bright yellow needles after five recrystallizations from ethanol; m.p. 145–146°.

Anal. Calcd. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57. Found: C, 60.47; H, 5.38.

Ethyl N-nitroso-N-(4-methoxybenzyl)-carbamate (ID) yielded (a) 64% of **4-methoxybenzyl methyl ether (IIID)**, b.p. 57–58° (0.4 mm.), 125° (30 mm.), $n^{25}D$ 1.5107. *Anal.* Calcd. for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 70.88; H, 8.02.

(b) 7% of **2-(4'-methoxyphenyl)-cycloheptanone (IID)** obtained, after several recrystallizations from petroleum ether (b.p. 63–69°), as colorless needles, m.p. 57–58° (reported[21] 60°). **The 2,4-dinitrophenylhydrazone of IID** was obtained, after several recrystallizations from ethanol–ethyl acetate, as bright yellow plates, m.p. 174–175°. *Anal.* Calcd. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57. Found: C, 60.60; H, 5.64.

Ethyl N-nitroso-N-(2-methylbenzyl)-carbamate (IF), yielded (a) 37% of **2-methylbenzyl methyl ether (IIIF)**, b.p. 93° (30 mm.), $n^{25}D$ 1.5047. *Anal.* Calcd. for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.10; H, 9.04.

(b) 29% of **2-(2'-methylphenyl)-cycloheptanone (IIF)**, b.p. 113° (0.25 mm.), $n^{25}D$ 1.5348. *Anal.* Calcd. for $C_{14}H_{18}O$: C, 83.12; H, 8.97. Found: C, 83.29; H, 8.95.

The 2,4-dinitrophenylhydrazone of IIF was obtained after several recrystallizations from ethanol as bright yellow blades, m.p. 188–189°. *Anal.* Calcd. for $C_{20}H_{22}N_4O_4$: C, 62.81; H, 5.80. Found: C, 62.85; H, 5.75.

(21) M. Tiffeneau, P. Weill, J. Gutmann and B. Tchoubar, *Compt. rend.*, **201**, 277 (1935).

Ethyl N-nitroso-N-(4-methylbenzyl)-carbamate (IG) yielded (a) 45% of **4-methylbenzyl methyl ether (IIIG)**, b.p. 84° (20 mm.), $n^{25}D$ 1.4990. *Anal.* Calcd. for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.47; H, 8.75.

(b) 26% of **2-(4'-methylphenyl)-cycloheptanone (IIG)** as feathery, white needles after recrystallization from petroleum ether (b.p. 63–69°), m.p. 57–58°. *Anal.* Calcd. for $C_{14}H_{18}O_2$: C, 83.12; H, 8.97. Found: C, 83.30; H, 9.03.

The 2,4-dinitrophenylhydrazone of IIG was obtained, after several recrystallizations from ethanol–ethyl acetate, as glistening, bright orange plates, m.p. 200–201.5°.

Anal. Calcd. for $C_{20}H_{22}N_4O_4$: C, 62.81; H, 5.80. Found: C, 63.14; H, 5.91.

Ethyl N-nitroso-N-(2,4,6-trimethylbenzyl)-carbamate (IIH) yielded (a) 62% of **2,4,6-trimethylbenzyl methyl ether (IIIH)**, b.p. 56° (0.50 mm.), $n^{25}D$ 1.5098. *Anal.* Calcd. for $C_{11}H_{16}O$: C, 80.44; H, 9.83. Found: C, 80.47; H, 9.72.

(b) 11% of ethyl N-(2,4,6-trimethylbenzyl)-carbamate, m.p. 109–111°, resulting either from de-nitrosation during the reaction[22] or to incomplete nitrosation in the preparation of IH.

(22) F. W. Bollinger, F. N. Hayes and S. Siegel, THIS JOURNAL, **72**, 5592 (1950), and **75**, 1729 (1953), obtained ethyl N-cyclohexylcarbamate in 15% yield and ethyl N-cyclopentylcarbamate in 7% yield from the corresponding N-nitroso compounds in methanol in the presence of potassium carbonate.

ST. LOUIS, MISSOURI

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DEPARTMENT OF THE PROCTER & GAMBLE COMPANY]

Mono-α-aminoacyl and Mono-α-dipeptide Triglycerides

BY W. FREDERICK HUBER

RECEIVED JUNE 28, 1954

2-α-Aminoacyl-1,3-dilaurins, -dipalmitins, and -distearins have been prepared representing a combination of the acidic constituents of protein and fat in one molecule. They were obtained as the acetate salts by hydrogenation of the corresponding 2-α-azidoacyl glycerides in glacial acetic acid. Hydrolysis of the acetate salts by water-washing yielded free bases with fat-like properties. Treatment of the free bases with an α-azidoacyl chloride, followed by hydrogenation, produced mono-α-dipeptide glycerides. Due to ease of hydrolysis of the aminoacyl ester linkage, pure 1-mono-α-aminoacyl glycerides and di- or tri-α-aminoacyl glycerides were not obtained.

The substitution of α-aminoacyl or polypeptide groups for one or two of the fatty acid groups in a triglyceride was of interest since the products would combine components of protein and fat in one molecule.

Several syntheses of glycerides containing amino acids or both amino acids and fatty acids have been reported.[1] Compounds such as 1-glycyldipalmitin and 1-alanyldistearin have been reported to melt at 215 and 223°, respectively. The compounds were claimed to be soluble in water and insoluble in ligroin or ethyl acetate. These properties seemed unlikely for molecules containing a high percentage of fatty acid groups and the present work confirms this. All the mono-α-aminoacyl glycerides described herein are soluble in petroleum ether, ether or chloroform and insoluble in water. They melt in the range of 30 to 70° and are fat-like in character.

Confirming former workers, efforts to prepare α-aminoacyl glycerides by conventional esterification techniques failed. Similar attempts employing amino acids or their acid chlorides in which the amino group was blocked by an acyl, phthalyl or carbobenzoxy group also failed. 2-Phthalylalanyl-1,3-distearin was prepared, but removal of the phthalyl group could not be accomplished by reaction with hydrazine or phenylhydrazine or by hydrogenation. No 2-carbobenzoxyglycyl-1,3-dipalmitin could be identified in the reaction product from carbobenzoxyglycyl chloride with 1,3-dipalmitin.

The only successful method found for synthesis of aminoacyl glycerides introduced the amino group as the last step by hydrogenation of an azido group. An α-halogeno ester was treated with sodium azide to form an α-azido ester which on saponification formed the α-azido acid. This was converted to the acid chloride with thionyl chloride and reacted with glycerol, a monoglyceride or a diglyceride in chloroform–pyridine solution to form a tri-, di- or mono-α-azidoacyl triglyceride. Hydrogenation of the latter in glacial acetic acid solution converted the azido group to an amino group, liberating nitrogen and producing the corresponding α-aminoacyl glyceride acetate. The acetates, as typical amine salts, are more soluble in polar than non-polar solvents. They are quite unstable to

(1) (a) A. Fodor and M. Weizmann, *Z. physiol. Chem.*, **154**, 290 (1926); (b) M. Weizmann and L. Haskelberg, *Compt. rend.*, **189**, 104 (1929); (c) M. Weizmann and L. Haskelberg, *Bull. soc. chim.*, **51**, 59 (1932); (d) L. Haskelberg, *Compt. rend.*, **190**, 270 (1930); *Bull. soc. chim.*, **51**, 212 (1932); (e) B. R. Harris, U. S. Patent 2,406,329 (1946).

TABLE I

Chloride	Yield, %	B.p. °C.	Mm.	$n^{25}D$	d^{25}_4	M.R. Found[a]	Calcd.
Azidoacetyl	37	55–60	18[b]	1.4634	1.303	25.3	23.9
α-Azidopropionyl	73	49–50	25[c]	1.4581	1.2439	29.3	28.5
α-Azidobutyryl	89	69–74	31	1.4544	1.1824	33.8	33.2[d]
α-Azidoisovaleryl	90	69–71	20	1.4564	1.1425	38.5	37.8[e]
α-Azidolauroyl	78	150–154	4	1.4590	0.9915	71.6	70.1[f]

[a] Atomic refract. of N in N_3 taken as 2.90. [b] M. O. Forster and R. Müller, *J. Chem. Soc.*, **95**, 200 (1909), report b.p. 50° (20 mm.). [c] K. Freudenberg, W. Kuhn and I. Bumann, *Ber.*, **63**, 2380 (1930), report b.p. 46–47° (13 mm.). [d] Calcd. for $C_4H_6ON_3Cl$: C, 32.6; H, 4.10; N, 28.5; Cl, 24.0. Found: C, 33.5, 33.2; H, 4.45, 4.25; N(Dumas), 25.5, 25.2; Cl, 22.6, 23.0. [e] Calcd. for $C_5H_8ON_3Cl$: C, 37.2; H, 5.0; N, 26.0; Cl, 21.9. Found: C, 37.5, 37.8; H, 5.2, 5.2; N(Dumas), 24.9, 25.3; Cl, 21.4, 21.1. [f] Calcd. for $C_{12}H_{22}ON_3Cl$: C, 55.5; H, 8.6; N, 16.2; Cl, 13.6. Found: C, 57.5, 57.6; H, 8.7, 8.3; N(Dumas) 14.8, 14.9; Cl, 13.4, 13.6.

moisture, water-washing quantitatively removing the acetic acid to yield the free base.

The free-base mono-α-aminoacyl triglycerides resemble typical fatty acid glycerides in their solubilities in neutral organic solvents. They also resemble aliphatic esters of amino acids in that they gradually hydrolyze in moist air to yield amino acid and partial glyceride.

The hydrochlorides were prepared by passing dry hydrogen chloride into an ether solution of the free base. They are stable when stored in a dry state while the acetates, under the same conditions, gradually lose acetic acid.

The general synthesis outlined worked well for the preparation of the 2-α-aminoacyl triglycerides but did not yield pure compounds when applied to the synthesis of di- or tri-α-aminoacyl glycerides. Even though all the azido nitrogen was reduced, the products melted over a wide range and gave amino nitrogen values varying between 80 and 100% of theory. Often high-melting material, analyzing as free amino acid, could be isolated, indicating these materials to be unstable and sensitive to hydrolysis. It may be that further attempts using low temperature hydrogenation and purification could lead to success.

The introduction of a polypeptide chain, instead of a single aminoacyl group, into a glyceride molecule should give a product approaching a true protein–fat chemical combination. Attempts to introduce a di- or tripeptide into a glyceride by direct reaction were unsuccessful. Methods tried were (a) direct esterification of partial glycerides employing the di- or tripeptide and (b) reaction of phthalyl- or carbobenzoxyamino-blocked polypeptides with partial glycerides. Success was attained by treating an α-azidoacyl chloride with an α-aminoacyl glyceride to yield an α-azidoacylaminoacyl glyceride which, on hydrogenation, yielded the "dipeptide–glyceride."

Experimental[2]

Ethyl α-Azidoalkanoates.—The following compounds, reported in the literature, were prepared by treating sodium azide with the required α-halogeno ester in alcohol according to described methods[3]: ethyl azidoacetate, 75% yield, b.p. 74–75° (23 mm.), $n^{25}D$ 1.4341, d^{25}_4 1.1181; ethyl α-azidopropionate, 90% yield, b.p. 60° (17 mm.), $n^{25}D$ 1.4259, d^{25}_4 1.0498; ethyl α-azidobutyrate, 61.8% yield, b.p. 76–77° (16 mm.), $n^{25}D$ 1.4290, d^{25}_4 1.0278; ethyl α-azidoisovalerate, 70.5% yield, b.p. 75–80° (15 mm.), $n^{25}D$ 1.4338, d^{25}_4 1.0181.

Unreported before is ethyl α-azidolaurate, 61.8% yield, b.p. 143–145° (1.5 mm.), $n^{25}D$ 1.4463, d^{25}_4 0.938.

Anal. Calcd. for $C_{14}H_{27}O_2N_3$: azido N, 10.4; sapon. equiv., 208. Found: azido N,[4] 10.5; sapon. equiv., 216.

α-Azidoalkanoic Acids.—The preparation of α-azidopropionic acid is typical.

A mixture of 80 g. (0.56 mole) of ethyl α-azidopropionate and 38 g. (0.68 mole) of potassium hydroxide in 130 ml. of 10% ethanol was carefully warmed until a vigorous reaction started. The insoluble ester layer saponified rapidly, evolving sufficient heat to maintain reflux. After 10 minutes, when saponification appeared complete, the mixture was refluxed an additional one-half hour, cooled, poured over ice and acidified with concd. sulfuric acid. The solution was extracted three times with ether. The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure at 50° to yield 58 g. (90%) of α-azidopropionic acid as a pale yellow liquid.

Yields on the other homologs were: azidoacetic acid, 62%; α-azidobutyric acid, 74%; and α-azidoisovaleric acid, 78%.

α-Azidolauric acid, m.p. 50–51°, was obtained in 66% yield after recrystallization from 4 volumes of petroleum ether at −10°.

Anal. Calcd. for $C_{12}H_{23}O_2N_3$: C, 59.7; H, 9.6; N, 17.4; azido N, 11.6. Found: C, 59.7, 59.6; H, 9.01, 9.07; N, 17.5, 17.2 (Dumas); azido N,[4] 11.8.

α-Azidoacyl Chlorides.—All were prepared by adding 1.5 equivalents of thionyl chloride to 1 equivalent of the α-azidoalkanoic acid over a 0.5 to 1 hour period followed by refluxing 1.5 to 2 hours, after which no more hydrogen chloride evolved. Distillation through a 4-inch Vigreux column under reduced pressure yielded the products tabulated in Table I.

α-Azidoacyl Glycerides.—A 20% excess of the α-azidoacyl chloride was added over a 15- to 30-minute period at 0 to 10° to 0.05–0.1 mole of glycerol, a monoglyceride or a diglyceride in 5 volumes of chloroform containing a 30% excess (in equivalents for expected HCl) of dry pyridine. The solution was allowed to stand at room temperature for 2 days and then poured onto ice. Sufficient diethyl ether was added to put the organic layer in the upper phase. The latter was separated and washed successively with water and 5% hydrochloric acid to congo red, and again with water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to yield the crude azidoacyl glyceride.

The purification procedure varied slightly according to the type of azidoacyl glyceride which was prepared.

The 2-α-azidoacyl 1,3-diglycerides and the tri-α-azidoacyl glycerides were purified by crystallization from 10 volumes of acetone or petroleum ether at 0 to 20°. The tri-α-azidoacyl glycerides were liquid at room temperature.

The 1,2-di-α-azidoacyl 3-monoglycerides were first submitted to a solvent partititon to remove unreacted monoglyceride. The sample, dissolved in 3 to 5 volumes of *n*-heptane (Skellysolve C) was extracted with equal volumes of

(2) All boiling points and melting points are uncorrected. Carbon, hydrogen, chlorine and Dumas nitrogen analyses were made by the Oakwold Laboratories, Alexandria, Virginia.

(3) M. O. Forster and H. E. Fierz, *J. Chem. Soc.*, **93**, 669 (1908); M. O. Forster and R. Müller, *ibid.*, **95**, 191 (1909).

(4) Azido nitrogen content was measured by heating a 50-mg. sample of the compound in 5 ml. of concd. sulfuric acid to 50° for one-half hour. The nitrogen evolved was measured manometrically, each azido group evolving one mole.

TABLE II

α-Azidoacyl glyceride	Yield, %	M.p., °C.	$n^{25}D$	d^{25}_4	Sapon. equiv. Found (Calcd.)	N (Kjeldahl) Found (Calcd.)	N (Dumas) Found (Calcd.)	Azido N as N_2 Found (Calcd.)[a]	Carbon Found (Calcd.)	Hydrogen Found (Calcd.)
2-Azidoacetyl-1,3-distearin	47.0	57–59			240 (238)	1.92 (1.98)	6.00 (5.93)	3.53 (3.96)	69.8 (69.5)	11.0 (11.0)
2-α-Azidopropionyl-1,3-dilaurin	83.1	26.5–27			303 (304)	2.53 (2.53)	7.76 (7.59)	5.20 (5.06)	65.7 (65.1)	10.0 (10.0)
1-α-Azidopropionyl-2,3-dipalmitin	56.9	39.5–41			252 (253)	1.91 (2.10)	6.33 (6.31)	3.87 (4.21)	68.8 (68.5)	10.5 (10.7)
2-α-Azidopropionyl-1,3-dipalmitin	74.0	41–43			254 (253)	2.10 (2.10)	6.59 (6.31)	4.25 (4.21)	68.8 (68.5)	10.7 (10.8)
2-α-Azidopropionyl-1,3-distearin	85.0	49–51			234 (233)	1.93 (1.94)	5.56 (5.82)	3.88 (3.88)	70.1 (69.9)	10.5 (11.0)
2-α-Azidobutyryl-1,3-distearin	81.6	36–37			228 (229)	1.84 (1.90)	5.48 (5.71)	3.50 (3.80)	70.6 (70.2)	10.9 (11.1)
2-α-Azidoisovaleryl-1,3-distearin	53	37–39			223 (224)	1.84 (1.87)	5.96 (5.60)	3.33 (3.73)	70.7 (70.5)	11.3 (11.2)
2-α-Azidolauroyl-1,3-distearin	82	43–45			199 (198)	1.56 (1.65)	5.05 (4.95)	3.26 (3.30)	72.3 (72.2)	11.9 (11.5)
1,2-Di-α-azidopropionyl-3-monolaurin	94		1.4646	1.0749	353 (359)	6.12 (5.98)	18.3 (17.9)	12.0 (12.0)	54.6 (53.8)	7.7 (7.8)
1,2-Di-α-azidopropionyl-3-monostearin	72.5		1.4645	1.0314	304 (305)	5.00 (5.07)	14.7 (15.2)	10.0 (10.1)	58.7 (58.7)	8.5 (8.8)
1,3-Di-α-azidopropionyl-2-monopalmitin	71.8		1.4645	1.0451	314 (321)	5.29 (5.34)	15.7 (16.0)	10.7 (10.7)	57.5 (57.2)	8.3 (8.5)
Tri-α-azidopropionin[a]	..		1.4803	1.2603	417 (439)	10.6 (11.0)	32.1 (32.9)	20.9 (21.9)	38.2 (37.6)	4.7 (4.5)
Tri-α-azidobutyrin[a]	68		1.4790	1.2062	394 (396)	9.14 (9.88)	28.3 (29.6)	19.1 (19.8)	43.0 (42.4)	5.8 (5.5)
1-α-Azidopropionyl-2,3-isopropylidene glycerol[a]	98		1.4510	1.140	249 (245)	5.94 (6.11)	18.0 (18.3)	10.5 (12.2)	47.9 (47.2)	6.7 (6.6)

[a] Reported as the crude product. Attempts to crystallize from acetone or petroleum ether at low temperatures were unsuccessful.

80% ethanol in a 4-stage technique[5] using separatory funnels. The monoglyceride-free sample, obtained by evaporation of the heptane layers, was recrystallized from 5 to 10 volumes of acetone or petroleum ether at 0 to −20°. The crude 1,3-di-α-azidopropionyl 2-monopalmitin, without a solvent partition, was crystallized from 10 volumes of acetone at −60°. The products were liquid at room temperature.

The compounds were characterized by m.p., saponification equivalent, C, H and total N by Dumas. In addition, Kjeldahl and azido nitrogen analyses were made which determine 1/3 and 2/3 of the total nitrogen content, respectively.

All data on the α-azidoacyl glycerides are given in Table II.

D,L-α-Aminoacyl Glyceride Acetates.—A solution of 10 to 20 g. of the α-azidoacyl glyceride in 10 volumes of glacial acetic acid with 2.0% of platinum oxide catalyst[6] was hydrogenated in a Parr apparatus under 50 lb. of hydrogen for 2 to 3 hours at room temperature. Following hydrogenation, the mixture was warmed to 60° to dissolve any separated α-aminoacyl glyceride acetate and suction-filtered to remove the catalyst. The solvent was removed by distillation under reduced pressure. The solid residue was recrystallized by dissolving in 25 to 50 ml. of chloroform containing a few drops of glacial acetic acid and pouring into 250 to 500 ml. of petroleum ether. The resultant α-aminoacyl glyceride acetates were white crystalline solids having a bitter, sour flavor. The compounds were characterized by melting point, perchloric acid titration for amino group content,[7] and elemental analyses for C, H and N.

All data on the D,L-α-aminoacyl glyceride acetates are presented in Table III.

D,L-α-Aminoacyl Glycerides. (a).—The α-aminoacyl glyceride acetate dissolved in 10 volumes of chloroform was washed with a slight excess of ice-cold 2% potassium hydroxide solution, followed by water until neutral. The chloroform was removed at 25 to 35° under diminished pressure and the residue recrystallized from 10 to 20 volumes of petroleum ether or ether at 0 to −30°. The free bases were obtained in 38 to 92% yields and of 97 to 100% purity as evidenced by perchloric acid titration of the amino group.

(b).—A single pass of a solution of 0.5 g. of 2-alanyl-1,3-dipalmitin acetate in 10 ml. of chloroform through a column of 17 g. of Amberlite IRA 400 in the hydroxyl form followed by elution with 100 ml. of chloroform yielded 0.42 g. (90%) of pure 2-alanyl-1,3-dipalmitin, m.p. 49.5–50.5°.

(c).—Later in the work it was found that simple ice-water washing of a chloroform solution of an α-aminoacyl glyceride acetate quantitatively removed the acetic acid and yielded the free base.

Table IV gives the yield, m.p. and pertinent analytical data of the α-aminoacyl glycerides.

D,L-α-Aminoacyl Glyceride Hydrochlorides.—Anhydrous hydrogen chloride was passed into a solution of 0.5 to 1.0 g. of the α-aminoacyl glyceride in 10 ml. of anhydrous ether at 0° to precipitate the hydrochloride. The products were recrystallized from chloroform–petroleum ether and analyzed by perchloric acid titration of the amino group, and nitrogen and chlorine determinations. The data are given in Table V.

(5) L. C. Craig and D. Craig, "Technique of Organic Chemistry," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1950, p. 259.

(6) The catalyst used was prepared by fusion of chloroplatinic acid with potassium nitrate according to R. Adams, V. Voorhees and R. L. Shriner, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 463. It was superior to commercial platinum oxide, presumably made by sodium nitrate fusion.

(7) Titrations were conducted in glacial acetic acid solution to a methyl violet end-point or potentiometrically by the method of J. S. Fritz, "Acid-Base Titrations in Non-aqueous Solvents," The G. Frederick Smith Chemical Co., Columbus, Ohio, 1952, p. 13.

TABLE III

D,L-α-Aminoacyl glyceride acetate	Yield, %	M.p., °C.[a]	$HClO_4$ equiv.[b]	N(Kjeldahl) Found (Calcd.)	N(Dumas) Found (Calcd.)	Carbon Found (Calcd.)	Hydrogen Found (Calcd.)
2-Glycyl-1,3-distearin	55.6	89–90	0.976		1.76 (1.89)	67.1 (69.6)	11.1 (11.3)
2-Alanyl-1,3-dilaurin	92.2	77–78	.970	2.18 (2.38)	2.23 (2.38)	63.7 (65.4)	10.3 (10.5)
1-Alanyl-2,3-dipalmitin	74.8	66–67[c]	.973				
2-Alanyl-1,3-dipalmitin	87.1	86–87	.973	1.88 (2.00)	1.89 (2.00)	67.5 (68.6)	10.8 (11.1)
2-Alanyl-1,3-distearin	84.5	88.5–89.5	.971	1.84 (1.85)	1.85 (1.85)	68.1 (69.9)	11.0 (11.3)
2-α-Aminobutyryl-1,3-distearin	53.5	81–82	.991	1.80 (1.82)	1.75 (1.82)	70.3 (70.2)	11.4 (11.4)
2-Valyl-1,3-distearin	83	79–80.5	.994	1.80 (1.79)	1.95 (1.79)	70.6 (70.5)	11.4 (11.4)
2-α-Aminolauroyl-1,3-distearin	87.4	76–77	.992	1.61 (1.59)	1.62 (1.59)	73.0 (72.1)	12.3 (11.8)

[a] The m.p.'s were obtained in the usual manner on solvent crystallized samples. The compounds, like *n*-acyl glycerides, can exist in various polymorphic forms having different m.p.'s. These data will be reported in a forthcoming publication. [b] Theoretical $HClO_4$ equivalent for all compounds is 1.00. [c] This material melted cloudy. Recrystallization caused considerable decomposition; free alanine separated, the material developing a wider m.p. and lower $HClO_4$ equivalent.

TABLE IV

D,L-α-Aminoacyl glyceride	Yield, %	M.p.,[a] °C.	$HClO_4$ Equiv.[b]	Sapon. equiv.[c] Found (Calcd.)	N(Dumas) Found (Calcd.)	Carbon Found (Calcd.)	Hydrogen Found (Calcd.)
2-Glycyl-1,3-distearin	85.6	71–72	0.990	244 (247)	2.09 (2.05)	71.6 (72.2)	11.4 (11.7)
2-Alanyl-1,3-dilaurin	88.2	28–29	.995	321 (319)	2.88 (2.65)	68.7 (68.3)	11.5 (10.9)
2-Alanyl-1,3-dipalmitin	82.0	49–50	.975	251 (263)	2.10 (2.19)	71.4 (71.3)	11.4 (11.5)
2-Alanyl-1,3-distearin	92.1	56–57	.982	233 (242)	1.99 (2.01)	73.2 (72.5)	11.6 (11.7)
2-α-Aminobutyryl-1,3-distearin	38.0	43–45	.982	225 (237)	1.80 (1.97)	72.3 (72.7)	12.0 (11.8)
2-Valyl-1,3-distearin	64.8	40–41	.990	231 (232)	1.95 (1.93)	73.4 (73.0)	11.9 (11.8)
2-α-Aminolauroyl-1,3-distearin	39.3	39–40	.989	196 (205)	1.72 (1.70)	74.5 (74.5)	12.0 (12.1)

[a] Table III, ref. a. [b] Theoretical $HClO_4$ equivalent for all compounds is 1.00. [c] A sample of 50 to 100 mg. dissolved in a mixture of 10 ml. of 2% alcoholic potassium hydroxide and 40 ml. of neutral ethanol was refluxed 45 minutes. Phenolphthalein was added to the cooled solution which was titrated with 0.1 *N* hydrochloric acid until colorless. A 5 ml. excess of 0.1 *N* HCl and 3 ml. of 40% formaldehyde was added and the mixture was boiled 2 minutes. It was finally cooled and back-titrated with 0.1 *N* sodium hydroxide; sapon. equiv. = 56.1 [blank − (ml. HCl × *N* − ml. NaOH × *N*)]/sample wt.

TABLE V

D,L-α-Aminoacyl glyceride hydrochloride	Yield, %	M.p., °C.	$HClO_4$ equiv.[a,b]	N(Dumas) Calcd.	N(Dumas) Found	Cl Calcd.	Cl Found
2-Glycyl-1,3-distearin	75.9	148–149	1.00	1.95	1.89	4.93	4.56
2-Alanyl-1,3-dilaurin	72.0	77–78.5	1.00	2.48	2.47	6.28	6.17
2-Alanyl-1,3-dipalmitin	51.2	88–89	0.995	2.07	2.09	5.24	5.43
2-Alanyl-1,3-distearin	66.8	91–92	0.997	1.91	2.03	4.84	4.95
2-α-Aminobutyl-1,3-distearin	88.4	91–92	1.03	1.88	1.91	4.75	4.85
2-Valyl-1,3-distearin	83.4	93–94.5	1.00	1.84	1.98	4.66	4.70
2-α-Aminolauroyl-1,3-distearin	53.8	77–78	1.05	1.63	1.72	4.13	4.29

[a] Theoretical $HClO_4$ equivalent for all compounds is 1.00. [b] Titrations conducted by modification of the method of T. Higuchi and J. Concha, *Science*, **113**, 210 (1951). Excess 0.01 *N* perchloric acid was added to the sample in glacial acetic acid. After boiling 3 minutes to remove all hydrogen chloride, back-titration with 0.01 *N* sodium acetate to methyl violet gave the equivalence point.

2-α-Acetylaminopropionyl-1,3-dipalmitin.—Three grams (4.1 ml., 0.03 mole) of triethylamine was added gradually to a solution of 7 g. (0.01 mole) of 2-alanyl-1,3-dipalmitin acetate in 70 ml. of dry chloroform at 0 to 10°, followed by 1.18 g. (1.1 ml., 0.015 mole) of acetyl chloride. The mixture was permitted to warm to room temperature and stand overnight. Addition of petroleum ether precipitated triethylamine hydrochloride which was removed by filtration. The filtrate was water-washed and cooled to −20 to −30° to precipitate 5.1 g. (75%) of crude 2-α-acetylaminopropionyl-

1,3-dipalmitin. Two recrystallizations from petroleum ether at 0 to −10° yielded 4.0 g. (59%) of pure product, m.p. 67–69°.

Anal. Calcd. for $C_{40}H_{75}O_7N$: N, 2.05; NH_2, 0 mole %. Found: N, 1.95; NH_2,[8] 2.9 mole %.

2-α-Palmitoylaminopropionyl-1,3-dipalmitin.—This compound was prepared in exactly the same manner as 2-α-acetylaminopropionyl-1,3-dipalmitin, using 4.12 g. (0.015 mole) of palmitoyl chloride. The yield of crude product was 7.5 g. (85%) and of pure product 5.8 g. (66%), m.p. 74–75°.

Anal. Calcd. for $C_{54}H_{105}O_7N$: N, 1.59; NH_2, 0 mole %. Found: N, 1.54; NH_2,[8] 2.8 mole %.

The above acylated alanyldipalmitins were also prepared by adding 0.015 mole of the acid chloride at 0° to 0.01 mole of alanyldipalmitin dissolved in 125 ml. of petroleum ether containing 3 ml. of pyridine. After standing overnight, the reaction mixture was poured over ice. The petroleum ether layer was separated and washed with water, 5% hydrochloric acid and again with water. Two recrystallizations of the products from petroleum ether gave 87% and 73% yields of 2-α-acetylaminopropionyl-1,3-dipalmitin and 2-α-palmitoylaminopropionyl-1,3-dipalmitin, respectively.

2-(α-Azidopropionylalanyl)-1,3-distearin.—To 2 g. (0.0026 mole) of 2-alanyl-1,3-distearin acetate dissolved in 20 ml. of dry chloroform and cooled to 0° was added 0.5 g. of dry triethylamine followed by 0.35 g. (0.0026 mole) of α-azidopropionyl chloride. The mixture was allowed to stand at room temperature for one day after which it was poured onto ice. The chloroform layer was separated and washed successively with water, 5% hydrochloric acid and water. Evaporation of the sodium sulfate-dried chloroform layer yielded 1.5 g. (70%) of crude 2-(α-azidopropionylalanyl)-1,3-distearin as a white solid, m.p. 43–45°. After two successive recrystallizations from 20 ml. of acetone at −35° and 15 ml. of petroleum ether at −10°, the yield of product was 1 g. (47%), m.p. 54–57°.

Anal. Calcd. for $C_{45}H_{84}O_7N_4$: C, 68.1; H, 10.7; N, 7.06 (3.53); NH_2, 0 mole %; sapon. equiv., 212. Found: C, 69.6; H, 10.9; N,[9] 3.33; NH_2,[8] 2.5 mole %; sapon. equiv., 207.

2-(α-Azidoisovalerylalanyl)-1,3-distearin.—By a procedure analogous to the foregoing, 7.6 g. (0.01 mole) of 2-alanyl-1,3-distearin acetate was acylated with 2.4 g. (2.1 ml., 0.15 mole) of α-azidoisovaleryl chloride to yield 6.9 g. (84%) of crude 2-(α-azidoisovalerylalanyl)-1,3-distearin, m.p. 38–40°. Two recrystallizations from 10 volumes of acetone at 0 to −10° yielded 5.0 g. (61%) of product, m.p. 49–51°.

Anal. Calcd. for $C_{47}H_{88}O_7N_4$: C, 68.7; H, 10.8; N, 6.84 (3.42); NH_2, 0 mole %; sapon. equiv., 205. Found: C, 68.3; H, 10.2; N,[9] 3.12; NH_2,[8] 5.4 mole %; sapon. equiv., 212.

2-(α-Azidopropionylalanyl)-1,3-dipalmitin.—In this procedure 8.3 g. (0.013 mole) of 2-alanyl-1,3-dipalmitin, dissolved in 50 ml. of chloroform containing 2 ml. of pyridine, was acylated with 2.2 ml. (0.02 mole) of α-azidopropionyl chloride under the reaction conditions used above. The yield of crude 2-(α-azidopropionylalanyl)-1,3-dipalmitin was 7.1 g. (74%), m.p. 54–62°. One recrystallization from 10 volumes of petroleum ether and two from acetone at −30° yielded 5.0 g. (52%) of product, m.p. 61–62.5°.

Anal. Calcd. for $C_{41}H_{76}O_7N_4$: C, 66.8; H, 10.4; N, 7.61 (3.80); sapon. equiv., 228. Found: C, 67.1; H, 10.0; N, 7.67 (Dumas); N,[9] 3.64; sapon. equiv., 238.

2-Alanylalanyl-1,3-distearin Acetate.—A solution of 0.8 g. of 2-(α-azidopropionylalanyl)-1,3-distearin dissolved in 10 ml. of glacial acetic acid was hydrogenated 2 hours under 50 lb. pressure using 40 mg. of platinum oxide[6] catalyst. The crude product obtained by filtration and evaporation of the acetic acid solution was recrystallized from 20 ml. of petroleum ether at −10°. The yield of 2-alanylalanyl-1,3-distearin acetate analyzing 83% pure, was 0.4 g., m.p. 63–64°. Attempts to further purify the compound resulted in partial decomposition and loss of part of the titratable amino group.

Anal. Calcd. for $C_{47}H_{90}O_9N_2$: N, 3.39; $HClO_4$ equiv., 1.00. Found: N, 2.94; $HClO_4$ equiv., 0.83.

2-Alanylalanyl-1,3-dipalmitin acetate was similarly prepared, but only in 80% purity, m.p. 52–54°. 2-Valylalanyl-1,3-distearin acetate likewise could not be obtained pure. Hydrogenation of the azido compound was incomplete and the product decomposed during recrystallization.

Proof of Structure by Degradation.—2-Alanyl-1,3-distearin acetate (2.00 g.) was refluxed with 40 ml. of water for 2 hours. The cooled mixture was extracted three times with ether and the ether extracts were washed and evaporated to yield 1.20 g. (72.6%) of 1,3-distearin, m.p. 71–73°. After recrystallization from petroleum ether it melted at 74–76°. The mixed m.p. with an authentic sample was unchanged.

Anal. Calcd. for $C_{39}H_{76}O_5$: hydroxyl value, 90.0. Found: hydroxyl value, 86.1.

The aqueous solution was evaporated to yield 0.22 g. of a yellow oil which was stirred 3 hours with 5 ml. of *N* sodium hydroxide and 0.5 g. of *p*-toluenesulfonyl chloride in 8 ml. of ether. Acidification of the aqueous layer yielded 0.15 g. (23%) of N-*p*-toluenesulfonyl-D,L-alanine, m.p. 139–140°. The mixed m.p. with an authentic sample was 139–140°.

Anal. Calcd. for $C_{10}H_{13}O_4SN$: N, 5.76. Found: N, 5.76.

In another experiment, 1.5 g. of 2-alanyl-1,3-distearin acetate was saponified with a solution of 1.5 g. of potassium hydroxide in 40% ethanol to yield, after acidification, 1.09 g. (96.5%) of stearic acid, m.p. 68–69°. Mixed m.p. with an authentic sample was 65–68°.

Anal. Calcd. for $C_{18}H_{36}O_2$: neut. equiv., 197. Found: neut. equiv., 194.

The aqueous extract of alanine was converted to the *p*-toluenesulfonyl derivative as described above. The yield was 0.137 g. (28%), m.p. 136–138°. Mixed m.p. with an authentic sample was 138–141°.

Acknowledgment.—The author wishes to express his appreciation to Mrs. Ruth Davis for assistance in the experimental work.

CINCINNATI 31, OHIO

(8) Perchloric acid titration was used.[6] It indicates the maximum amount of unreacted α-aminoacyl glyceride which can be present as an impurity.

(9) Analysis by the Kjeldahl method which determines one-half of the total nitrogen in this compound.