed oil was dissolved in II. of petroleum ether and dried by adding a sufficient quantity of anhydrous sodium sulfate. To the dry ethereal solution of the oil, bromine was added from a burette, under constant stirring and thorough cooling until the color of bromine did not disappear. The stirring continued for several hours and finally the precipitates were gathered on a Buchner's funnel followed by thorough washing with a mixture of dry petroleum ether and glacial acetic acid.

1. Dilinoleo-linolenin bromide The ethereal solution of the brominated oils, which were with difficulty soluble in petroleum ether, evaporated to dryness. The white powder thus obtained was treated repeatedly with ether until the melting point and bromine content remained constant. The bromine was determined by Baubigny and Chavanne's method. The bromide melted at 78°C, Br: 56.21%. The bromine content corresponds to 14 atoms of the element in one mole of the glyceride. It is probable, therefore, that bromine atoms are contented in pairs of $2+2 \times 6$ or $2 \times 4+6$.

To determine which one of the two pairs really occurs in the glyceride, one part of the bromide was hydrolyzed with ten parts of hydrochloric acid. The resultant brominated fatty acids were separated into two parts, one easily soluble in ether and the other with difficulty soluble. Easily soluble acid, m. p.: 115°C, Br: 53.27% (53.33% as tetrabromostearic acid). Soluble with difficulty acid, m. p.: 179°C. Br: 63.59% (63.32% as hexabromostearic acid). The molecular proportion of the acids was 2.29:1.

2. Linoleo-dilinolenin bromide With difficulty soluble in ether but easily soluble in benzene. M. p.: 117-118°C, Br: 59.32% ($0+2 \times 8$ or $4+2 \times 6$) Tetrabromostearic acid, m. p.: 115°C, Br: 53.81% Hexabromostearic acid,

m. p.: 179°, Br: 63.64%, molecular proportion of the acids was 1:1.91.

3. Linoleo-dilinolenin bromide With difficulty, soluble in nearly all fat solvents. M. p.: 158°C, Br: 59.32%. By hydrolysis with hydrochloric acid tetrabromostearic acid (m. p.: 115°C, Br: 53.76%) and hexabromostearic acid (m. p.: 179°C, Br: 63.45%) were obtained in molecular proportion of 1:2.03.

It is worthy of notice that two isomerides of linoleo-dilinolenin bromide (2 and 3) differ markedly as to the melting point and behavior against solvents. If one of the isomerides has a symmetrical, the other must have a nonsymmetrical construction, being thus optically active. The rotatory powers of the two were estimated but both happened to be inactive or the activity was so slight that it was difficult to decide which one was really active.

4. Dilinoleo-olein bromide Soluble in petroleum ether but with difficulty soluble in hot alcohol. An oil, Br: 47.25% ($\beta + 2 \times 2$ or $2 + 2 \times 4$). By acid hydrolysis, dibromostearic acid (Br: 36.43%) and tetrabromostearic acid (m. p.: 115°C, Br: 53.79%) were obtained in molecular proportion of 1:1.86.

PART II. SOY BEAN OIL.

Soy bean oil was treated in ways similar to those in the case of the linseed oil. The previous scheme.

The following brominated glycerides were isolated and identified.

	m. p.
1. Dilinoleo-linolenin bromide	78°C
2. Linoleo-dilinolenin bromide	118°C
3. Trilinolenin bromide	166°C
4. Dilinoleo-olein bromide	oil

Experimental

1. Dilinoleo-linolenin bromide With difficulty soluble in petroleum ether but easily soluble in ether. M. p.: 78°C, $(\alpha)_D^{20}$: 0, Br: 56.36% (Calc.: 56.06). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p.: 178°C, Br: 64.04%) were obtained in molecular proportion of 1.80:1.

2. Linoleo-dilinolenin bromide Soluble in benzene but with difficulty soluble in ether. M. p.: 118°C, Br: 59.89% (Calc.: 59.37). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p.: 113°C, Br: 53.95%) and hexabromostearic acid (m. p.: 178°C, Br: 62.93%) were obtained in molar proportion of 1:2.2.

3. Trilinolenin bromide With difficulty soluble in cold but soluble in hot benzene. Crystalline plates (from carbon tetrachloride). M. p.: 166°C, Br: 61.99% (Calc.: 62.23). By hydrolysis with hydrochloric acid, hexabro-

mostearic acid, (m. p. : 178°C, Br : 63.91%) and no other acid, was obtained.

4. Dilinoleo-olein bromide Soluble in petroleum ether but with difficulty soluble in alcohol. An oil at room temperature. Br : 47.80 (Calc. : 47.56). By hydrolysis with hydrochloric acid, tetrabromostearic acid (m. p. 113°C, Br : 53.48) and dibromostearic acid [Br : 36.64 (Calc. : 36.18%)] were obtained in molar proportion of 2.03 : 1.

PART III. TRAIN OIL.

Train oil was treated in ways similar to the cases of part I and II.

The following brominated glycerides were isolated and identified :

	m. p.
(1) Arachidono-clupanodono-olein bromide	95°C
(2) Diclupanodono-olein bromide	132°C
* (3) Diarachidono-olein bromide	200°C (decomposing)
* (4) Diarachidono-olein bromide	216°C (decomposing)
(5) Dizoomaro-olein bromide	oil

* Isomeric to each other.

Experimental

The brominated oil was too viscous to allow the use of any filter so that precipitates were separated by centrifuging.

(1) Arachidono-clupanodono-olein bromide Soluble in ether but with difficulty soluble in petroleum ether. M. p. : 95°C, Br : 61.98% (Calc. : 62.60). The brominated fatty acids, obtained by hydrolysing the glyceride with hydrochloric acid, were treated with alcohol and the larger portions of them went into solution while a part remained in residue. The alcoholic solution was evaporated to dryness and the residue was treated with petroleum ether dividing it into two parts, one soluble and the other insoluble. Octabromo-arachidic acid, m. p. : 220°C (decomposing), Br : 66.50% (Calc. : 66.70). Decabromobehenic acid, m. p. : 161-162°, Br : 69.98% (Calc. : 70.75). Dibromostearic acid, an oil, Br : 36.52% (Calc. : 36.18). Molar proportion of three acids, 1 :

1.05 : 1.28.

(2) Diclupanodono-olein bromide Soluble in cold benzene but with difficulty soluble in ether. M. p.: 132°C, Br: 64.03 (Calc.: 64.19). Br hydrolysis with hydrochloric acid, decabromobehenic acid (m. p.: 161–162°C, Br: 69.67%) and dibromostearic acid (Br: 36.79%) were obtained in molar proportion of 1.83 : 1.

(3) Diarachidono-olein bromide With difficulty soluble in cold but soluble in hot benzene. M. p.: 200°C (decomposing), Br: 60 : 60.69% (Calc.: 60.76). By hydrolysis with hydrochloric acid, octabromoarachidic acid [m. p.: 220 (decomposing), Br: 67.01%] and dibromostearic acid (Br: 36.76%) were obtained in molar proportion of 1.8 : 1.

(4) Diarachidono-olein bromide With difficulty soluble in hot benzene. M. p.: 216°C (decomposing), Br: 61.23%. By hydrolysis with hydrochloric acid, octabromoarachidic acid [m. p.: 220°C (decomposing), Br: 67.07%] and dibromostearic acid (Br: 36.53%) were obtained in molar proportion of 1.85 : 1.

(5) Dizoomaro-olein bromide Soluble in petroleum ether but with difficulty soluble in alcohol. An oil. Br: 36.92% (Calc.: 36.64). The brominated fatty acids obtained by hydrolysis with hydrochloric acids could not be separated from each other, for both behaved similarly against solvents. The mixture was dissolved in glacial acetic acid, an excess of zinc dust was added to the solution and, provided with a reflux condenser, the flask was heated on a water bath. The contents of the flask were filtered, the filtrate was evaporated to dryness and the residue was dissolved in ether. A mixture of unsaturated fatty acids was obtained by evaporating the ethereal solution to dryness. The mixture was dissolved in a dilute soda solution and oxidized with a potassium permanganate solution (1.5%) under constant stirring and through cooling. The precipitates of mangan oxide were dissolved by passing sulphur dioxide and oxy acids, which floated on the surface of the solution, were gathered on a Buchner's funnel, recrystallized from alcohol and treated with ether. Part (dioxypalmitic acid) of them went into ethereal solution while the other (dioxystearic acid) remained as residue. Dioxypalmitic acid melted at 115°C. Dioxystearic acid melted at 132–133°C.

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