STUDIES ON THE LIPOLYTIC ENZYME ACTION

III. HYDROLYSIS OF TRIPROPIONYL GLYCEROL

by

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In previous publications[1,2] the authors have studied the kinetics of the hydrolysis of *rac.* 1-caprylyl glycerol by pancreatic lipase and liver esterase. The present report deals with the enzymatic hydrolysis of a water-soluble triglyceride, *i.e.*, tripropionyl glycerol by liver esterase and pancreatic lipase.

Our results seem to indicate that by the action of *liver esterase* one acid group corresponding to a primary alcoholic group is split off very quickly involving the formation of 1,2-dipropionyl glycerol. Subsequently this compound splits off preferentially the propionyl group in position 1 at a relatively slower rate, forming 2-monopropionyl glycerol which again is decomposed very slowly compared with tri- and 1,2-dipropionyl glycerol. In the present case the kinetics of liver esterase are complicated by the fact that the hydrolysis proceeds stepwise and the principles of stationary states cannot be applied directly. It will be treated in detail in a following paper.

The kinetics of *pancreatic lipase* action for tripropionyl glycerol offer a different picture and are relatively simple. The reaction course can be ascribed to transformation of the triglyceride into 1,2-dipropionyl glycerol which is very slightly decomposed by the amounts of pancreatic lipase used in the experiments.

PROCEDURES AND SYNTHESES

Liver esterase was obtained by extracting chopped, acetone and ether dried liver tissue (rabbit) with 1% NaCl solution.

Pancreatic lipase extracts were prepared in a similar manner from pig pancreas tissue. The extracts were filtered repeatedly through cotton wool. pH of the extracts was brought to about 7.15 before they were added to the substrate mixture.

The enzymatic hydrolysis was followed at pH about 7.1 (temp. 22°) by means of the *continuous titration technique*[1]. The amounts of substrate used and ml base added are converted to mmol per litre enzyme-substrate mixture.

In order to elucidate the path of the hydrolysis of tripropionyl glycerol it was necessary to prepare the five different propionyl glycerols some of which have not been synthesized previously. In this connection other propionyl esters were also prepared.

The molecular weight of the esters was determined by saponification. An amount of ester corresponding to 1.5–2 m.equivs. of acid was refluxed for 2–3 hrs with 5 m.equivs. of KOH in alcoholic medium (about 60% alcohol). Excess of base was titrated with 0.1 *N* aqueous HCl. Duplicate determinations were performed together with two blanks.

Rac. 1-monopropionyl glycerol. 1-monopropionyl glycerol was prepared according to the method given for the analogous optically active compounds by BAER AND FISCHER[3], 1-monopropionyl acetone glycerol being prepared as an intermediate. Acetone glycerol was prepared by the method of VERKADE AND VAN DER LEE[4].

Propionyl chloride was obtained from column distilled, pure propionic acid, refluxed for two hours with half its volume of phosphorus trichloride. After cooling in the ice-box the supernatant was poured from the sirupy H_3PO_3 and fractionated, b.p. (atm. pressure) 78–84°. The main fraction

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was refluxed with sodium propionate in order to remove traces of phosphorus compounds, filtered and redistilled.

Acetone glycerol (117.2 g) was dissolved in pyridine (77.3 g) and the solution cooled in the ice-bath. To this solution propionyl chloride (82.2 g) was added slowly. The mixture was kept at room temperature for two days and then dissolved in chloroform (900 ml). The solution was washed thoroughly twice with water (50 ml) and dried with anhydrous sodium sulphate. The chloroform was distilled off and *rac.* 1-*propionyl acetone glycerol* obtained by fractional distillation, b.p. (12–13 mm) 94–96°; mol.wt., 192.9 ± 1 (calculated 188.1); yield 88%.

1-monopropionyl glycerol was obtained from the acetone compound (146 g) by hydrolysis for two hours with 10% acetic acid (622 ml) at 60°. At the end of the hydrolysis the clear solution was cooled to room temperature and, in order to remove traces of propionyl acetone glycerol, extracted with low boiling petroleum ether. The aqueous solution of 1-monopropionyl glycerol was concentrated *in vacuo* and the residue dissolved in ether (300 ml). The ethereal solution was then dried with sodium sulphate, filtered and concentrated to about 200 ml. Then it was treated in a separating funnel with petroleum ether (950 ml) and allowed to stand for one hour. The bottom layer was treated twice with ether (190 ml) and petroleum ether (950 ml), concentrated *in vacuo* and purified by fractional distillation, b.p. (4 mm) 129–130° and later (0.10 mm) 98–101°; mol.wt., 150.0 ± 1 (calculated 148.1); yield 72.4%.

As compounds used to identify 1-monopropionyl and 2-monopropionyl glycerols the dibenzoates were found suitable as they could be obtained in pure crystalline state with a difference in melting points of about 10°. FISCHER[5] found essentially higher melting point for 2-acetyl-1,3-di(*p*-nitrobenzoyl) glycerol than for 1-acetyl-2,3-di (*p*-nitro-benzoyl) glycerol.

1-*propionyl-2,3-dibenzoyl glycerol.* 1-monopropionyl glycerol (4.0 g) was dissolved in dry pyridine (5.1 g) and the solution cooled in ice-bath. To this solution there was added slowly benzoyl chloride (8.74 g) dissolved in dry chloroform (25 ml). After standing two days at room temperature ether (300 ml) was added. The ethereal solution was filtered from pyridine hydrochloride and washed several times with 0.25 *N* sulphuric acid, 5% sodium bicarbonate and water. The ether solution was dried with anhydrous sodium sulphate, filtered and concentrated under reduced pressure. A sirupy residue was dried *in vacuo* with P_2O_5, and the glyceride purified by recrystallization several times from abs. alcohol. The white crystals melted at 45.0–46.0°; mol.wt., 357.2 ± 0.4 (calculated 356.36); yield 92%.

Analysis calculated for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.44; H, 5.85.

1,3-*dipropionyl glycerol.* Propionyl chloride (10.1 g) was added dropwise, with shaking, to 1-propionyl glycerol (15.7 g), dissolved in pyridine (9.3 g) at −3°. The mixture was allowed to stand at room temperature for two days and then dissolved in ether and filtered from pyridine hydrochloride. The ethereal solution was washed successively with 1 *N* sulphuric acid, saturated sodium bicarbonate solution and water, and finally dried with anhydrous sodium sulphate. The ether was evaporated and the residue purified by fractional distillation, b.p. (0.10 mm) 99–101°; mol. wt., 200.8 ± 1 (calculated 204.3); yield 56%. The supposed 1,3-dipropionyl glycerol distilled at 99–101° at 0.10 mm, whereas the 1,2-dipropionyl glycerol (see later) distilled at 95–96° (0.10 mm) under exactly identical conditions (same day, same micro-distillation apparatus).

The authors find it justified to assume that the substance obtained is chiefly the symmetrical dipropionyl glycerol. In the preparation 0.1 mol of propionyl chloride was employed to 0.1 mol of 1-propionyl glycerol, and it is known that it is easier to esterify the 1-hydroxyls in glycerol than the 2-hydroxyl. The compound has been treated with 1 *N* H_2SO_4 during the preparation. If some 1,2-dipropionyl glycerol were formed this substance most likely would be converted into 1,3-dipropionyl glycerol owing to migration of the 2-acyl group in acid medium (DAUBERT AND KING[6]). It can further be demonstrated that under exactly identical experimental conditions there is an essential difference in the rate of the enzymatic hydrolysis of the substance prepared and 1,2-dipropionyl glycerol (Table I).

2-*monopropionyl glycerol* was prepared by the method given by BERGMANN AND CARTER[7] for analogous compounds, employing 2-propionyl-1,3-benzylidene glycerol as an intermediate. 1,3-benzylidene glycerol (20.5 g), prepared by the method of VERKADE AND VAN ROON[8], was dissolved in dry pyridine (28.9 g) and the solution cooled in an ice-bath. To this solution there was added slowly propionyl chloride (9.65 g). After the mixture was allowed to stand 12 hours at room temperature, ice water (200 ml) was added and the product separated as a solid mass which was minced. After washing several times with 4 *N* HCl and ice water to remove pyridine, the mass was dried *in vacuo* over concentrated sulphuric acid. The white crystals melted at 75–76°; yield 76%. The melting point found for the C_3-benzylidene compound lies almost on the low carbon branch of the melting point curve, reported by DAUBERT, FRICKE, AND LONGENECKER[9]. These authors apparently only cite data for acetyl and butyryl glycerols.

2-propionyl-1,3-benzylidene glycerol (14.4 g) was dissolved in absolute alcohol (70 ml) and palladium, prepared from $PdCl_2$ (2 g) and norite (8 g), was added. The mixture was transferred to the hydrogenation bottle and the air evacuated. The reduction was carried out at room temperature

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as described by DAUBERT, FRICKE, AND LONGENECKER[9] in their preparation of 2-monocaproin and 2-monocaprylin. The alcoholic solution containing 2-propionyl glycerol and toluene was concentrated *in vacuo* to a thick syrup at a low bath temperature. The 2-propionyl glycerol prepared was distilled at 0.10 mm, b.p. 96–97°; mol.wt., 147.5 ± 0.5 (calculated 148.1); yield 43%. When the activity of liver esterase was measured towards distilled and nondistilled 2-monopropionyl glycerol no difference was found, and the rate of hydrolysis (initial velocity) was lower than with the isomer 1-monopropionyl glycerol (Table I). It may thus be assumed that purification of 2-monopropionyl glycerol by distillation *in vacuo* (0.1 mm) has not caused migration of the acyl group.

For identification of 2-monopropionyl glycerol, *2-propionyl-1,3-dibenzoyl glycerol* was prepared. 2-monopropionyl glycerol (1 g) was dissolved in dry pyridine (1.5 g) and chloroform (1 ml), and the solution cooled in ice-bath. To this solution there was added slowly benzoyl chloride (2.2 g) dissolved in chloroform (6 ml). The mixture was allowed to stand two days at room temperature. The solution was taken up in ether and the isolation of 2-propionyl-1,3-dibenzoyl glycerol was carried out as described for the unsymmetrical isomer. The white crystals melted after several recrystallizations at 54.5–55.5°; mol.wt. 356.9 ± 0.5 (calculated 356.36); yield 90%.

Analysis calculated for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.59, H, 5.64.

1,2-dipropionyl glycerol was prepared with 1,2-dipropionyl-3-trityl glycerol as an intermediate. The intermediate was prepared in analogy with 1,2-distearyl-3-trityl glycerol, VERKADE, VAN DER LEE, AND MEERBURG[10]. Monotrityl glycerol (36.2 g) was dissolved in dry pyridine (56.2 g) and the solution cooled in an ice-bath. To this solution, propionyl chloride (24.1 g) dissolved in chloroform (33.6 ml) was added slowly. The mixture was allowed to stand for two days and then dissolved in ether (700 ml). The ether was washed successively with 2 *N* sulphuric acid, saturated sodium bicarbonate solution and water. The ether solution was dried with anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue, a viscous oil, was dried to constant weight in a vacuum dessicator over P_2O_5. It was not possible to purify the impure 1,2-dipropionyl-3-trityl glycerol by distillation or crystallization, mol.wt. 423.4 ± 1 (calculated 446.4); yield 81%.

The somewhat impure trityl compound was hydrogenated in alcoholic solution with palladium black as a catalyst in order to split off the trityl group. 3-trityl-1,2-propionyl glycerol (16.2 g) was suspended in abs. alcohol (100 ml). To this was added 0.6 g $CaCO_3$ + palladium black (from 16 g norite + 4 g $PdCl_2$). The reduction was carried out at 70° at which temperature the trityl compound dissolved completely. When no more hydrogen was taken up, the solution was cooled in the hydrogen atmosphere. The triphenylmethane was filtered off together with norite, Pd and $CaCO_3$. This precipitate was washed with hot abs. alcohol containing a little ether. The filtrate was concentrated under reduced pressure (bath 30°) until a precipitate of triphenylmethane occured. The solution was cooled and triphenylmethane filtered off. This procedure was repeated three times. At last the alcohol was distilled off almost completely. The remaining triphenylmethane was removed from the residue by addition of ice-cold water (60 ml). The triphenylmethane precipitated was filtered off, washed with a little water, and the combined aqueous solutions extracted with ether. The ethereal solution was dried with anhydrous sodium sulphate, filtered, and the ether evaporated under reduced pressure. The residue was dried to constant weight in a vacuum dessicator and purified by distillation, b.p. (0.10 mm) 95–96°; mol.wt., 205.7 ± 1 (calculated 204.3); yield 65%. It should be noted that no change in the activity of liver esterase (initial velocity) towards 1,2-dipropionyl glycerol before and after vacuum distillation (0.10 mm) could be stated. It is therefore assumed that the distillation did not involve an acyl migration.

Tripropionyl glycerol was prepared by slowly adding propionyl chloride (159.2 g) to a cooled solution of glycerol (52.8 g) in pyridine (148.7 g). The stiff solid mass was broken up with a stirring rod and pyridine (3 ml) was added after standing two days at room temperature. Water was then added (80 ml) and the tripropionyl glycerol taken up in ether. The ethereal solution was washed with 1 *N* sulphuric acid, 5% sodium bicarbonate and water, dried with anhydrous sodium sulphate and filtered. The residue obtained on evaporation was distilled *in vacuo*, b.p. (0.10 mm) 107–108.5°; mol.wt., 264.4 ± 2 (calculated 260.3); yield 80.6%.

1,2-dipropionyl ethylene glycol. Commercial ethylene glycol was purified by distillation at normal pressure. Propionyl chloride (58.1 g) was slowly added to an ice-cold solution of ethylene glycol (18.6 g) in pyridine (54.8 g). The mixture was kept at room temperature two days. Then it was extracted with ether (600 ml). The ether solution was filtered from pyridine hydrochloride, washed with 1 *N* sulphuric acid, saturated sodium bicarbonate and water, and finally dried over anhydrous sodium sulphate. The ether was evaporated and the residue purified by distillation, b.p. (2–3 mm) 77–79°; mol.wt. 177.3 ± 2.4 (calculated 174.2); yield 70.2%.

1-propionyl ethylene glycol was prepared by catalytic detritylation of the esterified 1-trityl ether of ethylene glycol. Monotrityl glycol (m.p. 103.5–104.5°) was prepared by the method of VERKADE, TOLLENAAR, AND POSTHUMUS[11]. The propionyl trityl glycol was synthesized in analogy with the method given by the same authors for the preparation of stearyl-, tridecanoyl- and lauryltrityl glycols. To an ice-cold solution of monotrityl glycol (35.5 g) in pyridine (92 g) there was added slowly a solution of propionyl chloride (11.7 g) in dry chloroform (92 ml). The mixture was kept

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at room temperature two days. The trityl compound was taken up in ether and the filtered ether solution washed with 2 *N* sulphuric acid, saturated sodium bicarbonate and ice water. Finally it was dried over anhydrous sodium sulphate. The ethereal solution was filtered and concentrated *in vacuo* leaving an oil which was dried to constant weight. On standing in the ice-box the material solidified. The substance (40 g) was dissolved in 350 ml alcohol (96%). The solution was cooled and water (50 ml) added slowly. By this procedure white particles and a yellow oil separated. The supernatant, containing the white particles was poured off. The oil was dissolved in warm alcohol and treated in the same way as described above. The alcohol-water mixtures were combined and water was further added. After cooling in the ice-box for one night, the white precipitate was filtered with suction and dried *in vacuo*; m.p. 65–66°; mol.wt. 369.7 ± 2 (calculated 360.4). The yield of the somewhat impure propionyl trityl glycol was 54%.

The hydrogenation of propionyl trityl glycol was carried out at 60° as described for 1-trityl-2,3-dipropionyl glycerol. The 1-propionyl ethylene glycol prepared boiled at 52° (3 mm); mol.wt. 120.5 ± 1.2 (calculated 118.1).

2-propionyl-1,3-dimethyl glycerol, b.p. (10–11 mm) 84°, mol.wt. 173.5 ± 0.7 (calculated 176.2) was prepared from dimethyl glycerol and propionyl chloride in the usual way. Professor HAKON LUND, Chemical Institute, Aarhus University, kindly prepared dimethyl glycerol, b.p. (9 mm) 66–66.5° and (750 mm) 168–169°, by the method of GILCHRIST AND PURVES[12]. According to FAIRBOURNE, GIBSON, AND STEPHENS[13] there is no doubt that the substance prepared by methylation of 1,2-dibromohydrin with sodium methoxide is identical with 1,3-dimethyl glycerol.

Propionyl-n-propyl was prepared from *n*-propyl alcohol (16.2 g) in pyridine (23.5 g) and propionyl chloride (25.2 g). The ester was isolated in the usual way; b.p. (760 mm) 121–123°; mol.wt. 116.4 (calculated 116.2).

Propionyl-iso-propyl was prepared in the same way from exactly the same amounts of *iso*propyl alcohol (b.p. 82.0–82.4°, atm. pressure), pyridine and propionyl chloride. The ester boiled at 108.5–110° (atm. pressure); mol.wt. 115.1 (calculated 116.2).

RESULTS

Experiments with liver esterase

Curve A in Fig. 1 illustrates the course of hydrolysis of tripropionyl glycerol. It is seen that an amount of acid corresponding with $^1/_3$ of the total amount is split off in about 16.5 min, whereas it takes about 255 min to liberate $^2/_3$ of the possible acid. Then the hydrolysis proceeds very slowly. Immediately after the experiment described equimolar amounts of 1,3- and 1,2-dipropionyl glycerols (Curves B and C) together with 1-monopropionyl glycerol (Curve D) were hydrolyzed under exactly the same experimental conditions. It appears that one of the two acid groups is split off from the two dipropionyl glycerols whereupon the rate of hydrolysis is very small. There is a considerable difference between the times taken by the two dipropionyl glycerols to attain the same degree of hydrolysis, and it should further be noted that by displacement Curve C can be made to cover approximately that part of Curve A which corresponds to liberation of the second acid equivalent. This does not hold for Curve B.

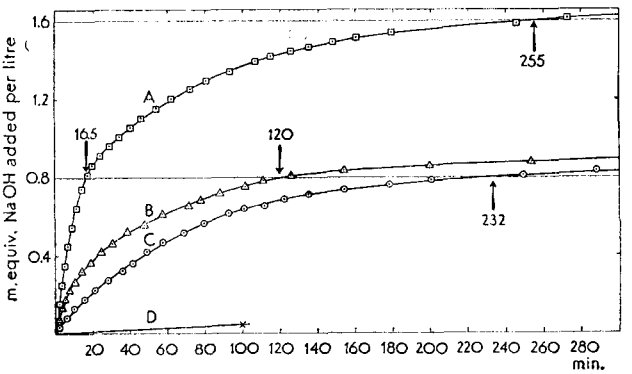

Fig. 1. Hydrolysis of different propionyl glycerols (0.804 mmol per litre) in the presence of the same amount of liver esterase
Curve A, tripropionyl glycerol; Curve B, 1,3-dipropionyl glycerol; Curve C, 1,2-dipropionyl glycerol; Curve D, 1-monopropionyl glycerol

If one assumes that the main part of the triglyceride is very quickly converted to

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1,2-dipropionyl glycerol and is further decomposed with this substance as an intermediate, then the time for splitting off $^2/_3$ of the total acid would be about 16.5 + 232 = 248.5 min. If tripropionyl glycerol were converted to 1,3-dipropionyl glycerol then it could be expected that $^2/_3$ of the acid were split off in about 136.5 min. The close agreement observed between the times 248.5 and 255 min. is best explained by assuming that tripropionyl glycerol is converted to 1,2-dipropionyl glycerol. Perfect agreement between found and computed times cannot be expected, as a complete conversion of tri- to dipropionyl glycerol has not occured when the degradation af the latter begins.

It is obvious that if the acid groups in tripropionyl glycerol are split off with the same degree of probability then there must be produced twice as much 1,2- as 1,3-dipropionyl glycerol. If on the other hand the acyl group in position 2 is hydrolyzed at a slower rate than the acyl groups in position 1 (or 3) the result will be, that more than $^2/_3$ of the produced diglyceride is 1,2-dipropionyl glycerol. In order to evaluate this possibility experiments were set up in which the relative rates of hydrolysis of equivalent amounts of different propionyl esters of primary and secondary alcohols were compared in the presence of the same amount of enzyme. As seen from Table I the rates of hydrolysis, determined graphically as the slope of the tangent to the first part of the reaction curve, are very different for the various substances. v_0 per equivalent of acid in the esters is referred to tripropionyl glycerol = 100.

TABLE I

RELATIVE INITIAL VELOCITY OF HYDROLYSIS OF DIFFERENT PROPIONYL ESTERS BY RABBIT LIVER ESTERASE AND PIG PANCREAS LIPASE

(Temp. 22°, pH 7.10. Same amount of enzyme in each series)

Ester 2.49 m.equivs. per l	Liver esterase. Initial velocity referred to tripropionyl glycerol = 100	Pancreatic lipase. Initial velocity referred to tripropionyl glycerol = 100
Tripropionyl glycerol	100	100
1,3-Dipropionyl glycerol	52.4	46
1,2-Dipropionyl glycerol	14.4	2.7
Dipropionyl ethylene glycol	99.4	8.9
1-Monopropionyl glycerol	2.7	<0.5
2-Monopropionyl glycerol	1.6	<0.5
1-Propionyl ethylene glycol	3.6	<0.5
Propionyl-*n*-propyl	48.8	2.2
Propionyl-*iso*-propyl	16.7	0.7
1-Propionyl acetone glycerol	67.5	8.9
2-Propionyl-1,3-dimethyl glycerol	30.4	5.0

It appears that v_0 is found larger when the propionyl radical is substituted in a primary than in a secondary alcoholic group, *cf.* 1-monopropionyl-/2-monopropionyl glycerol, propionyl-*n*-propyl/propionyl-*iso*-propyl and 1,3-dipropionyl-/1,2-dipropionyl glycerol. These results confirm the assumption that 1,2-dipropionyl glycerol is by far the most important primary intermediate in the hydrolysis of tripropionyl glycerol.

By considering the results given in Table I it is striking that the presence of free hydroxyls seems to involve a considerable inhibition of the liver enzyme activity, and conversely the blocking of free hydroxyls leads to a strongly increased rate of hydrolysis, *cf.* the glycerols and glycols. Apparently it makes little difference in which way the free

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hydroxyls are substituted (ester, ether, acetone). The experiments with the propyl esters show that when the hydroxyls in 1- and 2-monopropionyl glycerol are replaced by hydrogen this also causes a considerable increase in v_0.

Whether 1,2-dipropionyl glycerol is converted to 1- or to 2-monopropionyl glycerol, it can be expected that the hydrolysis comes almost to a standstill when $^2/_3$ of the acid in tripropionyl glycerol has been split off, *cf.* Curve D in Fig. 1 and Table I. In agreement with the above mentioned statements it is natural to assume that the propionyl group bound to the primary alcoholic group in 1,2-dipropionyl glycerol is split off preferentially. In order to verify this assumption the following experiment was carried out.

Hydrolysis of 1,2-dipropionyl glycerol by liver esterase

1.128 g 1,2-dipropionyl glycerol (5.52 mmol) were dissolved in 300 ml 1% NaCl and 2 ml veronal buffer added. The solution was adjusted to p_H 7.20 and 3 ml liver enzyme were added. The solution was kept at about p_H 7.10 by continuous addition of 0.203 *N* NaOH. After 390 min 27.3 ml base, corresponding to the liberation of one equivalent acid, had been added. Then the solution was saturated with NaCl and extracted 6 times with ether (2 litres in all). The ether extract was dried over anhydrous sodium sulphate, filtered and concentrated at reduced pressure. The faintly yellow, syrupy residue was dried *in vacuo* with P_2O_5 to constant weight, 0.45 g (corresponding to 55% of the possible amount of monopropionyl glycerol). For identification the residue was benzoylated as described above. The white crystals separated were recrystallized several times, m.p. 54.2–54.8; mol.wt. 358.5 (calculated for monopropionyl-dibenzoyl glycerol 356.36); yield 0.74 g (69%). The melting point of the product was not depressed by admixture with 2-propionyl-1,3-dibenzoyl glycerol. A melting point-composition diagram was constructed for mixtures of the two isomeric monopropionyl-dibenzoyl glycerols. From the diagram it seems justified to assume that at least 90% of the substance extracted from the reaction mixture is identical with 2-monopropionyl glycerol.

The results obtained are believed to support the view that by the action of liver esterase on tripropionyl glycerol, the degradation under the given experimental conditions proceeds via 1,2-dipropionyl and 2-monopropionyl glycerol. As the hydrolysis proceeds stepwise and the reaction products are not degraded at the same rate of hydrolysis, the kinetics of the hydrolysis of tripropionyl glycerol by liver esterase are not a simple matter. Investigations on the kinetics are now in progress.

Experiments with pancreatic lipase

The experiments in Fig. 2 offer an entirely different picture from those with liver esterase. It appears that when pancreatic lipase is employed only one acid equivalent is liberated from tripropionyl glycerol (Curve A_1); then the process comes almost to a standstill. It is further seen from Curves A_1 and B that the ratio of the initial velocities for tripropionyl glycerol and 1,3-dipropionyl glycerol is about 2:1, whereas 1,2-dipropionyl glycerol is practically not hydrolyzed (Curve C). In view of this result it is most natural to assume that 1,2-dipropionyl glycerol is the primary cleavage product with pancreatic lipase also. This assumption was confirmed by comparing the reaction curve obtained by adding liver esterase (Curve A_2) at the time 60 min, (when the reaction catalyzed by pancreatic lipase had almost stopped) and Curve D (1,2-dipropionyl glycerol + same amount of liver esterase). From the uniform course of the two curves it seems justified to assume that the amount of 1,2-dipropionyl glycerol is the

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same. If there had been perceivable amounts of 1,3-dipropionyl glycerol at the moment when the Curve A_1 has flattened, then an essentially larger rate of cleavage could be expected.

In the same way as with liver esterase the relative initial velocity of the degradation by pancreatic lipase for the propionyl esters mentioned was determined. Table I shows that the esters examined are hydrolyzed slowly compared with tri- and 1,3-dipropiony glycerol. Also here it is observed that the presence of a free primary alcoholic group in the ester inhibits the enzyme activity considerably. This inhibition seems to be especially marked when a low molecular acid is substituted in glycerol, as the monoglycerides of higher acids, *e.g.*, caprylic acid are easily hydrolyzed by relatively small amounts of pancreatic lipase[1].

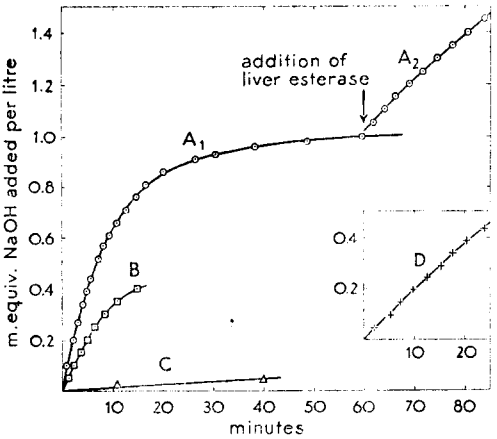

Fig. 2. Hydrolysis of different propionyl glycerols (1.0 mmol per litre) in the presence of the same amount of pancreatic lipase
Curve A_1, tripropionyl glycerol; A_2, addition of liver esterase after 60 min; Curve B, 1,3-dipropionyl glycerol; Curve C, 1,2-dipropionyl glycerol. Curve D, 1,2-dipropionyl glycerol in the presence of the same amount of liver esterase as in A_2

Kinetics of pancreatic lipase towards tripropionyl glycerol

In Paper I[1] in this series we have shown that the hydrolysis of *rac.* 1-caprylyl glycerol by pancreatic lipase may be explained by a reaction mechanism corresponding to a closed sequence consisting of 3 partial reactions. By means of the principles of stationary state an equation of the form

$$t = A \ln \frac{a}{a-x} - Bx + C\left(\frac{1}{a-x} - \frac{1}{a}\right) \qquad (1)$$

was put forward. A, B and C are expressed by the velocity constants, k_1, k_{-1}, k_2, k_{-2} and k_3 in the partial reactions, a = substrate concentration.

It appears from Table II that by means of equation (1) it is possible to render the course of the hydrolysis of tripropionyl glycerol by pancreatic lipase, assuming that only one acid equivalent is split off. Experiments were carried out at two different concentrations, and the mathematical treatment of the results was carried out in exactly the same way as described in Paper I[1]. From the substrate concentration a and A, B and C the following values were calculated: $k_{-1}/k_1 = 0.088$ mmol and $k_{-2}/k_3 = 0.140$. Inserting k_{-1}/k_1 and k_{-2}/k_3 in equation (19), Paper I, using $B = 2.48$ we calculate $1/k_2 = 19.4$ ($k_2 = 0.052$ min^{-1}·mmol^{-1}) in good agreement with the values found from the equation $1/k_2 = a/v_0 = A - Ba + C/a$. From Exp. VI we calculate $1/k_2 = 19.07$ and from Exp. VII $1/k_2 = 19.17$, average 19.12. The authors[14] have previously published experiments showing, that there is proportionality between v_0 and concentration of tripropionyl glycerol and this is in full agreement with the reaction scheme which is supposed to be valid for the process.

DESNUELLE, NAUDET, AND ROUZIER[15] have recently discussed the degradation of *emulsified* triolein and other naturally occurring fats by means of pancreatic lipase under

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TABLE II

COMPARISON OF OBSERVED AND COMPUTED t VALUES IN EXPERIMENTS WITH 2 DIFFERENT SUBSTRATE CONCENTRATIONS. EQUATION (1)

Exp. VI Substrate: 0.569 mmol tripropionyl glycerol per l. Enzyme: 12.3 ml pancreatic lipase per l reaction mixt. $A = 18.9$; $B = 2.48$; $C = 0.9$			Exp. VII Substrate: 1.012 mmol tripropionyl glycerol per l. Enzyme: 12.3 ml pancreatic lipase per l reaction mixt. $A = 19.9$; $B = 2.48$; $C = 1.8$		
$t_{obs.}$ min	x m.equiv. NaOH added	$t_{calc.}$ min	$t_{obs.}$ min	x m.equiv. NaOH added	$t_{calc.}$ min
1.09	0.032	1.11	1.22	0.062	1.21
1.99	0.057	2.02	2.27	0.111	2.25
3.43	0.094	3.48	3.37	0.160	3.36
4.44	0.118	4.52	4.57	0.209	4.57
5.60	0.143	5.64	5.85	0.259	5.84
6.73	0.167	6.84	7.19	0.308	7.23
8.10	0.192	8.12	8.74	0.357	8.74
9.45	0.217	9.50	10.54	0.406	10.40
10.90	0.241	11.01	12.42	0.455	12.23
12.56	0.266	12.64	14.50	0.505	14.27
14.51	0.291	14.45	16.70	0.554	16.56
16.45	0.315	16.45	19.48	0.603	19.17
18.55	0.340	18.90	22.55	0.652	22.20
21.30	0.364	21.27	26.00	0.702	25.82
24.31	0.389	24.22	29.75	0.751	30.22
28.06	0.414	27.76	35.45	0.800	35.86
32.40	0.438	32.08	42.00	0.849	43.55
37.98	0.463	37.54	53.25	0.899	55.50
44.20	0.487	45.10			
	$a_{max.} = 85.6$			$a_{max.} = 88.9\%$	

different conditions. The authors are of the opinion that the degradation of a triglyceride takes place in three steps, one acid group being split off in each step. The authors claim that the pancreatic lipase has no specificity with regard to the position of the acid groups in the different oleins and find that two molecules of 1-mono-olein are formed to one molecule of 2-mono-olein. Our findings seem to indicate that the results obtained by the french group cannot be transferred to the enzymatic hydrolysis of tripropionyl glycerol in aqueous solution, whether the hydrolysis is catalyzed by liver esterase or pancreatic lipase.

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SUMMARY

The hydrolyzing action of liver esterase and pancreatic lipase on tripropionyl glycerol in homogeneous aqueous solution has been studied.

1. By the action of liver esterase tripropionyl glycerol is degraded via 1,2-dipropionyl glycerol and monopropionyl glycerol, especially the 2-compound. The intermediates are hydrolyzed at highly different rates. Studies on the kinetics of the reaction are in progress.

2. By the action of pancreatic lipase tripropionyl glycerol is degraded to 1,2-dipropionyl glycerol;

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then the reaction practically comes to a standstill. The kinetics of this cleavage are relatively simple, the process being explained by a chain reaction consisting of three partial reactions. Putting the amount of enzyme = 1, then $k_2 = 0.052$ min^{-1}·mmol^{-1}, $k_{-1}/k_1 = 0.088$ mmol and $k_{-2}/k_3 = 0.140$.

3. Several propionyl esters were synthesized and the results of the enzymatic hydrolysis of these esters are able to elucidate the importance of ester constitution to the rate of hydrolysis. The presence of free alcoholic groups, especially primary, seems to involve a strong decrease in the rate of hydrolysis, as calculated per equivalent of acid that may be liberated.

RÉSUMÉ

Nous avons étudié l'action de l'estérase hépatique et de la lipase pancréatique sur l'hydrolyse du tripropionyl glycérol en solution aqueuse homogène.

1. Par l'action de l'estérase hépatique le tripropionyl glycerol est dégradé en passant par le 1,2-dipropionyl glycérol et le monopropionyl glycérol, particulièrement le 2-propionyl glycérol. Les glycérides partiels intermédiaires se décomposent à des vitesses fort différentes. Des études de la cinétique de la réaction sont en cours.

2. Par l'action de la lipase pancréatique le tripropionyl glycérol est transformé en 1,2-dipropionyl glycérol; ensuite la réaction s'arrête pratiquement. La cinétique de cette dégradation est assez simple. La réaction peut être expliquée par une chaîne composée de trois réactions partielles. En admettant que la quantité d'enzyme = 1, nous pouvons calculer $k_2 = 0.052$ min^{-1}·mmol^{-1}, $k_{-1}/k_2 =$ 0.088 mmol et $k_{-2}/k_3 = 0.140$.

3. Nous avons préparé plusieurs esters propioniques et les résultats de l'hydrolyse enzymatique de ces esters permettent d'élucider l'importance de la structure des esters pour la vitesse d'hydrolyse. La présence de groupes alcool libres, particulièrement de groupes primaires, diminue considérablement la vitesse d'hydrolyse, calculée par équivalent d'acide libérable.

ZUSAMMENFASSUNG

Die lipolytische Einwirkung von Leberesterase und Pankreaslipase auf Tripropionylglycerol in wässriger, homogener Lösung wurde studiert.

1. Durch die Einwirkung von Leberesterase wird Tripropionylglycerol über 1,2-Dipropionylglycerol und Monopropionylglycerol, besonders die 2-Verbindung, abgebaut. Die Zwischenprodukte werden mit sehr verschiedener Geschwindigkeit hydrolysiert. Studien über die Reaktionskinetik sind im Gang.

2. Durch die Einwirkung von Pankreaslipase wird Tripropionylglycerol zu 1,2-Dipropionylglycerol hydrolysiert. Dann hört die Reaktion so gut wie völlig auf. Die Kinetik dieser Spaltung ist verhältnismässig einfach. Der Reaktionsmechanismus wird als eine geschlossene Kette, bestehend aus drei Teilreaktionen, erklärt. Nimmt man an, dass die Enzymmenge = 1, so kann man die folgenden Konstanten berechnen: $k_2 = 0.052$ min^{-1}·mmol^{-1}, $k_{-1}/k_1 = 0.088$ mmol und $k_{-2}/k_3 = 0.140$.

3. Mehrere Propionsäureester wurden synthetisiert, und die Ergebnisse der enzymatischen Hydrolyse dieser Ester sind im Stande die Bedeutung der Esterkonstitution für die Hydrolysegeschwindigkeit zu erläutern. Die Anwesenheit freier Alkoholgruppen, besonders von primären, scheint eine starke Herabsetzung der Hydrolysegeschwindigkeit, berechnet pro Äquivalent Säure die abgespaltet werden kann, zu verursachen.

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