[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, TEXAS AGRICULTURAL EXPERIMENT STATION, TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM]

Syntheses of Derivatives of Dihydroxyacetone and of Glycerides[1,2]

BY HERMANN SCHLENK, BEVERLY G. LAMP AND B. WALLACE DEHAAS

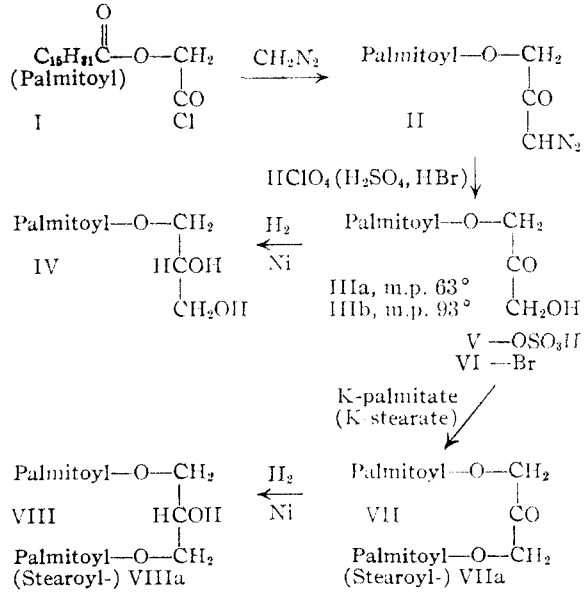
RECEIVED DECEMBER 26, 1951

A synthesis of glycerides is reported in which the alcoholic groups of the glycerol successively are built up and become available to react with individual fatty acids. Dihydroxyacetone esterified with fatty acids is an intermediate in the procedure. Palmitoxyhydroxyacetone is found to occur in a monomer and a dimer form. In the further reactions the keto group is catalytically reduced and forms the β-hydroxy group of the glycerol. The scope of applications for synthesizing glycerides will be comparable with that of the methods so far known. The new procedure is supplementary inasmuch as radioactive labeling of the glycerol is made possible. The relative positions of the fatty acids with respect to this label will then be known.

In a recent paper a synthesis of glycerol was outlined which was developed primarily for a possible preparation of radioactive glycerol.[3] The principles of this procedure were the preparation of 1-chloro-3-diazoacetone according to Arndt and Eistert,[4] subsequent replacement of the diazo group by hydrochloric acid followed by the reduction of the keto group and hydrolysis of the resulting 1,3-dichlorohydrin.

The same reactions when applied to acylated glycolic acid chloride provide a method for the preparation of esterified dihydroxyacetones and finally glycerides. The intermediates as well as the method of synthesizing glycerides are of interest even without the special aspect of tracer research. The reactions are shown in Table I.

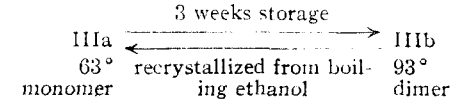
TABLE I



Palmitoxyacetic acid is accessible in satisfactory yields by the common procedure of esterification by treating acid chlorides with alcoholic groups in pyridine. This is preferable to the method of Grün and Wittka who prepared stearoxyacetic acid from the potassium salts of chloroacetic acid and stearic acid.[5] The Arndt–Eistert reaction[6] although carried out with a large excess of diazomethane will yield some 1-palmitoxy-3-chloroacetone and the desired diazoketone II is obtained in a yield not much higher than 60%. The diazo compound can be stored in the refrigerator for weeks without any decomposition. In light and at room temperature the m.p. is lowered within several days.

The catalytic decomposition of the diazo group to form 1-palmitoxy-3-hydroxyacetone (III) is carried out best by utilizing perchloric acid in aqueous dioxane. For this type of reaction aqueous sulfuric acid commonly is used as the catalyst[7] together with ethanol or dioxane as the organic solvent. In this instance, however, a sulfuric acid half ester, *i.e.*, 1-palmitoxyacetonyl-3-sulfate (V), will form as a by-product besides two substances having m.p. 63 and 93°. These two compounds (IIIa,b) are also the products of the reaction with perchloric acid. Their analyses are identical and in agreement with the values of palmitoxyhydroxyacetone. The chemical structure of IIIa and IIIb is established by the reaction with periodic acid.[8] The oxidizing titration shows 1 ketol group per molecule and allows the isolation of nearly 1 mole of formaldehyde-bismethone in both cases. Thereby, the structure of a derivative of glyceroldehyde or other tautomers is excluded for both isomers.

The further observations suggest the following interrelationship for the two substances



Attributing the higher melting point to a dimer form is justified by the molecular weight of IIIb. It was found to be twice the value of a palmitoxyhydroxyacetone. For dihydroxyacetone itself, the monomer–dimer interconversion was shown in particular by Bertrand[9] and Fischer.[10] The hydrogenation of IIIa, as well as IIIb, results in monopalmitin.

(1) This investigation was supported partly by a grant from the United States Atomic Energy Commission.

(2) Presented in part at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September, 1951.

(3) H. Schlenk and B. Lamp, THIS JOURNAL, **73**, 5493 (1951).

(4) F. Arndt and B. Eistert, *Ber.*, **61**, 1124 (1938).

(5) A. Grün and F. Wittka, *ibid.*, **54**, 273 (1921).

(6) B. Eistert, in "Neuere Methoden der präparativen organischen Chemie," 3rd ed., Verlag Chemie, 1949, p. 378.

(7) B. Eistert, *ibid.*, p. 381.

(8) E. Handschumaker and L. Linteris, *J. Am. Oil Chem. Soc.*, **24**, 143 (1947); the end value for oxidizing the ketol is reached after 2 min. shaking.

(9) G. Bertrand, *Compt. rend.*, **129**, 341 (1899).

(10) H. O. L. Fischer and H. Mildbrand, *Ber.*, **57**, 707 (1924).

The diazo group in 1-palmitoxy-3-diazoacetone is easily replaced by HBr. The bromoketone VI in turn reacts with potassium palmitate yielding 1,3-dipalmitoxyacetone (VII). The same substance is accessible also from dichloroacetone in a similar reaction, or from 1-chloro-3-diazoacetone by reacting first with melted palmitic acid which replaces the diazo group. The intermediate 1-palmitoxy-3-chloroacetone (not isolated) forms the second ester group with potassium palmitate.

Dipalmitoxyacetone can be hydrogenated to 1,3-dipalmitin (VIII) which then may be acylated further to form a triglyceride according to the usual procedures.

In attaching the second fatty acid to the glycerol skeleton (converting VI to VII) it is possible to vary this component and so finally to arrive at symmetrical diacid diglycerides, as is shown in the preparation of 1-palmito-3-stearin (VIIa and VIIIa). This opens the way for syntheses of triacid glycerides of known structure.

In suggesting a new synthesis for individual glycerides critical consideration must be given to the structure of the resulting compounds. In Table II the melting points of the substances synthesized and those of the possible isomers are compiled.

TABLE II

Melting point of	Found,[a] °C.	Literature, °C.
1-Palmitoxy-3-hydroxyacetone (IIIa,b)	63, 93	---[l]
α-Monopalmitin (IV)	72–73.5	77[f]
α-Monopalmitin[b]	72–73	
β-Monopalmitin		68[e] 68.5[f]
1,3-Dipalmitoxyacetone (VII)	77–78	---[l]
1,3-Dipalmitin (VIII)	69.5–70.5	72.5[g]
1,2-Dipalmitin		64,[h] 65[i]
1-Palmitoxy-3-stearoxyacetone (VIIa)	75–77	---[l]
1-Palmito-3-stearin (VIIIa)	65.5–66.5	---[l]
1-Palmito-2-stearin		60.5–61[j]
2-Palmito-1-stearin		68.5–69.5[j]
2-Lauro-1,3-palmitin[c]	50–50.5	53.5[k]
2-Lauro-1,3-palmitin[d]	51.5–52.5	

[a] Fisher–Johns melting point apparatus. [b] E. Fischer, M. Bergmann and H. Baerwind, *Ber.*, **53**, 1589 (1920); E. Baer and H. O. L. Fischer, THIS JOURNAL, **67**, 2031 (1945); C.P. grade palmitic acid was used for this synthesis as for the preparation of IV. [c] O. E. McElroy and C. G. King, THIS JOURNAL, **56**, 1192 (1934); VIII was used for this synthesis. [d] See (*c*); 1,3-dipalmitin (m.p. 70–71°) was furnished by Proctor and Gamble. [e] B. F. Daubert, THIS JOURNAL, **62**, 1713 (1940). [f] L. J. Filer, S. S. Sidhu, B. F. Daubert and H. E. Longenecker, *ibid.*, 66, 1335 (1944). [g] T. Malkin, M. R. el Shurbagy and M. L. Meara, *J. Chem. Soc.* 1411 (1937). [h] B. F. Daubert and C. G. King, THIS JOURNAL, 61, 3329 (1939). [i] E. Baer and M. Kates, *ibid.*, 72, 942 (1950). [j] P. E. Verkade, W. D. Cohen and A. K. Vroege, *Rec. trav. chim.*, **59**, 1123 (1940). [k] T. Malkin and M. L. Meara, *J. Chem. Soc.*, 1143 (1939). [l] New compound.

The glycerides synthesized here have different melting points from their isomers; they are low, however, compared with those reported in the literature for the same compounds. The fatty acids used in the commercially available grade may account for this. Nevertheless it is desirable to prove the purity of the glycerol and the structure of the compounds with regard to the position of the substitutions.

Although the structure of III is well established the subsequent hydrogenation to monopalmitin (IV) does not exclude *a priori* acyl migration. The reduction of the keto group is not necessarily comparable with the hydrogenolysis, which is generally accepted in fat syntheses.[11] Some analytical values shown in Table III prove that practically all the product of this reduction is α-monopalmitin as formulated in Table I and that the product compares well with those from other sources.

TABLE III

	M.p., °C.	Glycol[a] (periodic acid) α-Monopalmitin = 1	Total glycerol[b]
Total crude monopalmitin IV (solvent and catalyst removed, A. V. = 2)	67–72	0.93	0.92, 0.91
Recrystallized IV	72–73.5	.98	
α-Monopalmitin[c]	72–73	.98, 0.97	.97
α-Monopalmitin[d]	72–74	.99	

[a] See (8); the end value of the oxidation is reached after 3 min. shaking and 10 min. standing. [b] D. J. Bell, *J. Chem. Soc.*, 992 (1948). [c] See Table II (b). [d] Furnished by Proctor and Gamble, to whom the authors express their appreciation.

No chemical method is considered to be reliable for proving the positional structure of diglycerides. So the identity of VIII as being 1,3-dipalmitin is based upon the melting point only. 2-Lauro-1,3-dipalmitin prepared from VIII is identical with a preparation from 1,3-dipalmitin.[12]

These critical evaluations give no reason to assume any structural change for the final products. In applying the method to unsaturated fatty acids similar restrictions are to be expected as they are found in the known methods. The variations probably will include the use of ethers as intermediates in protecting free hydroxy groups from acylation.

A new aspect is given, however, in that the procedure consists of a stepwise building up of the glycerol and of the ester groups. This allows the placing of a certain fatty acid, possibly radioactive, in a known position relative to a radioactive C-atom used in building up the glycerol. Such detailed structural knowledge of a synthetic fat may become of interest in the further progress of the biochemistry of fats.

Experimental Part

Materials Used.—Lauric, palmitic and stearic acid (C.P., Eastman Kodak Co.) and glycolic acid (Matheson Co.) are used without further purification. Palmitoxyacetic acid is prepared by the reaction of glycolic acid in pyridine with palmitoyl chloride. The crude product is recrystallized from Skelly B and has a m.p. of 83–84°, which cannot be further raised; A.V. calcd. 178; found 176, 178.

Palmitoxyacetyl chloride (I) is prepared from the acid by refluxing with a two molar excess of oxalyl chloride and an equal weight of benzene. After evaporation the acid chloride is solid at room temperature; it is used directly for the subsequent reactions.

(11) A. W. Ralston, "Fatty Acids and their Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1948.

(12) See Table IId.

The diazomethane solutions[13] are titrated and usually are 0.7 to 0.8 molar. The solvents used for the reactions are purified and water-free except when stated otherwise. This applies also to most of the recrystallizations.

1-Palmitoxy-3-diazoacetone (II).—To 27.0 g. (0.0814 mole) of I dissolved in 400 ml. of ether, 0.252 mole of diazomethane in ether is added within one hour. The flask is fitted with a stirrer and kept at −2°. A precipitate is formed which will dissolve at room temperature. The excess of diazomethane is removed by flushing with air under slight vacuum and at the same time the ether is concentrated to about one-third of its volume. After crystallization at 4° the yellowish diazoketone is filtered and recrystallized from ether to yield 16.6 g. of pure II, having a m.p. of 57–59°.

Anal. Calcd. for $C_{19}H_{34}O_3N_2$: N, 8.28. Found: N, 8.57, 8.54.

The mother liquors may be used for the preparation of crude VI.

1-Palmitoxy-3-hydroxyacetone (III).—For the decomposition, 4.0 g. of II is dissolved in 40 ml. of dioxane. To 3 g. of perchloric acid in 20 ml. water 40 ml. of dioxane is added; both solutions are heated and poured together at 80°. Upon further warming by immersion in a water-bath the nitrogen evolution is finished within one minute. The virtually colorless solution is cooled rapidly and poured into 350 ml. of ice-cold water. After separation by centrifuging or filtering the wet precipitate is taken up in ether, any mineral acid washed out and the dried ethereal solution (Na_2SO_4) concentrated to about 30 ml. The concentrate is warmed to dissolve all crystals and poured into 100 ml. of Skelly F. The crystallization (2.4 g.) is completed overnight in the refrigerator. Products obtained in this manner soften and melt between 58 and 93°. By extracting this crude material with hot Skelly B the component having m.p. 93° (IIIb) is left behind and the component having m.p. 63° (IIIa) crystallizes out of the solvent. IIIa may be recrystallized from Skelly B or ethanol; IIIb is purified by repeating the extraction and by final recrystallization from a large amount of hot Skelly B.

Recrystallization of IIIb from ethanol in several experiments yielded IIIa; whereas, after three weeks storing, analytically pure IIIa has a m.p. of 90–93° and is identical with IIIb isolated formerly. The mixture shows no depression of the melting point.

Anal. Calcd. for $C_{19}H_{36}O_4$: C, 69.44; H, 11.05; mol. wt., 328. Found for IIIa: C, 69.46; H, 10.93. Found for IIIb: C, 69.53; H, 10.84; mol. wt., 645, 656 (camphene). Periodate titrations[8]: $C_{19}H_{36}O_4$ = 1 mole ketol; IIIa, 0.99 mole ketol; IIIb, 0.96 mole ketol.

The chloroform is removed from the titrated solution and an excess of dimethyldihydroresorcinol is added. The yields of formaldehydebismethone (m.p. 190–191°) are 0.85 and 0.88 mole, respectively, calculated again on the basis of $C_{19}H_{36}O_4$.

Both IIIa and IIIb having the correct melting points yield identical products in the hydrogenation.

Potassium Salt of 1-Palmitoxyacetonyl-3-sulfate (V).—In utilizing sulfuric acid instead of perchloric acid in the above procedure the salt is isolated in the following manner: Upon decomposition the resulting solution is extracted with ether and then shaken with 1 *N* potassium carbonate in water. The potassium salt forms a layer of microscopic needles between the two phases. After recrystallization from ethanol the m.p. is 128–129°.

Anal. Calcd. for $C_{19}H_{35}O_7SK$: C, 51.34; H, 7.94; S, 7.21; ash ($^1/_2K_2SO_4$) 19.60. Found: C, 51.14, 51.39; H, 7.88, 7.88; S, 7.49, 7.32; ash (CH), 20.08, 19.99.

From the neutral ether solution IIIa and b can be isolated in the described manner.

(13) "Organic Syntheses," John Wiley and Sons, Inc., Coll. Vol. II, 1943, p. 165.

1-Palmitoxy-3-bromoacetone (VI).—A mixture of 12.5 ml. of 40% aqueous hydrobromic acid and 15 ml. of acetic acid is added slowly at room temperature to a solution of 5 g. of II in 53 ml. of acetic acid. The nitrogen evolution is completed by slightly warming the solution and VI crystallizes upon cooling to room temperature. The m.p. of the substance after recrystallization from 95% acetic acid is 63–64°. The yield is 4.4 g., 76%.

Anal. Calcd. for $C_{19}H_{35}O_3Br$: Br, 20.42. Found: Br, 20.74.

1,3-Dipalmitoxyacetone (VII).—A solution of 3.45 g. of VI in 14 ml. of ethanol is mixed with the equivalent amount of palmitic acid previously neutralized with alcoholic potassium hydroxide solution. After refluxing for one hour the hot solution is filtered from potassium bromide. At room temperature VIII crystallizes out; it is further purified by recrystallization from cyclohexane to the m.p. 77–78°. The yield is 2.4 g., 48%.

Anal. Calcd. for $C_{35}H_{66}O_5$: C, 74.15; H, 11.74. Found: C, 74.16; H, 11.73.

1-Palmitoxy-3-stearoxyacetone (VIIa) is prepared in the same way, m.p. 75–77°.

Anal. Calcd. for $C_{37}H_{70}O_5$: C, 74.69; H, 11.86. Found: C, 74.79; H, 11.85.

For the preparation of VII also the mother liquor of II can be used. After evaporating the solvent the resulting mixture of II and VI (containing chlorine instead of bromine) is treated as described for the preparation of VI and the haloketones are then converted to VII, m.p. 76–77°. 1,3-Dichloroacetone is less stable in alkaline medium, which explains the low yield of VII (15%) obtained from this starting material.

These substituted acetone compounds reduce Fehling solution. It was found, however, that a spot test using the dry substances and a few drops of concentrated KOH under heating requires smaller amounts and is more reliable. A yellow color is developed, indicating the presence of a substituted acetone. This test was used to prove completeness of the subsequent hydrogenation.

Reduction to Glycerides. α-Monopalmitin (IV).—Since IIIa and b yield identical products upon hydrogenation it is permissible to use their mixture as it is obtained from Skelly F. Raney nickel freshly prepared is very satisfactory for catalyzing the hydrogenation. Ten grams of the alloy (Central Scientific Co.) is treated in the hydrogenation flask (similar to the Parr apparatus) with NaOH and water[14] until the aqueous washings have approximately *p*H 9. The water is then replaced by tetrahydrofuran and 0.82 g. of III in 50 ml. of tetrahydrofuran is added. Under a pressure of 25 lb./inch^2 the hydrogenation is finished after two hours. The catalyst is centrifuged off and the solution is evaporated to dryness. The palmitin is recrystallized from cyclohexane and ether to the constant melting point of 72–74°.

Anal. Calcd. for $C_{19}H_{38}O_4$: C, 69.05; H, 11.59. Found: C, 69.15; H, 11.45.

1,3-Dipalmitin (VIII) and 1-palmito-3-stearin (VIIIa): The reductions are carried out as described, taking the lower solubility of VII and VIIa into account.

VIII: m.p. 69.5–70.5° (recryst. from cyclohexane, ether and ether + ethanol). *Anal.* Calcd. for $C_{35}H_{68}O_5$: C, 73.89; H, 12.03. Found: C, 74.18; H, 12.36.

VIIIa: M.p. 65.5–66.5° (recryst. from cyclohexane and ether). *Anal.* Calcd. for $C_{37}H_{72}O_5$: C, 74.44; H, 12.16. Found: C, 74.49; H, 12.05.

In some of the hydrogenations dioxane or cyclohexane and PtO_2 were used; the described conditions, however, proved to be preferable in numerous experiments.

COLLEGE STATION, TEXAS

(14) R. Schröter, in "Neuere Methoden, etc.," p. 80.