

TRIOLEIN AND TRILINOLEIN

BY

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SECTION I

INTRODUCTION

Most of the natural fats and oils which form one of the important classes of food, and which are of great importance for industrial purposes, are composed largely of the glycerides of the higher fatty acids. In natural fats and oils, these glycerides are very complex mixtures, and our knowledge of their structure is still in an early stage of development. " Schonfeld and Hefter¹ give an excellent review of this phase of fat chemistry.

The triglycerides of many of the saturated fatty acids have been synthesized in pure form, and adequately characterized by a number of workers, notably Clarkson and Malkin². " Schonfeld and Hefter³ have summarized much of the work on these compounds. The glycerides of the saturated higher fatty acids are crystalline compounds, which can be purified by crystallization, and the saturated acids from which they are synthesized are comparatively easily obtained in a pure state.

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1. H. Schönfeld and G. Hefter, *Chemie und Technologie der Fette und Fetteproducte*. I. *Chemie und Gewinnung der Fette*. pp.194-228 (Wien; Julius Springer, 1936).
 2. C. E. Clarkson and T. Malkin, *J. Chem. Soc. (London)* 1934, 666-71.
 3. Schönfeld and Hefter, *op. cit.* pp. 229-262.

The triglycerides of the unsaturated acids, on the other hand, apparently have not been prepared in any great degree of purity. There are several possible reasons for this fact. The unsaturated acids are not easily obtained in a pure state, for starting material. This is especially true of oleic acid, one of the most important acids in fats and oils. They are subject to oxidation by the oxygen of the air, especially at elevated temperatures. The unsaturated acids and their triglycerides are liquids at ordinary temperatures, and hence present greater difficulties in purification by crystallization. The triglycerides cannot be distilled undecomposed under ordinary conditions of vacuum distillation. In view of these difficulties, it is not surprising that their preparation and purification has not been adequately reported to the present time.

Several preparations of triolein are described in the literature, but the purity of the oleic acid used is open to question, and the reported properties of the product are very meagre.

Berthelot⁴ prepared triolein by heating monoolein with an excess of oleic acid at 200° for four hours. No properties were recorded other than the fact that it remained liquid "down to 10° and lower". Guth⁵ prepared triolein by heating sodium oleate and tribromohydrin at

4. M. M. Berthelot, *Ann. Chim. et Phys.* 41:243-5 (1854).

5. F. Guth, *Z. Biol.* 44: 78-110 (1902-3).

180° for 10 hours. He stated that it solidified at -4° to -5°. After standing several weeks, it solidified at "ordinary temperatures", but after melting, it again solidified at the lower temperature. The iodine number was reported as 85.1 (86.1=theory) and saponification number was close to theory. Pottevin⁶ made triolein by reacting an excess of oleic acid with monoolein at 36° with 1% of pancreatic tissue to supply lipase catalyst. The product was stated to solidify "around 0°" and to have a density of 0.915 at 15°. Belluci⁷ heated oleic acid and glycerine at 215° to 220° under 30 to 40 mm. pressure and obtained triolein which he purified by extraction with alcohol. The product had an iodine number of 85.7 and saponification equivalent close to theory. None of these preparations can be considered satisfactory as adequately characterizing triolein nor as representing a product of proven purity.

Izar⁸ reported the preparation of trilinolein by the action of potassium linoleate on trichlorohydrine at 160°. He stated that it was a reddish yellow oil, and gave no analysis or other properties. At the time of this

6. H. Pottevin, *Compt. Rend.* 138:378-80 (1904).

7. I. Belluci, *Gazz. Chim. Ital.* 42 (II): 283-304 (1912).

8. G. Izar, *Biochem. Z.* 60: 320-9 (1914).

writing, the announcement of a paper to be given by Black and Overly⁹ on mono- and trilinolein was noticed. They formed the acid chloride of the tetrabromo addition product of linoleic acid and from it synthesized the glycerides of the tetrabromo acid. They then removed the bromine to generate the unsaturated acid glyceride. Further details of the purification and properties of their product are not available to the writer at this time.

The unsaturated acids occur as glycerides in practically all fats and oils, and their presence is of the greatest importance in determining properties such as consistency, digestibility, nutritional value, tendency to oxidize and polymerize, as in paint films. It, therefore, seemed desirable to find a suitable method of preparation of the unsaturated triglycerides and to prepare the triglycerides of oleic and linoleic acids, the two most important unsaturated acids occurring in the common fats and oils.

Linoleic acid ($\Delta^{9,12}$ octadecadienoic acid) is fairly easily obtained in pure form by debromination of the crystalline tetrabromostearic acid which is obtained by brominating the mixed acids of an oil rich in linoleic acid, such as cottonseed oil. The tetrabromide can be

9. H. C. Black and C. A. Overly, Abstracts of Papers, 98th Meeting, American Chemical Society, Boston, Mass. Sept. 11 to 15, 1939. (Wash. D.C., A.C.S. News Service) Div. Org. Chem. pp. 33-4.

purified by crystallization, and the linoleic acid regenerated by treatment with zinc powder. This was the method used to obtain linoleic acid, as described below. The linoleic acid thus formed appears to be identical with the original acid of the oil, as has been shown by Rollet¹⁰, Biroesel¹¹, Brown and Frankel¹², Hilditch¹³ and McCutcheon¹⁴.

Oleic Acid (Δ^9 octadecenoic acid) presents much greater difficulty in its purification. A number of methods have been proposed for the preparation of pure oleic acid or its esters. Holde and Gorgas¹⁵ were able to get a crystalline bromide, with considerable difficulty, from which they regenerated the oleic acid. Bertram¹⁶ made use of the solubility of a complex mercury compound of oleic acid to eliminate saturated acids down to 0.5%, and removed more highly unsaturated acids by crystallization from acetone at -15° . He made many references to previous attempts to prepare pure oleic acid, and claims to have tried many of them with limited success. Scheffers¹⁷

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10. A. Rollet, *Z. Physiol. Chem.* 62: 410 (1909).
 11. D. M. Biroesel, *Natural and Applied Sci. Bull. Univ. Manila, Phillipine Is.* 2: 103 (1932).
 12. T. B. Brown and J. Frankel, *J. Am. Chem. Soc.* 60:54-6 (1938).
 13. T.P. Hilditch and H. Jasperon, *J. Soc. Chem. Ind.* 58: 233 (1939).
 14. J.W. McCutcheon, *Can. J. Research* 16B: 158-75 (1938).
 15. D. Holde and A. Gorgas, *Z. Angew. Chem.* 39: 1443 (1926).
 16. S. H. Bertram, *Rec. Trav. Chim.* 46: 397 (1927).
 17. H. W. Scheffers, *Rec. Trav. Chim.* 46: 293 (1927).

removed saturated acids by the Twitchell lead salt method, and then removed more highly unsaturated acids by crystallization of the lithium salts from dilute alcohol. Raymond¹⁸ also used the lead salt separation, followed by crystallization of the acids at -20° from alcohol. Skelton¹⁹ used a modified lead salt method, followed by crystallization of the barium salt from moist benzene and alcohol, and also of the lithium salts from alcohol. He finally fractionated the methyl esters in vacuo. Keffler and McLean²⁰ also used the lead salt separation, followed by crystallization of the lithium salts (12 times) from 80% alcohol. They then removed the remaining saturated acids down to 0.3% by repeated fractional vacuum distillation of the esters. Brown and Shinowara²¹ prepared oleic acid by direct crystallization of the mixed acids of olive oil from acetone.

The criticism of all of the above methods is that the iodine number is the only analytical constant of much significance which is used as a criterion of purity. Linoleic and saturated acids (stearic and palmitic) would be the most probable impurities, and equal amounts of each of these would yield a product of theoretical iodine number. Saponification equivalent would be of little value in

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18. E. Raymond, *Chimie & Industrie*, Special no. Feb. 1929:523.
19. J. H. Skelton, *J. Soc. Chem. Ind.* 50: 131T (1931).
20. L. Keffler and J. H. McLean. *J. Soc. Chem. Ind.* 54: 362T (1935).
21. J. B. Brown and G. Y. Shinowara, *J. Am. Chem. Soc.* 59: 6 (1937).

detecting small quantities of impurities of similar molecular weight, and physical constants are of value only when compared with a compound of known purity.

There are two analytical methods of determining the composition of a mixture of oleic, linoleic and saturated acids. The first is by determination of the iodine number and the thiocyanogen number. The iodine number gives a measure of the total unsaturation of the sample, the reagent adding completely to both double bonds of linoleic acid and to the one double bond of oleic acid. The thiocyanogen reagent, however, adds to only one of the double bonds of linoleic acid, but adds completely to the double bond of oleic acid. From these two values, the composition of such a mixture can be calculated. The second method consists of determining the iodine number, and then determining the saturated acids by an independent method. The Bertram procedure²² for saturated acids is probably the most exact method, although rather tedious. Its application to the detection of small amounts of saturated acids was examined by Riemenschneider and Wheeler²³, who showed that it could be used to determine as little as 0.1% of saturated acid. When the percent of saturated acid is known, the composition

22. S. H. Bertram. Z. Untersuch. Lebens. 55: 180 (1928)

23. R. W. Riemenschneider and D. H. Wheeler, Paper presented to the American Oil Chemists' Society, May 6, 1939, to appear in Oct. or Nov. 1939 number of Oil and Soap.

can be calculated from the iodine number. Hartsuch²⁴ used the iodine number and the thiocyanogen number to make a comparison of the low temperature crystallization method of Brown (loc.cit.) with several others. He finally arrived at a method involving crystallization of olive oil acids at three different temperatures from acetone, precipitation of the lead salts, and fractional distillation of the acids. The purest fraction he reported as 97.8% pure, with 1.9% linoleic and 1.2% saturated acids. He concluded that "this probably represents as pure a sample of oleic acid as has thus far been obtained". Wheeler and Niemenschneider²⁵ used both of the above mentioned methods of analysis of mixtures of oleic, linoleic and saturated acids in a study of the preparation of pure methyl oleate from the mixed esters of the acids of olive oil. They found that low temperature crystallization from acetone of the methyl esters was more effective than with the free acids. Their method consisted of a preliminary fractional distillation in vacuo to remove most esters of the lower acids, followed by four crystallizations at suitable temperatures from acetone, to remove linoleic and the bulk of the saturated esters. Traces of palmitic ester still

24. P. J. Hartsuch, J. Am. Chem. Soc. 61: 1142 (1939).

25. D. H. Wheeler and R. W. Niemenschneider, Paper presented to American Oil Chemists' Society, May 6, 1939, to appear in Oct. or Nov. 1939 number of Oil and Soap.

remaining were removed by a second careful fractional distillation in vacuo, and the product finally purified by two more crystallizations from petroleum ether. Their final product had theoretical iodine number, thiocyanogen number 0.2 unit below theory, and showed less than 0.1% of saturated acid by the Bertram method. They followed each step by analysis and felt certain that their product contained not over 0.2%, and probably not over 0.1% of linoleic or saturated acid esters as impurities. This material was used as the source of oleic acid for the synthesis of triolein to be described.

It was found that in the strict absence of oxygen, oleic and linoleic acids could be esterified with glycerol directly, using p-toluene sulfonic acid as catalyst, to give good yields of the triglycerides. Further purification of the products was obtained by low temperature crystallization and molecular distillation, as described below.

SECTION II

ANALYTICAL METHODS AND APPARATUS

Iodine Numbers. Iodine numbers were determined by the official Wijs Method²⁶. The excess of iodine over chlorine in the reagent was adjusted to 2%, and the size of the sample regulated so that not over 33% of the total available halogen was consumed, as recommended by Keffler and Maiden²⁷, in their study of the high precision determination of iodine numbers by the Wijs method. Duplicate determinations usually checked within 0.1 to 0.2%, while analyses of the same product from one day to another rarely varies over 0.2 to 0.3%.

Thiocyanogen Numbers. Thiocyanogen numbers were determined by the modified Kaufman method²⁸, except that the temperature was controlled, and the reaction time was varied as described. The excess of reagent was 100 to 150%.

Saturated Acids. The Bertram method (loc. cit.) was used essentially as described, except that sulfur dioxide was used in place of bisulfite, and the magnesium salts were decomposed directly on the filter paper, as mentioned by Riemenschneider and Wheeler (loc. cit.).

26. Official and Tentative Methods of the Assoc. of Official Agr. Chem. (Wash. D.C. Assoc. of Official Agr. Chem. 1935) p. 410.

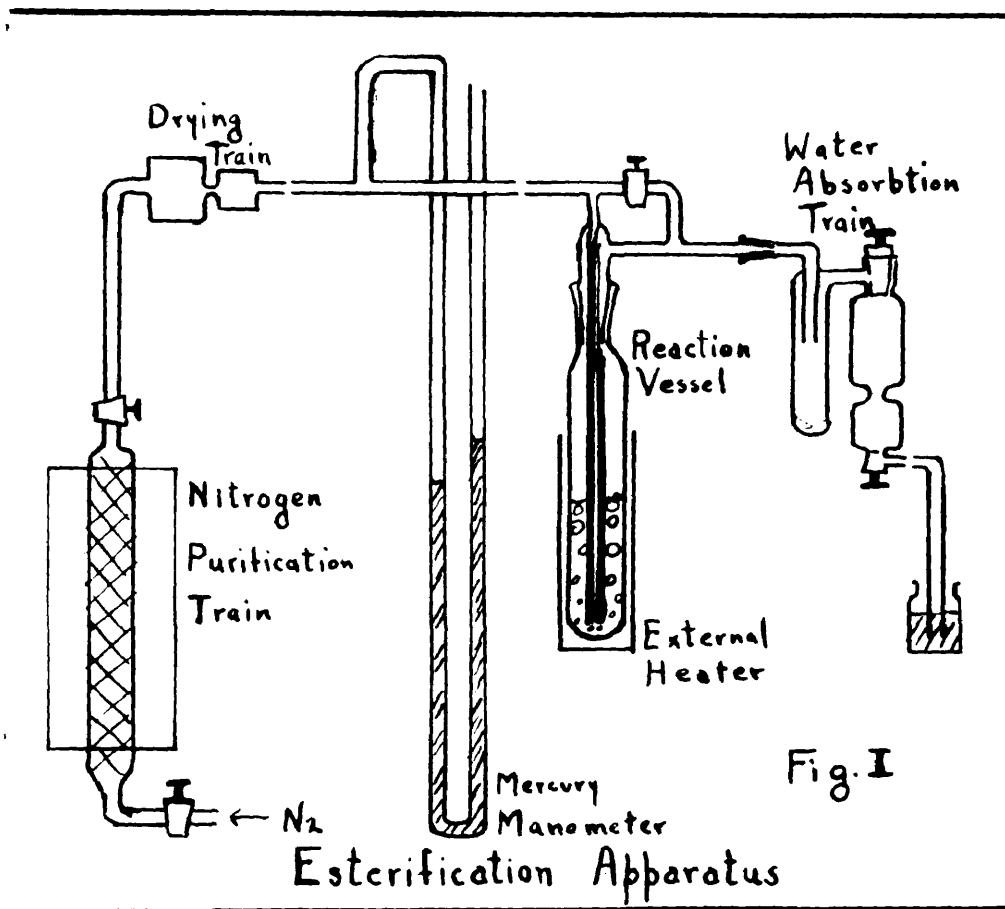
27. L. J. P. Keffler and A. M. Maiden, J. Soc. Chem. Ind. 52: 242-245T (1933).

28. J. Assoc. Official Agr. Chem. 21: 87 (1938).

Saponification Numbers. These were determined on a sample of 0.15 to 0.18 gm. by refluxing for one half-hour with 10 cc. of 0.1 N sodium hydroxide in n-propanol (made by dissolving sodium in n-propanol and adding 1% of water). The excess of caustic was titrated with 0.1 N standard acid in a micro-burette, using phenolphthalein indicator. Duplicates usually agreed within one unit or less on substances such as the triglycerides (0.3% error).

Free Acid. Free acid was determined by addition of a weighed sample to neutralized n-propanol, and titration with 0.1 N caustic, using a micro-burette and with phenolphthalein indicator.

Esterification Apparatus. The esterification apparatus was relatively simple, consisting of a purification train for nitrogen, manometer, reaction vessel, and water absorption train. (Fig. 1). The nitrogen was freed from oxygen by passing it over reduced copper (by reduction of wire form copper oxide) at a very dull red heat. The manometer was useful in checking on the slight positive pressure under which the apparatus was operated, and in checking on evacuation of the apparatus, which preceded the beginning of a run. After several evacuations and releases with nitrogen, the nitrogen was passed through the reaction mixture at a rate sufficient to give vigorous agitation, and it carried off the liberated water to a large extent, although during the first hour or two frequent flaming of the upper



portions and tubes of the reaction vessel was necessary to prevent excessive condensation. Completion of the reaction was judged by almost complete cessation of evolution of water, determined by weighing the absorption train.

Low Temperature Crystallization. The glycerides were crystallized by cooling their acetone solutions with an alcohol-carbon-dioxide external bath, with mechanical stirring. Filtrations were made with suction, in a room at -18° to -20° , and were fortunately quite rapid.

Molecular Distillation. The molecular distillations were carried out in a cyclic still, similar to the one described by Hickman.²⁹ (Fig. 2) The liquid is pumped by the magnetic pump from the lower reservoir through the tube which delivers it onto the heated distilling column. As it flows down the surface of the column, the more volatile portions are distilled or evaporated onto the condenser and drain into the receiver. The undistilled portion runs through the cooler to the upper reservoir. At the completion of a cycle, the liquid in the upper reservoir is dropped to the lower reservoir by lifting the steel ball magnetically, and another cycle is begun. The high speed diffusion pump attached directly to the still has a capacity rating of 220 liters per second at about one micron. After degassing of the sample and elimination of traces of volatile material, the pressure dropped to 2 microns or lower, and in the

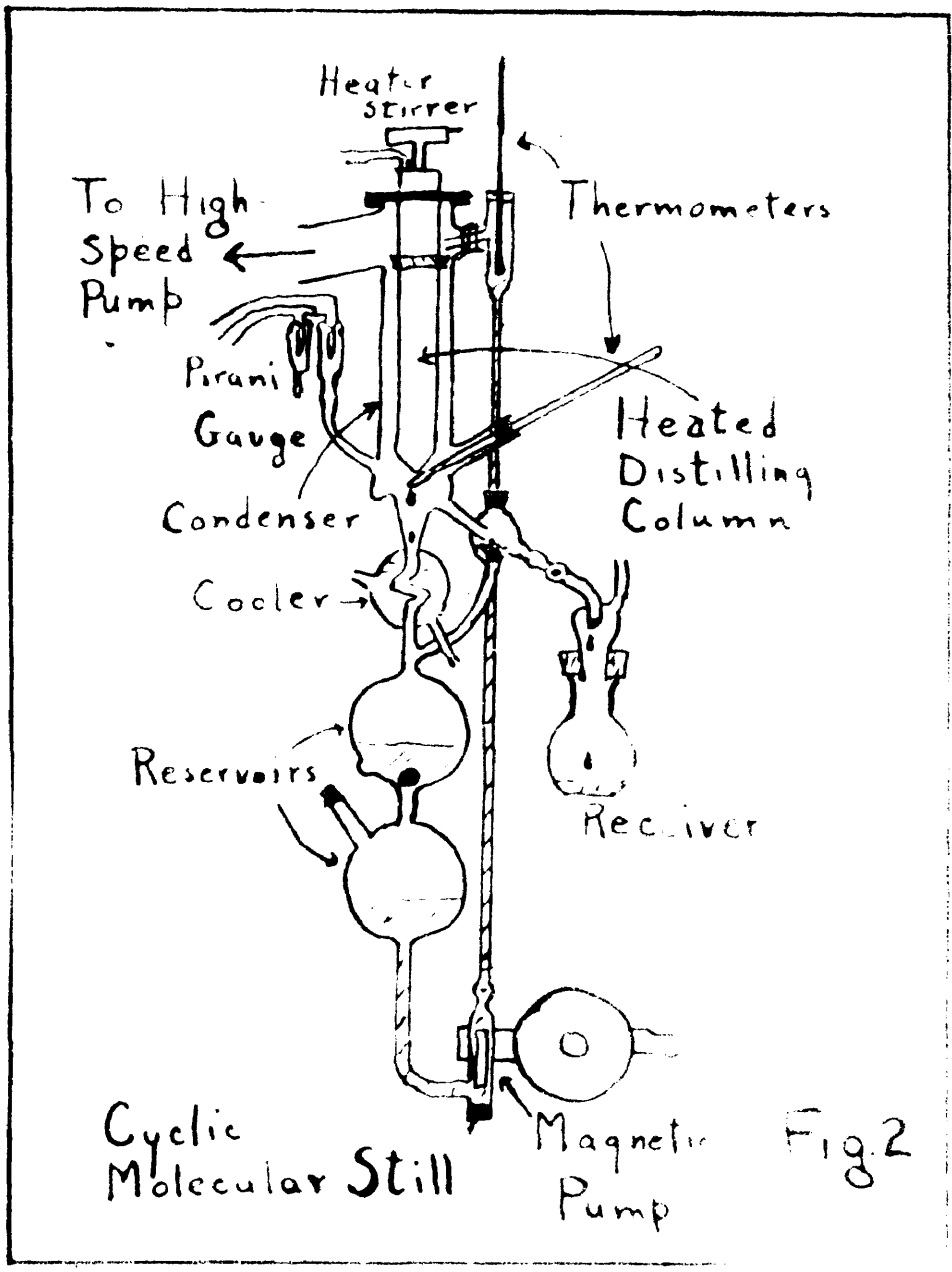


Fig. 2

particular still used, triolein and trilinolein distilled at a rate of one drop every five seconds with a column temperature of 245° to 250° . The liquid to be distilled was pumped over the column at a rate of about 15 cc. per minute.

Melting Points and Freezing Points. Capillary melting points were taken at the lower temperatures with a toluene filled thermometer which was checked against the calibrated thermocouple at the temperatures involved. An evacuated double-walled vessel, equipped with a mechanical stirrer served as a bath. It was filled with alcohol which was cooled to the desired temperature by direct addition of solid carbon dioxide. The liquid samples were introduced into an ordinary capillary melting point tube with a very fine capillary and the tube was then sealed off with a micro burner.

Cooling and warming curves were taken with a single thermocouple, placed in the middle of a 5 cc. sample in a test tube 13 x 130 mm., and fitted with a glass stirrer, operated by hand. This tube was fitted into an outside tube 23 x 130 mm. to afford an air space to serve as lagging. The external temperature was adjusted according to the material, as described. Supercooling was quite marked, and usually made stirring impossible after crystallization once started. It is felt that the freezing and melting points of the stable higher melting forms are probably accurate to 0.3° .

The potentiometer was a portable type from the Brown Instrument Company, calibrated by the U. S. Bureau of Standards, graduated to 0.05 millivolts, permitting estimation to 0.01 millivolt. The thermocouple was a special alloy which gave rather larger voltage change per degree than the usual thermocouple. It was calibrated for the range of temperatures needed by taking freezing points of pure compounds in the same apparatus used for the glycerides. Carbon tetrachloride (F. -22.9°), chlorobenzene (F. -45.2°), chloroform (F. -63.5) and solid carbon dioxide (V.P. -78.5°C) were used. Ice and steam points were also checked. By means of a calibration curve, temperatures could be estimated to 0.1 to 0.2 $^{\circ}$.

Refractive Index. This constant was determined with a Bausch and Lomb Company Abbe type refractometer, permitting estimation of the fourth decimal of index.

Density. A Sprengel type pycnometer of 4 cc. capacity was used. Weighings were corrected to vacuo in the usual manner.

SECTION III

TRIOLEIN

Methyl Oleate and Oleic Acid. The methyl oleate used as a source of oleic acid was that prepared by Wheeler and Hiemenschneider (loc.cit.)²⁵ and described in the introduction. The acid was recovered from the ester by refluxing for one-half hour with a 50% excess of N potassium hydroxide in ethanol, cooling and treating with an excess of dilute mineral acid. The liberated acid was extracted with petroleum ether, washed and dried and distilled in vacuo. 152.5 gm. of methyl oleate gave 135.0 gm. of distilled acid (93% yield) with iodine number 89.8, 90.0, (theory - 89.9.)

Synthesis of Triolein. The triglyceride was formed by direct esterification of redistilled glycerol with a slight excess of oleic acid, and p-toluene sulfonic acid catalyst in the apparatus previously described, at a temperature of 120° to 125°. Evolution of water was quite rapid during the first hour or two, and then slowed down as the amount of evolved water approached the theoretical amount. The reaction was continued until the amount of water collected in one-half hour was less than 1% of the total. The reaction mixture was then dissolved in 2 volumes of petroleum ether, extracted once with 70% ethanol to which enough dilute potassium hydroxide was added to make a slight excess (to

phenolphthalein) over the amount required to neutralize the catalyst and excess acid. The ether solution was then washed seven or eight times with 70% ethanol, followed by an equal number of washings with water. The ether solution was dried, and the solvent removed, finally under vacuum (.2 mm.) with warming. A number of preparations were made on samples of oleic acid of considerable less purity than that described above. Yields around 90% of products with iodine numbers several units below theory were the usual result. The iodine number could usually be raised to within less than one unit of theory by crystallization from acetone at -40° . Molecular distillation further raised the iodine number to values approaching theory, according to the purity of the acid used in the synthesis. Details of these preparations will not be given, but only those of the triolein prepared from the purest oleic acid described above: 131.0 gm. (0.464 moles) of oleic acid, 13.44 gm. of glycerine (0.438 equiv.) and 1.4 gm. of p-toluenesulfonic acid were used. 7.81 gm. of water (7.89 gm.= theory) was collected in five hours, and 120.7 gm. (93.6% yield) of crude triolein with iodine number 85.2, 85.3. The crude triolein was crystallized from acetone (10 cc per gm.) at -45° to give 116.5 gm. of product, iodine number 85.5, 85.4, showing very little change in this case by crystallization. The product was further

purified by molecular distillation as described below.

Molecular Distillation of Triolein. The triolein just described was subjected to fractional molecular distillation in the still previously described. 109.2 gms. were distilled, with the results shown in table I.

TABLE I

Fraction	Wt. Gms.	Iodine No. (theory, 86.1)
1	7.5	83.6, 83.5
2	11.1	84.7, 84.8
3	8.4	85.7, 85.4
4	8.1	85.5, 85.8
5	48.1	86.1, 86.2, 86.1, 85.8
Residue	21.3	85.9, 85.8

The main fraction (5) agrees well with theory for iodine number (86.1 = theory) and was chosen for further examination, to be described later.

Another sample of triolein which had been prepared from a less highly purified methyl oleate whose iodine number was 85.3 (85.7 = theory) was also distilled. The iodine number of the product was 85.2 (86.1 = theory) before distillation. Results are shown in table II.

TABLE II

Fraction	Wt. Gms.	Iodine No.
1	11.5	85.1 , 85.1
2	9.5	85.4 , 85.6
3	8.2	85.6 , 85.9 , 85.5
4	8.4	85.8 , 85.7
5	8.7	85.9 , 85.7
6	26.5	85.6 , 85.5
7	8.0	86.0 , 85.7
Residue	15.7	84.3 , 84.2

Fractions 3,4,5,6 and 7 were combined and redistilled, with results shown in table III.

TABLE III

Fraction	Wt. Gms.	Iodine No.
1	3.4	85.7 , 85.5
2	4.3	85.5 , 85.9
3	3.9	85.9 , 86.1
4	19.4	85.8 , 86.0
Residue	19.4	85.8 , 85.9

The results of this redistillation indicate that triolein can be distilled without appreciable decomposition or polymerization.

Some Properties of Distilled Triolein. The pure triolein was a colorless, odorless, tasteless, rather viscous liquid. Fraction 5, table I was found to have a density of 0.8988 at 40°, and refractive index $n_D^{40^\circ} = 1.4621$, $n_D^{50^\circ} = 1.4586$. Molecular refraction calculated from data at 40° was 270.6, compared to 269.0 calculated from the sum of atomic refractions. Saponification equivalent was 295.8 (294.9 = theory) and free acid was 0.05% as oleic. Fraction 5, table II was found to contain 0.5% saturated acids (as glycerides) by the Bertram method, and probably also contained 0.1 or 0.2% of linoleic acid. Its physical properties, however, were quite similar to the purer fraction just described. Density at 40° was 0.8985 and $n_D^{40^\circ} = 1.4619$, $n_D^{50^\circ} = 1.4584$. Molecular refraction calculates to the same as above. Thiocyanogen absorption and freezing and melting points will be discussed in detail below.

Thiocyanogen Absorption of Triolein. The thiocyanogen absorption was determined at 20° to 23° on fraction 5, table I. The significance of the application of this value to fat analysis will be discussed later under trilinolein. The results were as shown in table IV.

TABLE IV

Hours Reaction Time	Thiocyanogen No. (theory, 86.1)
1	77.7
3	85.2, 85.6
4	85.8, 85.7, 85.7, 85.9
5	85.8, 85.8
10	85.8, 85.9
24	86.2, 86.2

Theory for triolein is 86.1. It will be seen that the value is essentially constant and quite close to theory from 4 hours on. Similar results on fraction 4, table III were obtained, except that the values were a few tenths of a unit lower.

Melting and Freezing Points and Polymorphism of Triolein. Polymorphism was exhibited by triolein, similar to that observed by Clarkson and Malkin (loc.cit.) on the saturated glycerides, except that the transitions from the lower melting forms appear to be much more rapid, and consequently determinations of their melting points are only approximate. From capillary observations, there appear to be three forms of triolein: I, the stable highest melting form with m.p. of $+4.7$ to $+5.0^{\circ}$, II. a form melting at about -11° or -12° , and III. a form melting around -32° .

The phenomena observed in capillary tubes was as

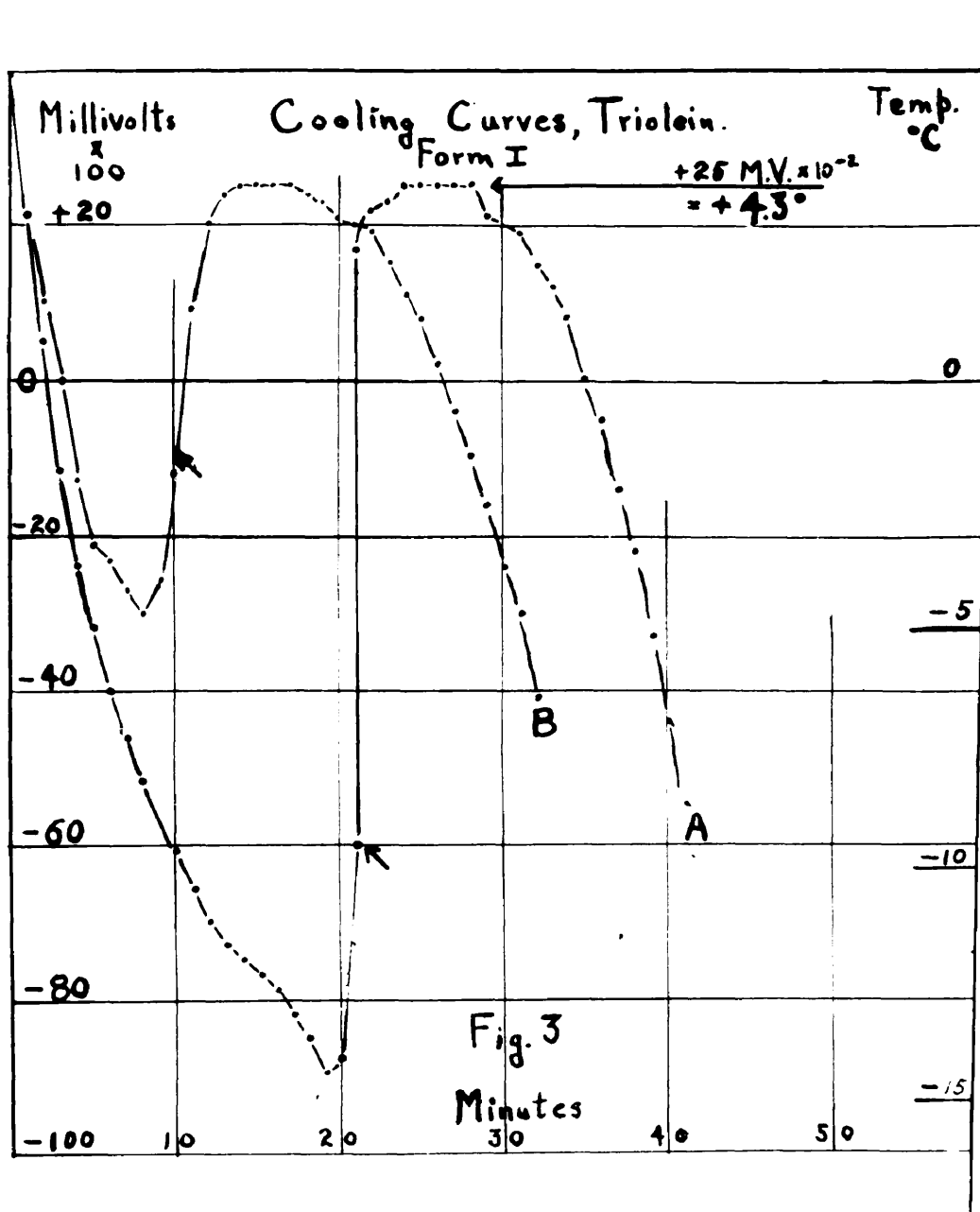
follows. If a sample was immersed in a bath at about -55° , it rapidly froze to a white opaque crystalline mass. If the bath were slowly warmed, no intermediate melting points would be observed, only that of form I. If, however, the sample, rapidly frozen at -55° , was quickly transferred to a bath at -32° or higher, it melted to a turbid liquid and then rapidly solidified again in about three seconds, to a white opaque mass. If the second bath was below -32° , there was a slight change in appearance, but no melting. If the bath was then warmed to -32° or above there was no melting as the change from III to II had occurred below the m.p. of III. If the sample which had melted and resolidified at -32° or above was now placed in a bath at about -12° or above, it again melted and then resolidified in 4 or 5 seconds. Similarly, if the bath were below -12° , no melting was observed, even if the bath was then raised to -12° or above, as the change from II to I has occurred below the m.p. of II. The sample now melts normally at $+4.7$ to $+5.0^{\circ}$. The melting points of the two lower forms are to be considered as only approximate since the transitions are so rapid that equilibrium between the bath and sample was far from realization before the change was complete.

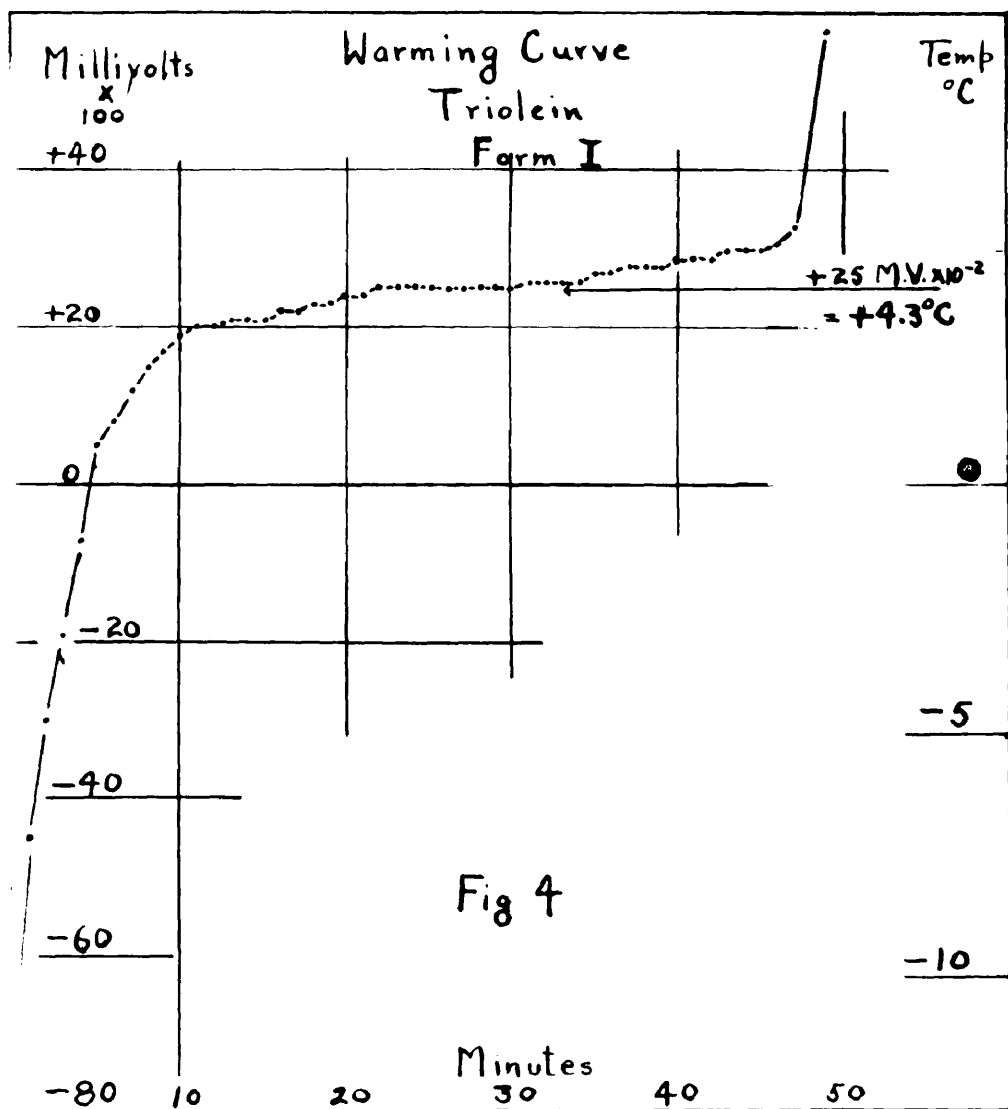
In view of the rapid transitions shown by the lower forms, and the observation that transition occurred below their melting points, it was not expected that cooling or warming curves would reveal much information on these forms

other than to confirm their presence.

The freezing and melting point of the stable form I could be determined fairly satisfactorily from the cooling and warming curves. Supercooling was quite pronounced (to -14°) without seeding (Curve A, Fig. 3, external bath -18°) and was still considerable (to -4°) with seeding (Curve B, Fig. 3, external bath -14°). Seeding was accomplished by allowing a portion of unmelted material to remain from a previous melting. The warming Curve (Fig. 4, external bath $+14^{\circ}$) shows good agreement with the cooling curve for form I. The freezing and melting point from these curves is $+4.3^{\circ}$ which is in good agreement with the capillary m.p. of $+4.7^{\circ}$ to $+5.0$, since capillary m.p.'s. are normally somewhat higher than values from freezing or melting curves.

Evidence for form II could be obtained by cooling at a rather rapid rate (external temperature -78°) Fig. 5 shows such a curve. Supercooling is again quite marked, and the hold for the melting point of form II is incomplete, followed by a rapid rise in temperature, due to heat of transformation into form I. This rise is almost to the m.p. of form I, and then is followed by a rapid drop, due to normal cooling effects. The fact that the incomplete hold (-8°) is considerably higher than the capillary m.p. of form II (-11 to -12) is no doubt due to the fact that some transition of II to I is occurring at the same time that II is melting,





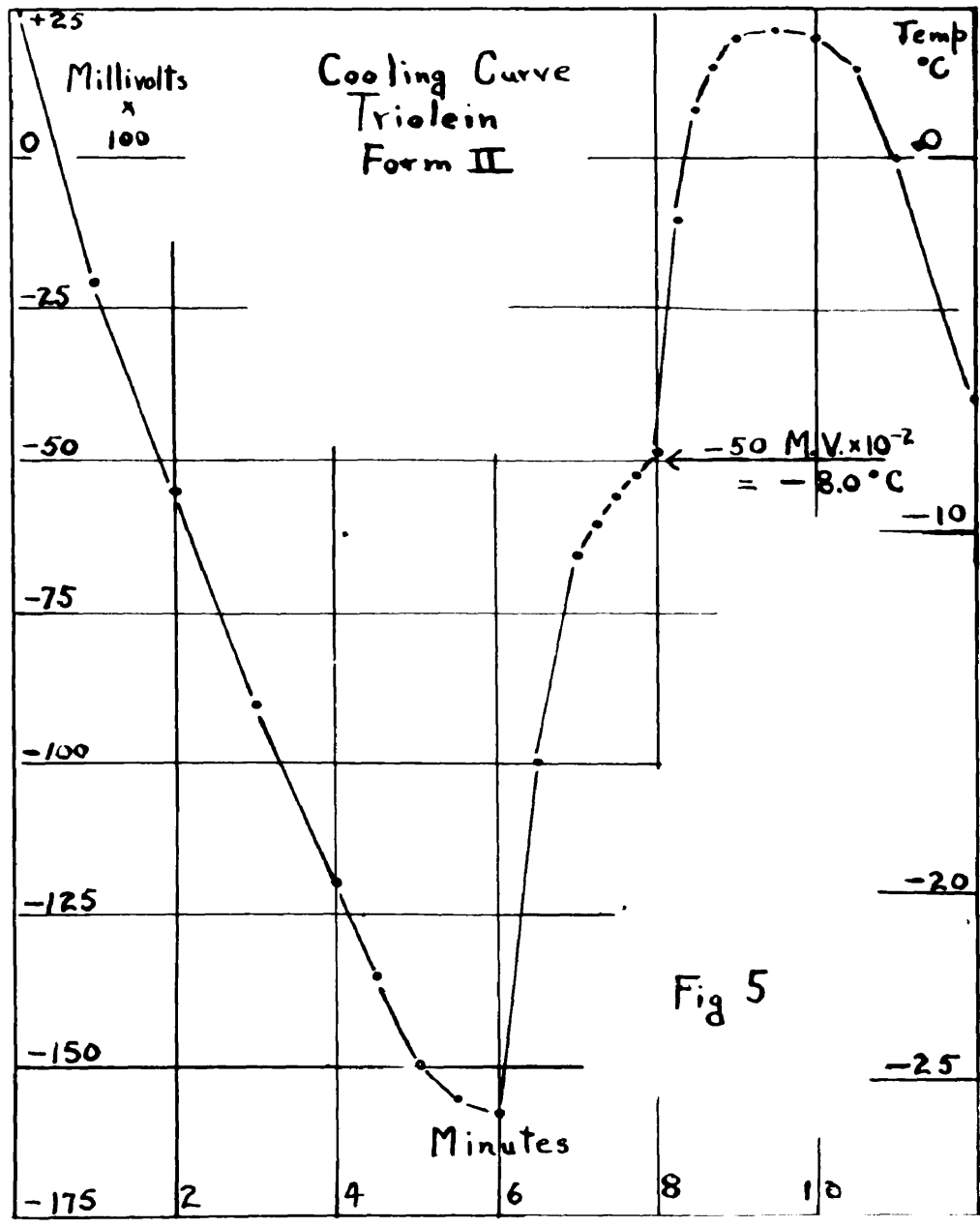
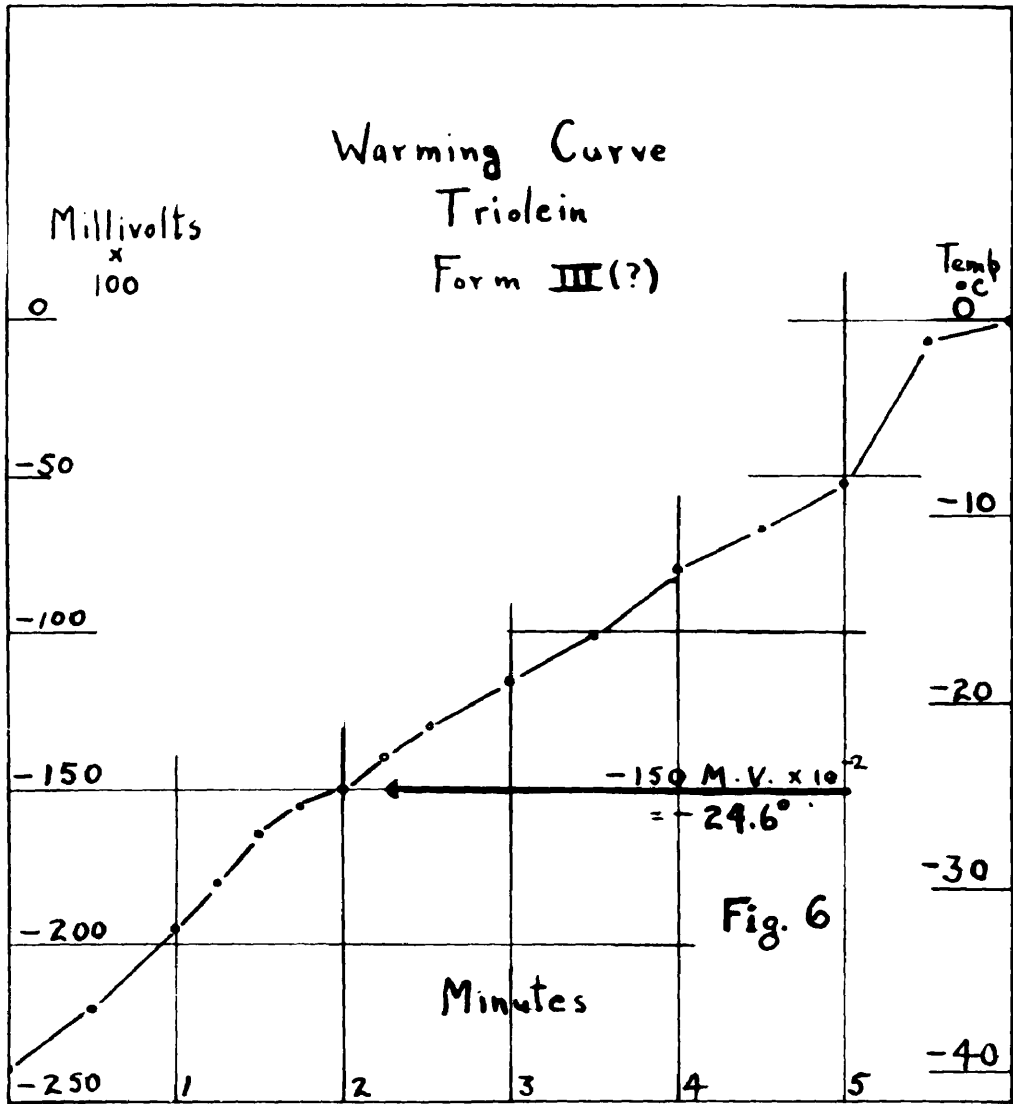


Fig 5

and the heat liberated displaces the incomplete hold to a higher temperature.

Evidence for form III was not very satisfactory, from cooling or warming curves. It was found that if the sample were frozen very rapidly by direct immersion of the tube (without the external tube) into an alcohol-carbon dioxide slush for three or four minutes, a warming curve with external bath at 0° , showed a slight inflection at -24.6° , as seen in Fig. 6. This was duplicable, but the inflection was slight, and probably represents a slight heat of fusion of this form, combined with the effect of transition to a higher melting form.

Fraction 4, table III which contained 0.5% saturated acids showed no detectible difference in melting point behavior.



Summary of Data on Triolein. The results on triolein are summarized in Table V.

TABLE V

	Found	Theory
Iodine No.	86.1	86.1
Phosphorus No.	85.6 (4 hrs.) 86.2 (24 hrs.)	86.1
Density	0.8988 at 40°	
Refractive Index	$n_{40}^{\circ} = 1.4621$	
	$n_{50}^{\circ} = 1.4586$	
Molecular refraction	270.6	269.0
Saponification equivalent	295.8	294.9
Free acid	0.05	0.0
	I +4.7° to 5.0° (cap.) +4.3 (curve)	
	II -11 to -12° " -8 " "	
Melting points	III -32° " -24.5 "	

SECTION IV
TRILINOLEIN

Methyl Linoleate and Linoleic Acid. The tetrabromostearic acid used was that described by Riemenschneider, Wheeler, and Sando³⁰ obtained by brominating the unsaturated acids of cottonseed oil in ether at -10° . The product was recrystallized from ether until it showed a melting point of 115.0° to 115.2° (corr.). The tetrabromide was debrominated according to the process described by Kimura³¹, except that the zinc was added in small portions to moderate the vigor of the reaction. The procedure regularly gave yields of about 95% of theory of a product of theoretical iodine number. A typical preparation was as follows: 222 gm. of tetrabromostearic acid was warmed with 660 cc. of absolute methanol, and 110 gm. of zinc powder added in small portions, so as to keep the mixture boiling. When all the zinc had been added, 44 gm. of sulfuric acid in 100 cc. of methanol was added, and the mixture refluxed for one-half hour. The cooled reaction mixture was diluted with water and extracted with petroleum ether. The ether solution was dried, evaporated and distilled in vacuo to give 104 gm.

30. R.W.Riemenschneider, D.H. Wheeler and C.E.Sando, J. Biol. Chem. 127:391-402 (1939).

31. W. Kimura, Pettechem. Umschan 42: 78 (1935).

(96% yield) of methyl linoleate. The acid was recovered from the ester in the same manner as described for oleic acid. 104 gm. of ester gave 96.8 gm. (97.7% yield) of distilled acid with iodine number 180.8, 181.3 (181.2 = theory).

Synthesis of Trilinolein. Trilinolein was prepared in exactly the same manner as triolein, using a slight excess of acid, and with p-toluenesulfonic acid catalyst. Recrystallization was from acetone at -60° to -65° . When 10 cc. of acetone per gm. of linolein was used, the compound started to separate as an oil at about -30° , and finally crystallized to granular aggregates at -55° to -60° , with stirring. The material could be caused to separate in much finer crystals by inoculation with a small portion of previously crystallized material as soon as separation at -30° started. The data on three different preparations were as follows:

Run 1. 39.1 gm. of linoleic acid (0.1395 moles), 4.12 gm. of glycerine (0.1342 equiv.) and 0.4 gm. p-toluenesulfonic acid were used. 2.35 gm. (2.42 gm. = theory) of water was collected in $3\frac{1}{2}$ hours, and 35.9 gm. of crude trilinolein (91.4% yield) with iodine number 171.2 were obtained. Crystallization from acetone (10 cc. per gm.) gave 33 gm. of product with iodine number 173.0. Recrystallized again, the recovery was 30 gm. of product

with iodine number 172.7, practically unchanged. Theoretical iodine number for trilinolein is 173.4.

Run 2. 41.1 gm. (0.1465 moles) of linoleic acid, 4.24 gm. (0.1381 equiv.) of glycerine and 0.4 gm. of p-toluenesulfonic acid were used. 2.36 gm. of water (2.49 gm.=theory) was collected in 3 hours, and 35.7 gm. of crude triolein (88% yield) with iodine number 172.0 was obtained. Crystallization from acetone at -60° gave 31.7 gm. of product with iodine number 172.9.

Run 3. 96.2 gm. (.343 moles) of linoleic acid 10.25 gm. (.335 equiv.) of glycerine and 1.0 gm. of p-toluenesulfonic acid were used. 5.77 gm. of water (6.05 gm.= theory) were collected in 5 hours, and 89.5 gm. (93.3% yield) of crude trilinolein with iodine number 171.5 was obtained. Crystallization as before gave 82.2 gm. of product with iodine number 172.7.

Molecular Distillation of Trilinolein. The trilinolein from runs 1 and 2 were combined, and 54.4 gm. were subjected to fractional molecular distillation, with the results shown in table VI.

TABLE VI

Fraction	Wt. Gms.	Iodine No.
1	5.1	169.5
2	7.8	172.7, 172.6
3	6.6	173.4, 174.2
4	11.2	173.7, 174.2
residue	20.0	173.8, 174.0

The trilinolein from run 3 was combined with a portion of fractions 3,4 and residue, table VI, and recrystallized from acetone to give 97.5 gm. of product, iodine number 172.9 which was distilled with the results shown in table VII.

TABLE VII

Fraction	Wt. Gms.	Iodine No.
1	9.5	168.4
2	7.7	172.8
3	7.0	172.6, 172.9
4	8.6	173.2, 173.3
5	46.7	173.5, 173.2
residue	18.0	168.0

Fractions 4 and 5 were combined and redistilled with results as shown in table VIII.

TABLE VIII

Fraction	Wt. Gms.	Iodine No.
1	3.2	173.8, 172.6
2	4.5	173.8, 173.2
3	3.8	173.9, 173.9, 173.3, 173.5
residue	20.1	173.9, 174.2

The results of the redistillation indicate that trilinolein can also be distilled in the molecular still without appreciable decomposition or polymerization. Fraction 4, table VI and fraction 4, table VIII are considered to represent pure material and were used in the following studies of its properties. Although the iodine number is slightly high (0.3%) it is not considered significantly so for the type of compound and method of analysis.

Some Properties of Distilled Trilinolein. The pure trilinolein was a colorless, odorless, tasteless, rather viscous liquid. Fraction 4, table VIII had a saponification equivalent of 292.4 (292.9 = theory), and free acid was 0.03% as linoleic. Refractive indices were $n_D^{40^\circ} = 1.4719$, $n_D^{50^\circ} = 1.4683$. Fraction 4, table VI had identical refractive index, and a density of 0.9184 at 40°. Molecular refraction

calculated from data at 40° was 267.9, compared to 267.4 for the sum of atomic refractions. Freezing points and thiocyanogen absorption will be considered in detail in the sections to follow.

Thiocyanogen Absorption of Trilinolein. The use of thiocyanogen absorption as an analytical means of determining mixtures of oleic, linoleic and saturated acids or their esters is based on the assumption that the thiocyanogen adds completely to oleic and quantitatively to only one of the two double bonds of linoleic acid, or at least gives values exactly half of the iodine number. Kaufman³², who has been largely responsible for development of its use, has summarized many of its applications, and gives many references. However, an examination of the results on which the method is based leave much to be desired. The method has not been properly tested on pure materials, and usually no mention is made of temperature. Kaufman (loc.cit.) merely states that "The titration was not on free linoleic acid, but on the triglyceride. Iodine number 169.1. Thiocyanogen number 83.5, remained constant after about five hours. It shows that the thiocyanogen number is one-half the iodine number". No information was given as to the source of the

32. H. D. Kaufman, Studien auf dem Fettgebiet. (Berlin, Verlag, Chemie, G.m.B., 1935) Chapter III.

linolein, nor temperature. This iodine number is 3.3 units below theory, and the thiocyanogen number is 3.2 units below theory for one double bond, or 2.1 units below one-half of the iodine number. Kimura³³ reported a thiocyanogen number of 89.9 for methyl linoleate (theory = 86.3), but the iodine number of the product was reported as 180.4 (theory = 172.5). Waterman, Bertram and Van Western³⁴ reported values on linoleic acid (from the tetrabromide) of 91.9 (29 hours) and 92.9 (70 hours) (90.6 = theory). Kaufman and Hartweg³⁵ recently described a semi-micro method, and reported a linoleic acid with iodine number of 182.0 (181.2 = theory) which gave thiocyanogen numbers from 90.9 at 4 hours to 91.5 at 12 hours. No statement of temperature was made. The Committee on Analysis of Commercial Fats and Oils, American Chemical Society³⁶ recommends a 0.2 N solution for 24 hours at 18° to 21°, but no results are given on pure materials. The Association of Official Agricultural Chemists²⁸ (loc.cit.) adopted as tentative a method using 0.1 N solution for 20 to 24 hours, with no statement of temperature. McKimney³⁷ reported a collaborative study on corn and cottonseed oil of the two preceding methods, and favored the latter as to

33. W. Kimura, J. Soc. Chem. Ind. Japan (suppl.) 32: 141B (1929).

34. H.I. Waterman and S. H. Bertram, J. Soc. Chem. Ind. 48: 50-51T (1929).

35. H.P.Kaufman and L.Hartweg, Fette u. Seifen 45: 356-9 (1938).

36. W.H. Irwin et al, Ind. Eng. Chem. Anal. Ed. 8: 233-7 (1936).

37. R.S. McKimney. J. Assoc. Official Agr. Chem. 21:443-5 (1938)

reproducibility and probable accuracy. Miemenschneider and Wheeler²³(loc.cit.) studied the course of the absorption of thiocyanogen by highly purified methyl oleate and methyl linoleate, and found that at 20-24°, a three hour reaction time was most accurate, particularly in mixtures containing rather large proportions of linoleic ester. A longer time, up to 24 hours gave values as much as 3 or 4 units too high for methyl linoleate.

It was therefore considered desirable to study the absorption of thiocyanogen by pure trilinolein to similarly establish the correct reaction time for this acid in the form of its triglyceride. The results were as shown in table IX. The analyses were made at 20° to 23°.

TABLE IX

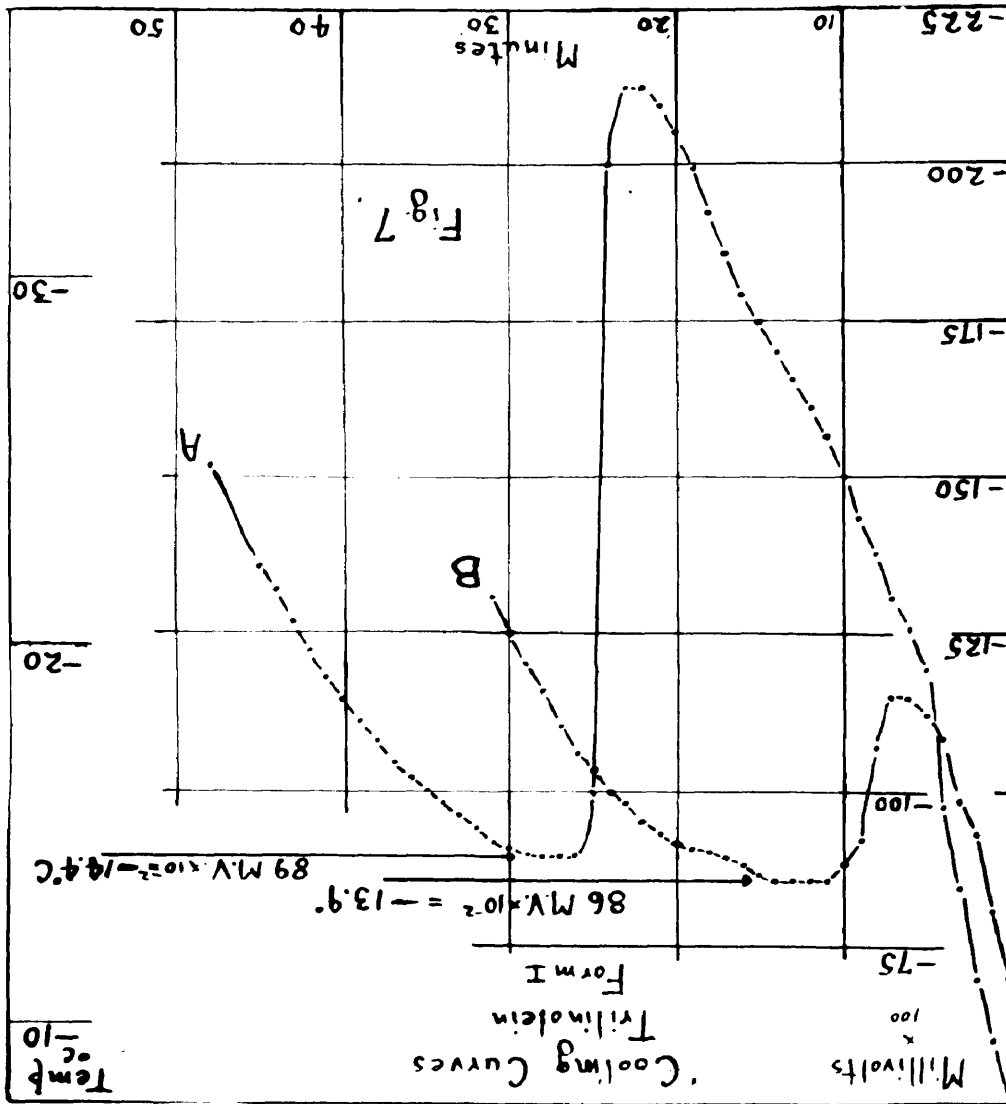
Hours Reaction Time	Thiocyanogen No.(theory 86.7)
1	73.9
2	83.9, 84.2
3	85.3, 85.4 85.2, 85.7
4	87.0, 87.2, 86.7,) ave.= 86.6, 86.7, 87.3) 87.1
5	87.2, 87.3
10	88.6, 88.5
24	90.6, 90.4

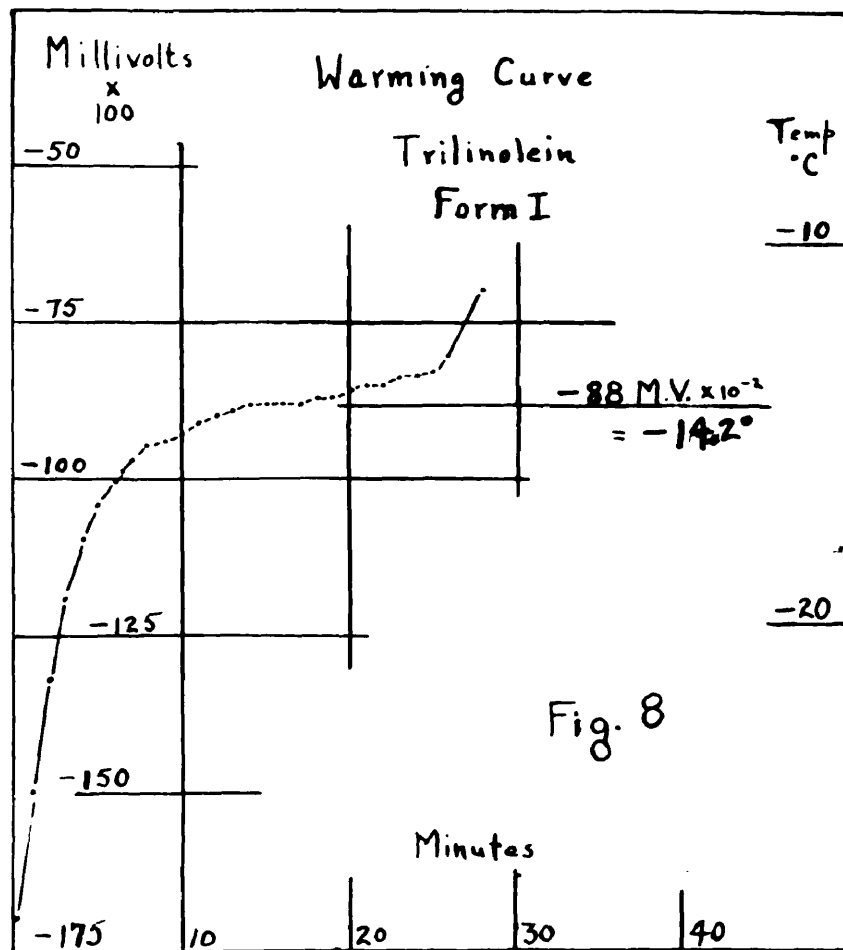
These data show that a 4 hour reaction time gives values very close to theory (86.7) for one double bond of linoleic acid in trilinolein, at a temperature of 20° to 23°. It will be recalled that triolein gave values close to theory from 4 hours on, at 20° to 23°. These facts establish 4 hours as the correct reaction time for oleic and linoleic glycerides, in determining the thiocyanogen number. Several determinations made at around 30° were several units higher than those shown above, at two and three hours, and they indicate the necessity for temperature control for reproducible and accurate results.

Melting and Freezing Points and Polymorphism

of Trilinolein. Only two forms were observed in the case of trilinolein, in contrast to the three forms found for triolein. These were a stable higher melting form I, m.p. -13.1° to -12.8° (capillary) and a lower melting form II, m.p. about -43° . The capillary observations are of a similar character to those reported for triolein, except that the changes seem to be slower. The sample had to be held at -70°C for about 15 minutes before it changed from the clear supercooled liquid to a slightly translucent crystalline appearance (form II). If the sample were now rapidly placed in a bath at -43° or above, it completely melted to a clear liquid and then changed to an opaque white solid (form I). This change to form I required 30 to 40 seconds in the capillary tubes. The melting point of the substance, taken in the usual manner was now -13.1° to -12.8° . No intermediate forms were found either by capillary observations nor by cooling and warming curves.

Cooling curves for Form I (Fig. 7) show extreme supercooling without seeding (curve A external temp. -45°), and the arrest is shorter and 0.5° lower than when supercooling is diminished by seeding, (curve B, external temp. -28°). The freezing point from the latter curve is seen to be -13.9° . The warming curve, (Fig. 8) shows a melting





point slightly lower, -14.2° .

Evidence for form II was not obtained on cooling curves, probably due to greater tendency to supercooling. By rapid cooling of the sample by direct immersion (without external tube) of the tube containing the sample into alcohol-carbon dioxide slush, it could be cooled to -75° as a very viscous supercooled liquid, which, after about fifteen minutes, changed to a white opaque solid, accompanied by a slight rise in temperature, followed by cooling back to -75° . A warming curve on the sample now showed an incomplete hold at -45.5° , followed by a sharp rise in temperature, often above the external bath temperature (Fig. 9, external bath -30°). The material now shows the m.p. of form I.

Summary of Data on Trilinolein. The results obtained on trilinolein are summarized in table X.

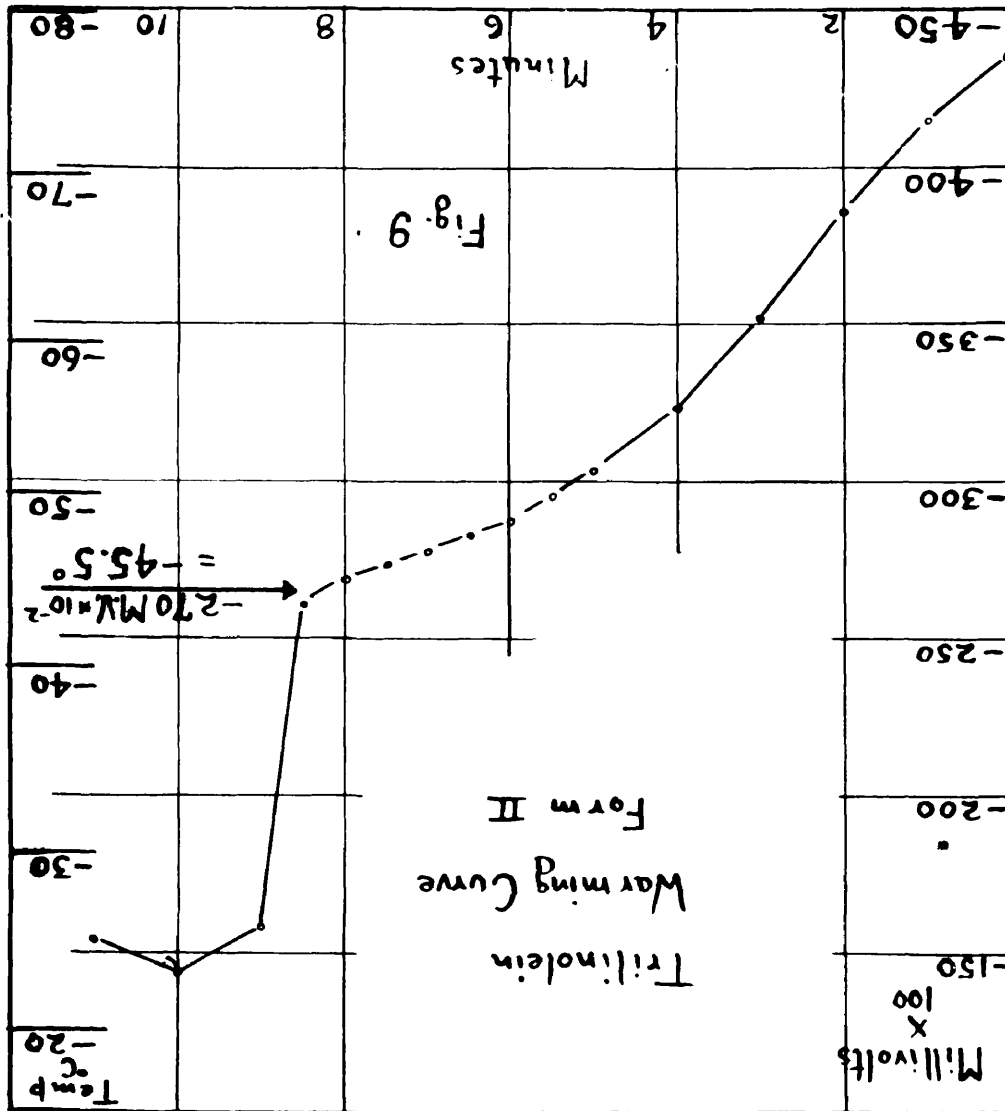


TABLE X

	Found	Theory
Iodine No.	173.7 \pm .2	173.4
Thiocyanogen no.	87.1 (4 hr.) 90.5 (24 hr.)	85.7
Density	0.9184 at 40 $^{\circ}$	
Refractive index	$n_D^{40^{\circ}} = 1.4719$, $n_D^{50^{\circ}} = 1.4683$	
Molecular refraction	267.9	267.4
Saponification equivalent	292.4	292.9
Free acid	0.05	0.0
Melting Points	I -13.1 $^{\circ}$ to -12.9(cap.) II -43 $^{\circ}$	-13.9 $^{\circ}$ (curve) " -45.5 $^{\circ}$

SECTION V

SUMMARY

1. Triolein and Trilinolein have been synthesized by direct esterification of glycerol with the acids and p-toluenesulfonic acid as catalyst, in oxygen-free nitrogen atmosphere at 120°-125°.
2. Triolein and Trilinolein have been purified by low temperature crystallization and molecular distillation to give products with analytical constants in good agreement with theoretical values.
Certain physical constants of the purified triglycerides have been determined.
3. Thiocyanogen absorption of triolein and trilinolein has been studied, and a 4 hour period at 20-23° found to be correct.
4. Evidence for three different crystalline forms of triolein has been obtained from capillary melting points and from cooling and warming curves.
5. Evidence for two different crystalline forms of trilinolein has been obtained from capillary melting points and from cooling and warming curves.

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GRADUATE SCHOOL RECORD

Name Donald Hyde Wheeler

MAJOR Organic Chemistry		Degree sought Ph.D.				
Year	Description of course	No. of course	1st Sem.	2nd Sem.	Sum-mer	Total
1927-28	Advanced organic chemistry	Ch.	4 SAT			4
1929-30	Advanced Organic Chemistry	Ch. 116y	4 B	2 A		6
	Seminar	Ch. 226	1 SAT	1 SAT		2
	Research	Ch. 210		2 SAT		2
1930	Research	Ch. 224S			6 SAT	6
1930-31	Microanalysis	Ch. 206s		4 B		4
1936-37	Special Topics	Ch. 203f	2 A			2
1938-39	Special Topics	Ch. 203f	2 B			2
	Research	Ch. 229f	4 I			4

MINORS 1. Physical Chemistry

2.

1928-29	Advanced Inorganic Chemistry	Ch. 103s		4 A		4
1929-30	Colloid Chemistry	Ch. 212y	2 A	2 A		4
1930-31	Physical Chemistry	Ch. 102y	3 A	3 A		6
	Catalysis	Ch. 215f	2 A			2
	Industrial Chemistry	Ch. 110y	3 A			3
	Advanced Inorganic Laboratory	Ch. 200 ByN	2 A			2
<u>Second Minor—Physics</u>						
1928-29	Advanced Physics	Phys. 108f	4 B			4
1937-38	Atomic Structure	Phys. 200Ny	3 A	3 A		6

Average B+

Undergraduate Transcript Oberlin College

Transfers None

Residence 11 semesters, part time

200 Courses Modern Languages, OK

Thesis Subject The oxidation of unsaturated fatty acids and their esters.

THE GRADUATE SCHOOL
University of Maryland

I beg to appoint the following committee to examine Mr. Donald H. Wheeler

Representing major subject Dr. Drake (Chairman)

Dr. Mozingo

Representing minor subjects Dr. Haring

Dr. White

Prof. Eichlin or Dr. Dickinson

Representing Graduate Faculty Dr. Broughton

DATE OF EXAMINATION: **May 13 to May 29, 1940**

The chairman of the committee will arrange with the other members of the committee, the exact date, hour and place for the examination. He will also notify the candidate. The report of the examination must be on file in the office of the Graduate School not later than **May 29, 1940.** A favorable report is signed by each member of the committee. The entire examination will ordinarily be oral or may be partly written as determined by the committee in charge. A typewritten copy of the thesis must be deposited in the office of the Graduate School not later than **May 11, 1940.**

C. O. Appleman,

Dean of the Graduate School.