

[CONTRIBUTION NO. 222 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

### SYNTHETIC GLYCERIDES. III. MIXED TRIGLYCERIDES OF THE DISTEARIN SERIES

By H. E. ROBINSON, J. N. ROCHE AND C. G. KING

RECEIVED AUGUST 28, 1931

PUBLISHED FEBRUARY 5, 1932

In previous communications<sup>1</sup> on synthetic glycerides it was pointed out that much of the information reported in the literature was faulty and that reliable constants were not available for the identification of natural fatty acid glycerides. Experimental work indicated that the melting points and refractive indices of symmetrical isomers were definitely higher than those of the unsymmetrical forms. In continuing the study, six sets of isomeric mixed triglycerides of the distearin series have been prepared and their melting points and refractive indices determined. Quantitative solubility determinations have also been made on representative pairs.

Progress in the study of the components of naturally occurring fats is in part dependent upon the synthesis of fatty acid glycerides of known constitution and the determination of their chemical and physical properties.

A critical study of methods used for the preparation of glycerides and the correlation of available data on their physical properties should bring out definite relationships between the constitution of the fats and their physical properties. In the past the methods of synthesis of the glycerides have varied greatly and the products of the reactions have not always been definitely characterized. In many cases where pure compounds were thought to have been formed, it is probable that mixtures or unexpected isomers were formed.

A review of the literature readily shows the need for the preparation and determination of physical constants of glycerides of proved configuration. Fischer's work<sup>2</sup> on the shifting of acyl groups in glycerides did much to stimulate interest in clarifying and extending the data on the preparation and properties of such compounds.

The greatest discrepancies in the synthesis of glycerides have appeared in the cases where so-called  $\beta$ -monoglycerides and  $\alpha,\beta$ -diglycerides were utilized as intermediate compounds. Fairbourne and Foster<sup>3</sup> have shown experimentally that  $\beta$ -monoglycerides prepared from  $\beta$ -monochlorohydrin and the potassium salts of fatty acids by the method reported by Grün,<sup>4</sup> or some modification thereof, are identical with the  $\alpha$ -compounds. The

<sup>1</sup> J. N. Roche, H. P. Averill and C. G. King, *THIS JOURNAL*, **51**, 866 (1929); **52**, 365 (1930).

<sup>2</sup> E. Fischer, *Ber.*, **53**, 1621 (1920).

<sup>3</sup> A. Fairbourne and G. E. Foster, *J. Chem. Soc.*, 3148 (1926).

<sup>4</sup> Grün, *Ber.*, **43**, 1288 (1910).

triglycerides prepared from such  $\beta$ -monoglycerides are therefore probably the unsymmetrical isomers.

Delaby and Dubois,<sup>5</sup> from a study of the reactions between glycerol and formic acid, reported the synthesis of  $\alpha,\beta$ - and  $\alpha,\alpha'$ -diformates of glycerol. The probable identity of these compounds is indicated by the investigations of Fairbourne<sup>6</sup> on  $\alpha,\beta$ - and  $\alpha,\alpha'$ -diglycerides. From the results of Fairbourne's investigations, it would seem very doubtful that an unsymmetrical diglyceride has ever been isolated in pure form. Syntheses of unsymmetrical triglycerides based on the use of  $\alpha,\beta$ -diglycerides would seem questionable in like degree.

Helferich and Sieber<sup>7</sup> have apparently made a true  $\beta$ -monobenzoate by a method which will probably be applicable to other  $\beta$ -type esters. Hill, Whelen and Hibbert<sup>8</sup> have synthesized the true  $\beta$ -methyl ether, probably the first  $\beta$ -ether of proven constitution ever isolated. It is noteworthy that the compound is reported to be comparatively stable.

From a consideration of the slight difference in the physical constants of the isomeric  $\alpha$ - and  $\beta$ -monomargarins reported by Thomson<sup>9</sup> and the doubtful method of preparation of the symmetrical compound, it is probable that these compounds are identical. The same criticism may be made regarding the  $\alpha,\beta$ - and  $\alpha,\alpha'$ -dimargarins reported by Thomson.

Fairbourne<sup>6</sup> has shown that all preparations of  $\alpha,\beta$ -distearin from  $\alpha,\beta$ -dichloro- and  $\alpha,\beta$ -dibromohydrins are identical with  $\alpha,\alpha'$ -distearin prepared in an analogous manner from  $\alpha,\alpha'$ -dihalohydrins. Fischer's work<sup>2</sup> on the symmetrical structure of distearin prepared from  $\alpha$ -iodo- $\alpha',\beta$ -distearin offers an explanation for the probable symmetrical structure of nearly all so-called unsymmetrical diglycerides of fatty acids which have been reported in the literature. The experiments of Grün and Limpacher<sup>10</sup> on the optical activity of  $\alpha,\beta$ - and  $\alpha,\alpha'$ -diglycerides do not appear to permit any definite conclusions, probably because adequate methods for the preparation of  $\alpha,\beta$ -diglycerides were not available.

The use of  $\alpha,\beta$ -acetoneglycerol which has been prepared by the condensation of acetone and glycerol in either neutral or acid medium, for the preparation of  $\alpha$ -monoglycerides, is justified by the work of Irvine, MacDonald and Soutar<sup>11</sup> and Hibbert and Marazain.<sup>12</sup> The isomeric  $\alpha,\alpha'$ -acetoneglycerol has been prepared and its configuration proved by Carter.<sup>13</sup>

<sup>5</sup> R. Delaby and P. Dubois, *Compt. rend.*, **187**, 767 (1928).

<sup>6</sup> A. Fairbourne, *J. Chem. Soc.*, 369 (1930).

<sup>7</sup> B. Helferich and H. Sieber, *Z. physiol. Chem.*, **175**, 311 (1928).

<sup>8</sup> H. C. Hill, M. S. Whelen and H. Hibbert, *THIS JOURNAL*, **50**, 2235 (1928).

<sup>9</sup> W. F. Thomson, *Trans. Roy. Soc. Canada*, **20**, 445 (1926).

<sup>10</sup> Grün and R. Limpacher, *Ber.*, **59**, 1350 (1926); **60**, 147 (1927).

<sup>11</sup> J. C. Irvine, J. MacDonald and C. W. Soutar, *J. Chem. Soc.*, **107**, 337 (1915).

<sup>12</sup> H. Hibbert and J. G. Marazain, *Can. J. Res.*, **2**, 35 (1930).

<sup>13</sup> N. M. Carter, *Ber.*, **63B**, 2399 (1930).

A new method may be available for the synthesis of mixed triglycerides through the preparation and identification of  $\beta$ -monopalmitin by Bergmann and Carter.<sup>14</sup>

In the field dealing with the separation and identification of naturally occurring mixed triglycerides, the work of Hilditch and associates<sup>15</sup> is outstanding, particularly in view of the applicability of their procedures to the problem of establishing the configuration of mixed glycerides containing both saturated and unsaturated fatty acids. Proof of the structures of such derived products will also be in part dependent upon the availability of reliable methods of synthesis.

### Experimental

The absolute glycerol, acetone, chloroform, ethyl alcohol, ether, ethyl acetate and petroleum ether used in this work, were prepared by standard methods. The purity of all reagents used during the course of the investigation was carefully checked.

Acyl chlorides were prepared by the use of thionyl chloride and fatty acids as recommended by McMaster and Ahmann.<sup>16</sup> Capryloyl chloride, b. p. 71–73° (6–7 mm.), capryl chloride, b. p. 105–107° (8–9 mm.), and stearyl chloride, b. p. 185° (3 mm.), were prepared by this method.

**Preparation of Intermediate Glycerides.**— $\alpha$ -Monocaproin was prepared by the method recommended by Schuette and Hale<sup>17</sup> for  $\alpha$ -monobutyryl. Anhydrous glycerol (25 g.) was treated with 30 g. of caproic acid and 2 g. of phosphoric acid (85%). The final distillate showed a boiling point of 162° (4 mm.); yield 75%.  $\alpha$ -Caproyl-acetone-glycerol, b. p. 124° (3 mm.), was prepared by the action of caproyl chloride on acetone-glycerol. On attempted hydrolysis with concentrated hydrochloric acid at –15° the compound decomposed.

An attempt to prepare  $\alpha$ -monocapryloin by the above method was unsuccessful. Direct esterification of glycerol with fatty acids of more than six carbon atoms as above described does not seem to be readily accomplished. The  $\alpha$ -monocapryloin used was prepared by Fischer's method. It distilled at 178–181° (5–6 mm.).  $\alpha$ -Monocaprin, m. p. 51.2°,  $\alpha$ -monolaurin, m. p. 63.2°,  $\alpha$ -monomyristin, m. p. 67.5°, and  $\alpha$ -monopalmitin, m. p. 77.0°, were prepared as described in a previous communication.

$\alpha, \alpha'$ -Distearin, m. p. 78.9°, was prepared from  $\alpha$ -iodo- $\alpha', \beta$ -distearin by hydrolysis with silver nitrite in boiling ethyl alcohol. In the purification of the  $\alpha, \alpha'$ -distearin, the addition of a small amount of sodium iodide solution was found to aid materially in the removal of traces of silver. This compound was also prepared by the action of sodium stearate on  $\alpha, \alpha'$ -dichlorohydrin. The melting points and mixed melting points of the two preparations were identical.

**Synthesis of Triglycerides.**—For the preparation of symmetrical mixed triglycerides the preparation of  $\beta$ -capro- $\alpha, \alpha'$ -distearin is used as an example.

This compound was prepared by the slow addition of 0.68 g. of capryl chloride with 0.50 g. of quinoline to 2.0 g. of  $\alpha, \alpha'$ -distearin contained in a glass-stoppered iodine flask. The reaction mixture was dissolved in 10 cc. of chloroform and allowed to stand in an oven at 55° for forty hours. The chloroform solution was then taken up with 100 cc.

<sup>14</sup> M. Bergmann and N. M. Carter, *Z. physiol. Chem.*, **191**, 211 (1930).

<sup>15</sup> T. P. Hilditch and C. H. Lea, *J. Chem. Soc.*, 3106 (1927); G. Collin, T. P. Hilditch and C. H. Lea, *J. Soc. Chem. Ind.*, **48**, 46T (1929).

<sup>16</sup> L. McMaster and F. E. Ahmann, *THIS JOURNAL*, **50**, 147 (1928).

<sup>17</sup> H. A. Schuette and J. T. Hale, *ibid.*, **52**, 1978 (1930).

of ether and 60 cc. of half normal sulfuric acid and placed in a separatory funnel. The ether solution was washed successively with half normal sulfuric acid, 10% potassium bicarbonate solution and finally with distilled water. After several hours of drying over sodium sulfate, the triglyceride was precipitated from ether solution by cooling in an ice-salt bath at  $-20^{\circ}$ . The white crystals were dried over phosphorus pentoxide *in vacuo*. After three recrystallizations from ether and from acetone the compound melted sharply at  $56.2^{\circ}$ ; yield, 72%.

For the unsymmetrical mixed triglycerides, the  $\alpha$ -monoglycerides were treated with two moles of stearyl chloride, using quinoline as a condensing agent. The unsymmetrical compounds were all prepared below  $35^{\circ}$ .

The melting points and refractive indices of the triglycerides prepared are listed in the following table.

TABLE I  
MELTING POINTS AND REFRACTIVE INDICES

Compound	M. p. found, $^{\circ}$ C.	$n_D^{20}$	M. p. and R. I. previously recorded
$\alpha$ -Caproylo- $\alpha',\beta$ -distearin	42.7	1.43985	New
$\beta$ -Caproylo- $\alpha,\alpha'$ -distearin	47.2	1.44019	New
$\alpha$ -Capryllo- $\alpha',\beta$ -distearin	47.6	1.44028	New
$\beta$ -Capryllo- $\alpha,\alpha'$ -distearin	51.8	1.44140	New
$\alpha$ -Capro- $\alpha',\beta$ -distearin	48.2	1.44058	New
$\beta$ -Capro- $\alpha,\alpha'$ -distearin	56.2	1.44153	New
$\alpha$ -Lauro- $\alpha',\beta$ -distearin	50.6	1.44098	47 and $49^{\circ}$ , <sup>a</sup> 49-50 <sup>b</sup>
$\beta$ -Lauro- $\alpha,\alpha'$ -distearin	59.8	1.44222	53.5 and $68.5^{\circ}$ <sup>c</sup>
$\alpha$ -Myristo- $\alpha',\beta$ -distearin	58.5	1.44122	52 and $62^{\circ}$
$\beta$ -Myristo- $\alpha,\alpha'$ -distearin	63.5	1.44300	55 and $57.5^{\circ}$ <sup>c</sup>
$\alpha$ -Palmito- $\alpha',\beta$ -distearin	62.6	1.44245	$63.2^{\circ}$ <sup>d</sup>
$\beta$ -Palmito- $\alpha,\alpha'$ -distearin	68.0	1.44374	$67.9^{\circ}$ , <sup>d</sup> $64.8^{\circ}$ , <sup>e</sup> $n_D^{70} - 1.4467^e$

Average  $dn/dt$  for six unsymmetrical isomers, 0.00040

Average  $dn/dt$  for six symmetrical isomers, 0.00041

Average difference in R. I. for six isomeric pairs, 0.00112

Average difference in m. p. for six isomeric pairs,  $6.0^{\circ}$

<sup>a</sup> *Ber.*, **40**, 1795 (1907); <sup>b</sup> *ibid.*, **53**, 1589 (1920); <sup>c</sup> *ibid.*, **40**, 1781 (1907); <sup>d</sup> *Biochem. Z.*, **130**, 252 (1922); <sup>e</sup> *Proc. Roy. Soc. Canada*, **13**, 255 (1919).

Consideration of the data presented shows that in every case the symmetrical compounds have a higher index of refraction and a higher melting point than their unsymmetrical isomers. The melting points and refractive indices of the compounds are seen to be in order in the series. An immersion refractometer mounted in a constant temperature bath as described previously was used for all determinations. The values for the refractive indices given in the table were derived from graphs plotted from index readings over a range of about  $10^{\circ}$ . A consistent difference in refractive index was found for the two isomeric types, the symmetrical form being slightly higher, as found previously.

The molecular weights of all triglycerides prepared were found to check the theoretical values within the limits of experimental error. An accuracy of 1 to 2% was attained in the determination of molecular weights

using the ebullioscopic procedure with the differential thermometer perfected by Menzies and Wright.<sup>18</sup> Ethyl acetate was found to be the best solvent for the determinations.

**Determination of Quantitative Solubilities.**—It has been stated previously<sup>1,2</sup> as a qualitative observation that unsymmetrical triglycerides were somewhat more soluble than their symmetrical isomers, but no quantitative data have been recorded. It is evident that there is an appreciable difference in the solubility of the isomeric forms in the usual organic solvents. This difference in solubility should be of considerable assistance in the separation and identification of isomers.

Ten cc. of the solvent used was saturated at 50° with the glyceride and placed in a thermostat at the indicated temperature for several hours with constant shaking. One cc. of the saturated solution was withdrawn and evaporated to dryness in a weighing bottle.

TABLE II  
SOLUBILITIES

Compound	Solvent <sup>a</sup>	T, °C.	Solubility in grams per 100 cc. of solvent
$\alpha$ -Capro- $\alpha'$ , $\beta$ -distearin	Acetone	29.0	39.45
$\beta$ -Capro- $\alpha$ , $\alpha'$ -distearin	Acetone	29.0	2.57
$\alpha$ -Capro- $\alpha'$ , $\beta$ -distearin	Alcohol	27.5	0.22
$\beta$ -Capro- $\alpha$ , $\alpha'$ -distearin	Alcohol	27.5	0.14
$\alpha$ -Lauro- $\alpha'$ , $\beta$ -distearin	Petr. ether	27.5	38.41
$\beta$ -Lauro- $\alpha$ , $\alpha'$ -distearin	Petr. ether	27.5	11.42
$\alpha$ -Myristo- $\alpha'$ , $\beta$ -distearin	Alcohol	29.0	0.59
$\beta$ -Myristo- $\alpha$ , $\alpha'$ -distearin	Alcohol	29.0	0.47
$\alpha$ -Palmito- $\alpha'$ , $\beta$ -dimyristin <sup>b</sup>	Alcohol	27.5	0.42
$\beta$ -Palmito- $\alpha$ , $\alpha'$ -dimyristin <sup>b</sup>	Alcohol	27.5	0.10
$\alpha$ -Palmito- $\alpha'$ , $\beta$ -dimyristin <sup>b</sup>	Acetone	27.5	1.82
$\beta$ -Palmito- $\alpha$ , $\alpha'$ -dimyristin <sup>b</sup>	Acetone	27.5	0.61

<sup>a</sup> All solvents used were anhydrous. <sup>b</sup> Prepared by Roche, Averill and King.<sup>1</sup>

### Summary

Isomeric triglycerides of the distearin series have been prepared containing the natural fatty acids from caproic to palmitic. Melting points, refractive indices, solubilities and molecular weights of the isomeric compounds have been determined.

It was found in every case that the melting point of the symmetrical compound is appreciably higher (average 6°) than that of the corresponding isomer. The melting points of the individual compounds are also in order in the series. The symmetrical isomers also have a measurably higher index of refraction (average 0.00112) and a lower solubility. The refractive indices of the isomers are also in order as a series.

The use of thionyl chloride for the preparation of acyl chlorides of

<sup>18</sup> A. W. C. Menzies and S. L. Wright, THIS JOURNAL, 43, 2312 (1921).

the fatty acids was found more satisfactory than the methods previously used. The removal of traces of silver from the diglycerides synthesized by Fischer's method has been simplified by the use of sodium iodide solution.

The synthesis and identification of the following compounds herein described have not been reported previously:  $\alpha$ -monocaproin,  $\alpha$ -monocaproylo-acetoneglycerol, and the isomeric caproylo-distearins, capryllo-distearins and capro-distearins.

PITTSBURGH, PENNSYLVANIA

[CONTRIBUTION FROM THE FERTILIZER AND FIXED NITROGEN DIVISION OF THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

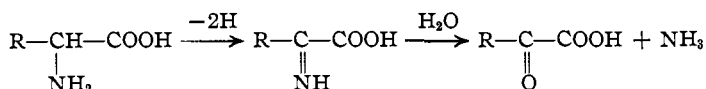
## THE DEHYDROGENATION OF CERTAIN AMINES AND HYDRAZINES BY UNSATURATED COMPOUNDS (PRELIMINARY PAPER)

BY L. A. PINCK AND GUIDO E. HILBERT

RECEIVED SEPTEMBER 2, 1931

PUBLISHED FEBRUARY 5, 1932

The two theories of biological oxidation that probably have received most attention in recent years are those of Warburg<sup>1</sup> and of Wieland.<sup>2</sup> Warburg's theory is expressed as being a process in which the oxidation is catalyzed by iron. The active agent, "the respiration enzyme," is an iron complex of a compound related to hematin. Wieland's theory, which is definitely contradictory to that of Warburg, is pictured as being a transfer of hydrogen atoms from one molecule to the other. The oxidation is thus essentially a dehydrogenation. Wieland and Bergel<sup>3</sup> studied the oxidation of amino acids with animal charcoal and adduced evidence that the degradation takes place in the following manner



in which the first step consists of a removal of hydrogen atoms. The oxidation of amines, which has been the subject of considerable work, has usually been carried out by such drastic reagents as the hypohalites<sup>4</sup> and potassium permanganate,<sup>5</sup> oxidizers which presumably do not exist in biological systems. Additional evidence has now been obtained in favor of Wieland's mechanism of biological oxidation. The dehydrogenation has

<sup>1</sup> Otto Warburg, "Über die katalytischen Wirkung der lebendigen Substanz," Berlin, 1928.

<sup>2</sup> Wieland, *Ergebnisse Physiol.*, **20**, 477 (1922).

<sup>3</sup> Wieland and Bergel, *Ann.*, **439**, 196 (1924).

<sup>4</sup> Hofmann, *Ber.*, **14**, 2725 (1881); Stieglitz, and co-workers, *THIS JOURNAL*, **36**, 272 (1914); **38**, 2046 (1916); Hellerman and Sanders, *ibid.*, **49**, 1742 (1927).

<sup>5</sup> Goldschmidt and Beuschel, *Ann.*, **447**, 197 (1926); Goldschmidt and Reichel, *ibid.*, **456**, 152 (1927).