Esterification of hydroxy compounds by fatty acid anhydrides

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SUMMARY Partial glycerides are rapidly and completely esterified at room temperature to triglycerides by fatty acid anhydrides in the presence of perchloric acid. This esterification occurs without isomerization of the partial glyceride. As a consequence, by the use of partial glycerides of the appropriate structure, triglycerides having a specific structure can be prepared by this means. Other strong acids also catalyze esterification at room temperature, but this is accompanied by isomerization of the partial glyceride. The effect of varying the reaction conditions on the nature and extent of the reaction with partial glycerides has been determined. Alcohols also can be esterified by fatty acid anhydrides in the presence of perchloric acid.

THE USUAL METHODS for the synthesis of triglycerides yield products in which the fatty acids are distributed randomly. As a consequence, the preparation of glycerides of specific structure requires the use of appropriate blocking groups or acylation of partial glycerides with a fatty acid chloride. These methods are discussed in the review articles of Malkin and Bevan (1), Hartman (2), and Mattson and Volpenhein (3).

Fritz and Schenk (4) have described a method for determining the hydroxyl value of a compound. In their procedure, acetylation is brought about using acetic anhydride with perchloric acid as the catalyst. The reaction takes place rapidly at room temperature. We have found that this reaction also takes place with the anhydrides of long chain fatty acids. Moreover, when the material that is acylated is a partial glyceride, the esterification takes place with little or no isomerization. This latter observation is surprising because Martin (5) has shown perchloric acid to be an excellent catalyst for the isomerization of monoglycerides.

EXPERIMENTAL

The purity of the lipid components used in these studies was established by appropriate applications of gas–liquid chromatography, thin-layer chromatography, and lipase hydrolysis (6). All solvents were purified prior to use. As part of this process the alcohol, present as stabilizer, was removed from the chloroform. The acid catalysts were purchased from commercial sources and used without further treatment.

The fatty acid anhydrides were prepared by refluxing the fatty acid with an equal weight of acetic anhydride for 3 hr. The bulk of the excess acetic anhydride was removed by distillation. Finally, the mixed acetic-fatty acid anhydride was converted to the fatty acid anhydride by heating for 30 min to 150° at 1–2 mm pressure. The asymmetric acetic-oleic anhydride was prepared by the method of Ralston and Reck (7).

Except where noted otherwise, the following method for esterification was employed. The partial glyceride and the acid anhydride in a molar ratio of 1–1.2 were dissolved in 2.5 volumes of chloroform. This was mixed at room temperature and 0.03 mole of $HClO_4$ was added. Stirring was continued at room temperature for 3 hr. The reaction was stopped by adding an equal volume of water. Ethyl ether was added and the resulting upper phase was washed three times with water and dried with sodium sulfate. The solvents were then removed by evaporation. The residue was crystallized three successive times from 20 volumes of acetone at 20°. In most experiments a diglyceride containing only saturated

TABLE 1 ESTERIFICATION OF 1,3-DIPALMITIN USING VARIOUS AMOUNTS OF OLEIC ANHYDRIDE*

Anhydride Added	Molar Ratio Anhydride: Diglyceride	Hydroxyl Value of Product	Esterification
mmoles			%
1.93	0.55	41.4	58
3.87	1.10	4.4	96
4.95	1.40	1.6	98

* Reactants: 3.52 mmoles of 1,3-dipalmitin, 1.26 mmoles of $HClO_4$ (70%), and anhydride stirred for 3 hr at room temperature.

TABLE 2 ESTERIFICATION OF 1,3-DISTEARIN WITH OLEIC ANHYDRIDE USING VARIOUS AMOUNTS OF PERCHLORIC ACID*

$HClO_4$ Added	Molar Ratio, $HClO_4$: Anhydride	Yield	Hydroxyl Value of Product	Esterification
mmoles		%		%
0.060	0.0016	80	44	51
0.117	0.0032	73	65	28
0.294	0.008	71	57	37
0.584	0.016	94	0	100
1.170	0.032	97	0	100

* Reactants: 29.5 mmoles of 1,3-distearin, 36.6 mmoles of oleic anhydride, and various amounts of 70% perchloric acid stirred for 10 min at room temperature.

acids and the anhydride of an unsaturated acid were used. Consequently this isolation procedure yields the newly formed triglyceride plus any unreacted diglyceride, but none of the anhydride or free fatty acid. Where monoglyceride was the starting material, the triglyceride was obtained in pure form by successive recrystallizations. The values for the yield of product are based on the amount of partial glyceride used. The product was characterized by standard chemical procedures (8) and lipase hydrolysis (6).

RESULTS AND DISCUSSION

The extent of esterification when various proportions of fatty acid anhydride and diglyceride were allowed to react is given in Table 1. The reaction goes to completion when a small excess of the anhydride is added. Since only 1 mole of anhydride reacts with 1 mole of diglyceride, only one fatty acid of the anhydride is used in the synthesis.

As shown in Table 2, the maximum rate of esterification is obtained when the quantity of perchloric acid is in excess of 1 mole per cent of the amount of anhydride added. In these particular experiments, a reaction time of 10 min was used, and where a sufficient amount of perchloric acid was present, the reaction went to completion in that time.

TABLE 3 THE RATE OF ESTERIFICATION OF 1,3-DIPALMITIN WITH OLEIC ANHYDRIDE USING VARIOUS ACID CATALYSTS*

Catalyst	Hydroxyl Value of Reaction Products — Reaction Time, Min					
	5	10	15	30	60	300
Perchloric	0.5	1.4	0	0.6	—	—
Fluorosulfonic	4.0	4.2	3.9	4.5	—	—
Sulfuric	10.5	3.7	5.7	9.5	—	—
p-Toluenesulfonic	54.6	69.9	—	36.6	13.9	9.6
Anhydrous HCl	—	—	—	—	96.2	61.1

* Reactants: 2.63 mmoles of 1,3-dipalmitin, 3.15 mmoles of oleic anhydride, and acid catalyst at 1% (v/v) concentration of the total stirred for the times indicated at room temperature.

TABLE 4 THE STRUCTURE OF TRIGLYCERIDES FORMED BY THE REACTION OF DIGLYCERIDES WITH FATTY ACID ANHYDRIDES*

Reactants		Product			
			Fatty Acid Composition of 2-Position		
Diglyceride	Anhydride	Yield	10:0	16:0	18:1
		%			
1,2-Diolein	Palmitic	97	—	6	92
1,3-Diolein	Palmitic	99	—	92	5
1,3-Dipalmitin	Oleic	94	—	2	97
1,3-Dicaprin	Oleic	65	1	1	98

* Reactants in molar proportion: diglyceride 1.0, anhydride 1.2, and perchloric acid 0.02 stirred at room temperature for 1 hr.

The rapidity of the reaction in the presence of $HClO_4$ is shown again in Table 3. With only 5 min reaction, the diglyceride was almost completely converted to triglyceride. The results in this table also show that strong acids other than perchloric are capable of rapidly bringing about esterification at room temperature. However, hydrochloric acid is not an efficient catalyst.

Hydrolysis with pancreatic lipase of the triglycerides formed by the perchloric acid catalyzed reaction revealed that esterification had taken place with little or no isomerization. This statement is supported by the results shown in Table 4, in which the composition of the fatty acids in the 2-position of the triglycerides resulting from the reaction between various diglycerides and anhydrides is given. The difficulty of obtaining diglycerides in an absolutely pure form makes it impossible to determine whether a small amount of isomerization accompanied the esterification. The results obtained when dipalmitin or dicaprin was the starting material point to no more than 2 per cent isomerization.

The results presented in Table 5 demonstrate that the amount of isomerization varied with the different catalysts. In the case of *p*-toluenesulfonic acid, where a

TABLE 5 COMPOSITION OF FATTY ACIDS ESTERIFIED AT THE 2-POSITION OF THE TRIGLYCERIDES FORMED BY THE REACTION OF 1,3-DIPALMITIN AND OLEIC ANHYDRIDE IN THE PRESENCE OF VARIOUS CATALYSTS*

Catalyst	Reaction Time	Weight Per Cent	
		Palmitic	Oleic
	min		
Perchloric	10	3.3	96.5
Fluorosulfonic	10	8.8	90.7
Sulfuric	10	29.6	69.5
p-Toluenesulfonic	300	49.0	49.2

* Reactants: as described in Table 3.

long reaction time was necessary in order to obtain an appreciable amount of esterification, the product equilibrated to a random distribution of fatty acids.

As shown in Table 6, the rate and specificity of esterification is dependent also on the nature of the solvent. In general we have found chloroform to be the most convenient of the suitable solvents for laboratory-scale synthesis, for although carbon tetrachloride and benzene are also suitable, diglycerides are more soluble in chloroform.

When a mixed anhydride is used, the two fatty acids do not react equally. For example, the product of the esterification of 1,3-dipalmitin with oleic-acetic anhydride consisted, in molar proportions, of 83 parts 2-oleoyl dipalmitin and 17 parts 2-acetyl dipalmitin. When equal molar amounts of acetic anhydride and oleic anhydride were allowed to react with 1,3-dipalmitin, the molar proportion of the products was 61 parts 2-oleoyl dipalmitin and 39 parts 2-acetyl dipalmitin. Thus, if fatty acid anhydrides are prepared by the use of acetic anhydride, as described here, all residual acetic anhydride or mixed anhydride must be removed. The method described above leads to a suitable preparation of fatty acid anhydrides.

The combination of fatty acid anhydride and perchloric acid can also bring about the complete esterification of monoglycerides as shown in Table 7. Here the yields were not as good as when diglycerides were the starting material. Moreover, the pattern of solvents that result in minimum isomerization differs from that seen with the diglycerides. Here the preferred solvent is benzene because of the higher yield and the minimum amount of isomerization.

Martin (5) has shown perchloric acid to be an excellent catalyst for the isomerization of monoglycerides. This acid also isomerizes diglycerides.[1] In spite of this property of perchloric acid, the results presented here demonstrate that esterification takes place with little or no isomerization. To determine whether isomerization could take place under these conditions, the esterification was carried out in two steps. For this purpose 3 mmoles of 1,3-dipalmitin, 1.5 mmoles of oleic anhydride, and 0.03 mmole of $HClO_4$ were mixed for 1 hr at room temperature. With this quantity of anhydride only half of the diglyceride would be esterified. An additional 1.5 mmoles of oleic anhydride was then added to the mixture and the remaining diglycerides thus acylated. The 2-position of the resulting triglycerides consisted of 76% oleic acid and 24% palmitic acid. This is about the composition that would be expected if half the diglyceride molecules were isomerized to the usual 60/40 mixture of 1,3- and 1,2-isomers. That esterification takes place without isomerization under the preferred conditions of the reaction is probably attributable to the esterification reaction being much more rapid than the isomerization reaction.

Although we used 70% perchloric acid in the studies described here, the concentration of the acid is not important. Similar rates and specificity were obtained with perchloric acid of as low as 20% concentration provided an adequate amount of total perchloric acid was added. A wide range of concentrations can be used because it is likely that any water that is added with the perchloric acid is immediately removed by reaction with the fatty acid anhydride. Thus the reaction is carried out under strictly anhydrous conditions. This absence of water probably accounts in part for the rapid esterification.

In an earlier study we established conclusively (6) that partial glycerides can be esterified by fatty acid chlorides without isomerization. A comparison of the fatty acid chloride method with the fatty acid anhydride method was carried out by esterifying mixtures contain-

[1] Unpublished observations.

TABLE 6 THE ESTERIFICATION OF 1,3-DIPALMITIN WITH OLEIC ANHYDRIDE IN VARIOUS SOLVENTS USING PERCHLORIC ACID*

Solvent	Reaction Time	Yield	Hydroxyl Value	Esterification	2-Position Fatty Acid Composition	
					16:0	18:1
	hr	%		%	%	
None (but excess anhydride)	1	92	0	100	1.8	97.4
Chloroform	3	91	0	100	4.0	95.5
Carbon tetrachloride	3	92	0	100	2.4	96.9
Pyridine	3	65	42	57	—	—
Benzene	3	93	0	100	2.1	97.3
Ethyl acetate	3	73	42	57	—	—
Tetrahydrofuran	3	82	30	69	15.6	83.0
Dimethylacetamide	3	63	98	0	—	—
Acetic acid	3	60	62	38	—	—
Acetone	3	61	71	28	—	—
Hexane	3	65	—	5	—	—
Ethyl ether	3	65	27	73	—	—

* Reactants: as described in Table 4.

ing various proportions of 1,2- and 1,3-diglycerides by both of these procedures. The structure of the resulting triglycerides as determined by lipase hydrolysis was found to be essentially identical regardless of the method of acylation that was employed.

Complete acylation of partial glycerides by fatty acid chlorides requires a rather long reaction time. The perchloric acid method is more rapid, requiring less than 3 hr and probably only a few minutes. In both methods there is the same problem of removing the last trace of residual free fatty acid. The best solution to this problem that we have found is repeated crystallization of the product where this method is applicable.

In combination with fatty acid anhydride, perchloric acid also catalyzes the room temperature esterification of materials other than mono- and diglycerides. For example, starting with the alcohols, the complete esters of glycerol, erythritol, pentaerythritol, glucose, and sucrose have been prepared. The rate of esterification of these polyols is much slower than that of the partial glycerides and is markedly influenced by the solvent. This is probably due to the different solubility characteristics of the alcohol and the fatty acid anhydride.

Cholesterol esters are readily prepared by the method described here and, moreover, there is no problem in finding a suitable solvent for the reactants. For example, 0.01 mole of cholesterol, 0.012 mole of oleoyl anhydride, and 0.00036 mole of $HClO_4$ were dissolved in benzene and mixed at room temperature for 30 min. After three recrystallizations, cholesteryl oleate in 75% yield was obtained. Thin-layer chromatography of the product resulted in but a single spot which had an R_F identical with that of authentic cholesterol oleate. Kuksis and Beveridge (9) were unable to prepare the sterol esters of long chain fatty acids by refluxing ergosterol and fatty acid anhydride in benzene. As we have shown, this reaction occurs readily at room temperature, if trace amounts of $HClO_4$ are present.

The method of esterification that is described here should be of particular value for the preparation of esters of polyunsaturated fatty acids because no heat is used in the reaction. The slight discoloration that appears in the product can be removed by a single treatment with activated charcoal or silica gel.

TABLE 7 THE OLEIC ANHYDRIDE–PERCHLORIC ACID ESTERIFICATION OF 2-MONOSTEARIN*

Solvent	Triglyceride Yield	Hydroxyl Value	2-Position Fatty Acid Composition	
			18:0	18:1
	%		%	
Chloroform	69	2	72	27
Carbon tetrachloride	66	0.1	86	13
Pyridine	53	1	99	1
Benzene	75	2	95	5
Ethyl acetate	53	15	90	10
Tetrahydrofuran	63	0	74	24
Dimethylacetamide	3	89	—	—
Acetic acid	92	0	88	12

* Reactants in molar proportion: 2-monostearin 1.0, oleic anhydride 2.4, and perchloric acid 0.04, stirred for 3 hr at room temperature.

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