Chemistry of Natural Compounds, Vol. 39, No. 4, 2003

ACID-CATALYZED INTRA- AND INTERMOLECULAR ACYL EXCHANGE IN MONO- AND DIGLYCERIDES

S. V. Isai,[1] A. A. Usol′tsev,[2] and E. N. Stiba[1]

UDC 547.426

Acid-catalyzed transformation of 1-monolauroylglycerine (1-MLG) was investigated. It has been demonstrated that 1-MLG disproportionates readily and quickly into a mixture of 1,2- and 1,3-diacylglycerines and glycerine. The transformation of 1,3-diglycerides into 1,2-diglycerides was studied.

Key words: monoglycerides, 1,2- and 1,3-diglycerides, kinetics, GC analysis.

The fact that the 1,2- and 1,3-diglyceride (DG) content in various specimens is used in medicine and biology and the food industry as an indicator characteristic of certain processes makes them interesting. For example, 1,2-DG is known to accumulate during the early stages of cardiac ischemia [1]. The 1,2-DG content changes during diabetes [2, 3]. Diglycerides are an integral part of anticancer pharmaceuticals [4]. 1,2-DG is used as a tracer to investigate circadian rhythms [5]. The ratio of 1,2- to 1,3-DG is an indicator characteristic of the quality of olive oil [6, 7].

DG, like monoglycerides, are difficult to analyze quantitatively primarily because of their ability to isomerize. Migration of the acyl moieties in mono- and diglycerides also causes problems with the synthesis of these compounds.

We studied the transformation under acidic conditions of mono- and diacylglycerines with saturated aliphatic acyl moieties.

The migration of acyl moieties in mono- and diglycerides under various conditions (in solution and the solid state, at room and elevated temperatures, with saturated and unsaturated acyl chains, with aliphatic chains of different length) has been well studied [8-12]. Thus, migration of the acyl moiety in 1,2-dipalmitoylglycerine at various temperatures and times has been investigated [10]. The uncatalyzed intramolecular transfer of an acyl moiety has been reported [13]. Also, a variety of methods has been used to monitor the isomerization. GC is mentioned more than the others. However, various analysis conditions, phases, and derivatives were used. For example, separation of isomeric mono- and diglycerides as the acetate and trimethylsilyl derivatives with saturated and unsaturated aliphatic chains over cyanosiloxane (Silar 10C) gave positive results for the isomers with unsaturated chains whereas the separation of isomers with saturated chains gave partially overlapping peaks [14]. The HPLC separation of mono- and diglyceride isomers as the 3,5-dinitrophenylurethane derivatives has been reported [15]. A separation method for mono- and diglyceride isomers that uses supercritical extraction has also appeared [16].

The interconversion of 2- and 1-monoacylglycerines was studied by PMR and differential scanning calorimetry [17].

We propose using intra- and intermolecular exchange of acyl moieties in glycerides to prepare different diglycerides. The course of the interconversions and the reaction products were monitored by GC using the silyl derivatives. It has been found that silylation of the 1,2- and 1,3-diglycerides inhibits the isomerization and disproportionation to form tri- and monoglycerides. These results agree with those in the literature [8, 18].

1) Pacific Institute of Bioorganic Chemistry, Far-East Division, Russian Academy of Sciences, 690022, Vladivostok, pr. 100-Letiya Vladivostoku, 159, e-mail: piboc.@stl.ru; 2) Far-East State University (FESU), Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 260-263, July-August, 2003. Original article submitted August 11, 2003.

0009-3130/03/3904-0325$25.00 ©2003 Plenum Publishing Corporation

TABLE 1. Glyceride Content in the Reaction Mixture Resulting from Transformation of 1-MLG into 2-MLG and the Diglyceride Isomer Mixture in Petroleum Ether under Acid-Catalysis Conditions

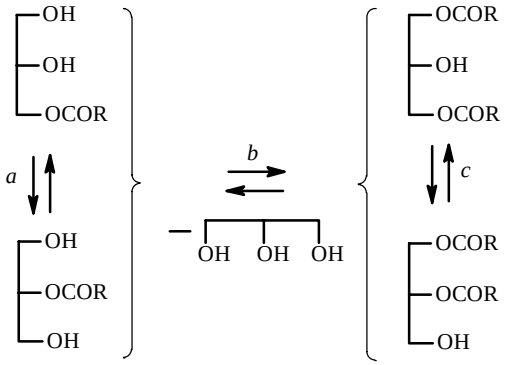
Reaction time, min	Reaction component content ($M \times 10^{-6}$)*					K*** ≈
	1-MLG	2-MLG	1,2-DLG	1,3-DLG	Gro**	
0	36.4	0.1	-	-	-	
10	33.6	1.9	0.2	0.3	0.4	N.d.
20	28.4	2.2	1.1	2.5	2.2	N.d.
30	16.6	2.2	3.0	7.6	4.0	2.9
40	11.0	2.0	2.7	11.3	9.4	8.3
50	14.1	2.5	7.1	4.8	7.9	4.2
60	12.9	2.7	2.8	9.7	8.3	5.1
120	11.9	4.1	3.9	8.4	8.1	N.d.
180	10.6	4.5	4.1	8.7	8.5	5.6

*Reaction component content in micromoles.

**Glycerine was determined from the difference between the initial 1-MLG content in the reaction and the total glycerides formed during the reaction.

***Equilibrium constant (see the text).

N.d. = not determined.



Scheme 1

Transformation of 1-Monolauroylglycerine. Table 1 lists quantitative data for the transformation kinetics of 1-monolauroylglycerine (1-MLG) into 2-monolauroylglycerine (2-MLG) and into a mixture of isomers of diglycerides, 1,2- and 1,3-dilauroylglycerine (DLG) under acid-catalysis conditions. The kinetic results are expressed in moles. This makes it possible to follow the consumption of 1-MLG and the formation of other products during the reaction. The transformation of 1-MLG catalyzed by TsOH proceeds with a gradual decrease of the 1-MLG concentration during the first 30-40 min. The concentrations of all reaction products, including an increase in the concentration of starting 1-MLG, change during the next 20 min. This can be explained by the simultaneous occurrence of competing reactions (Scheme 1). Then the reaction again proceeds with a decrease of the 1-MLG concentration and an increase of the concentrations of all products formed (Table 1).

We calculated the equilibrium constant for the transformation of 1-MLG at certain times using the results from GC analysis of the reaction products and the formula:

$$K = [DLG_{1,2-}]\,[DLG_{1,3-}] / [1\text{-MLG}]^2$$

Based on the GC data (Table 1), we propose the reaction pathway shown in Scheme 1. The GC results detected the formation of 2-MLG, from which 1,2-DLG then forms.

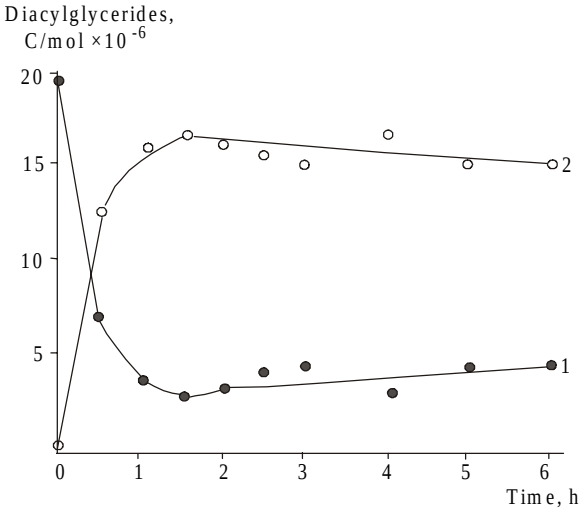


Fig. 1. Transformation of pure 1,3-dimyristoylglycerine in boiling anhydrous benzene and TFA. Initial 1,3-DMG, $C_0 = 19.5 \times 10^{-6}$ M (1), concentration of 1,2-DMG formed (2).

It should be noted that only peaks for the mono- and diglycerides are detected in the chromatograms. According to Scheme 1, glycerine forms in addition to mono- and diglyerides. The glycerine is insoluble in the reaction medium, petroleum ether, and settles to the bottom of the reaction vessel. It is not incorporated into the analytical aliquot. Its content (Table 1) was determined by the difference between the amount of 1-MLG added to the reaction and the total products formed.

Transformation of 1,3-Dimyristoylglycerine (1,3-DMG). Figure 1 shows the kinetics of 1,3-dimyristoylglycerine transformation (1) using anhydrous benzene and TFA and the formation of 1,2-dimyristoylglycerine (2) under these same conditions. The concentration of the 1,2-diglyceride increases rapidly during the first 30 min (up to 80% of the starting 1,3-DMG added to the reaction). The system reaches equilibrium after 5-6 h, (1,2):(1,3) = 77:23. The transformation of 1,3-DMG into 1,2-DMG follows Scheme 1c. The equilibrium constants were calculated using the formula:

$$K = [DMG_{1,2-}]/[DMG_{1,3-}]$$
$$K(1.5\ h) = K(4\ h) = 5.6$$
$$K(3\ h) = K(6\ h) = 3.2$$

According to the literature [19], the 1,3- and 1,2-DG are in equilibrium in solution (Scheme 1c). Therefore, it seemed interesting to determine the migration pathways using the formation of 1,2-DMG. The following hypotheses were tested in carrying out the transformation of 1,3-DMG. TFA is known to react autocatalytically with alcohols to form trifluoroacetates [20]. It is also known that TFA does not catalyze the hydrolysis of its own esters. Therefore, it seemed prudent to attempt the transformation of 1,3-DG into 1,2-DG using an excess of TFA in order to shift the equilibrium. It should be considered that two different competing esterifications occur upon reaction of DG and TFA. These are intra- and intermolecular. Intramolecular esterification is the shift of an acyl moiety of DG from the 3-position to the 2-position. Intermolecular esterification is trifluoroacetylation. The primary alcohol is more reactive than the secondary. Therefore, it is esterified first and the reaction shifts to the side of the 3-trifluoroacetate of 1,2-DG.

We also attempted to isomerize 1,3-DG in an excess of TFA. The reaction proceeds much less, K = 0.7-0.9. The yield of the 1,2-isomer is less than 58%. GC analysis was performed by the method described above.

Thus, the study of the transformations of 1-MLG and 1,3-DMG gives hope that they will find applications in analysis and synthesis of glycerides.

EXPERIMENTAL

Pure 1-monolauroylglycerine (mp 61-62°C) and chromatographically pure 1,2-DMG and 1,3-DMG (mp 52°C and 64°C, respectively) were synthesized in the Department of Bioorganic Chemistry of FESU using the method of Lok et al. [21]. They were freshly distilled and free of impurities and water.

GC analysis was performed on a GC-9A chromatograph (Shimadzu, Japan) with parallel capillary quartz columns (25-30 m × 0.25 mm, OV-101 5 μm thick, thermostat temperature 295°C, vaporizer 300°C, carrier gas He, 1 μL samples). All analyses were repeated in duplicate. A flame-ionization detector was used.

Acetate and silyl derivatives were investigated for GC monitoring of the interconversion of the reaction products. The acetate derivative of 1,2-DMG (retention time $\tau = 34.3$ min) appears in the chromatograms earlier than 1,3-DMG ($\tau = 35.7$ min).

Silyl derivatives of the isomeric DMG are separated well under these conditions: $\tau_{1,2} = 30.3$ min; $\tau_{1,3} = 32.5$ min.

1,3-DMG has an R_f value much greater than that of 1,2-DMG on TLC plates [22]. TLC was performed on KSK silica gel using petroleum ether (40-70°C):Et_2O:AcOH (80:20:1) (1), heptane:isopropyl ether:AcOH (60:40:4) (2), and hexane:Et_2O:AcOH (80:20:1) (3). The developer was H_2SO_4 in MeOH and heat [22].

Synthesis of Trimethylsilyl Derivatives. Method 1. A solution of DG or mixed DG in hexane (10 μL) was treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (10 μL). The tube was tightly sealed, stored for 30 min at 50°C, and analyzed without further workup.

Method 2. A solution of glyceride (1 mg) in anhydrous pyridine (100 μL) was treated with BSTFA (0.3 mL), stored at room temperature for 1-2 h, and evaporated to dryness. The solid was dissolved in hexane (100-200 μL, 1-2 drops). This method was more convenient because it requires evaporation of pyridine, which interferes with the chromatography.

Synthesis of Acetate Derivatives. Method 1. A solution of glyceride (1 mg) in anhydrous pyridine (200 mL) was treated with Ac_2O (200 μL) and thoroughly mixed. The tube was tightly closed and left in a desiccator overnight. The volatile components were distilled to dryness under Ar or in vacuum. The solid was dissolved in hexane (100-200 μL).

Method 2. A solution of glyceride (20-50 mg) in $CHCl_3$ (1 mL) was treated with Ac_2O (0.5 mL), shaken for 30 s, treated with conc. $HClO_4$ (0.1 mL), shaken an additional 10 s, cooled to 0-5°C, and extracted with cold $CHCl_3$:MeOH:H_2O (1.5:2.1:2 by vol). The lower phase was removed. The upper phase was extracted again with $CHCl_3$:MeOH:H_2O (2.5:2.3:2). The organic extracts were combined and evaporated with benzene:MeOH (3:2) as an azeotrope. The solid was dissolved in hexane (200-400 μL). The retention times of the acetates were 5-10 min.

Transformation of 1-MLG. A solution of 1-MLG (20 mg) in petroleum ether (20 mL) (36.4×10^{-6} M) was treated with *p*-toluenesulfonic acid (1-2 mg) and boiled on a water bath. Samples (10 μL) were collected 10, 20, 30, 40, 50, 60, 120, and 180 min after the start of the reaction, placed in small glass tubes with tight-fitting stoppers, and immediately treated with BSTFA (10 μL). The samples were analyzed by GC after 30 min.

Transformation of 1,3-DMG. A mixture (10 mg, 19.5×10^{-6} M) of chromatographically pure 1,3-DMG, anhydrous C_6H_6 (0.5 mL), and anhydrous TFA (1 mL) was boiled on a water bath for 6 h. Samples (10 μL) were collected 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after the start of the reaction. The volatile components were removed. The solid was dissolved in hexane (5 μL) and treated with a MeOH:pyridine mixture (6:1, 4 μL) to remove the TFA groups. Samples were evaporated to dryness after 5-10 min. The solid was dissolved in hexane (5 μL) and treated with BSTFA (10 μL). GC analysis was performed after 30 min.

REFERENCES

1. T. Kawai, K. Okumura, H. Hashimoto, T. Ito, and T. Satake, *Mol. Cell. Biochem.*, **99**, 1 (1990).
2. K. Okumura, T. Nishiura, Y. Awaji, J. Kondo, H. Hashimoto, and T. Ito, *Diabetes*, **40**, 820 (1991).
3. K. Okumura, N. Akiyama, J. Kondo, Y. Shirai, H. Hashimoto, and T. Ito, *The Diabetic Heart*, M. Nagano and N. S. Dhalla, eds., Raven Press Ltd., New York (1991).
4. S. Sakurai, K. Asahi, N. Takahashi, H. Hibino, and N. Fukuda, Jpn. Pat. No. 02 11,516 (1990); *Chem. Abstr.*, **113**, 103420t (1990).
5. M. Ramsdale and P. L. Lakin-Thomas, *J. Biol. Chem.*, **275**, 27541 (2000).

6. A. M. Leone, M. Santoro, V. A. Liuzzi, E. La Notte, and G. Gambacorta, *Riv. Ital. Sostanze Grasse*, **65**, 613 (1988); *Chem. Abstr.*, **112**, 20009u (1990).
7. G. Amelotti, A. Daghetta, and A. Ferrario, *Riv. Ital. Sostanze Grasse*, **66**, 681 (1989); *Chem. Abstr.*, **113**, 76704e (1990).
8. W. T. DeGroot, *Lipids*, **7**, 626 (1972).
9. C. M. Lok and J. P. Ward, *Chem. Phys. Liquids*, **39**, 19 (1986).
10. D. R. Kodali, A. Tercyak, D. A. Fahey, and D. M. Small, *Chem. Phys. Lipids*, **52**, 163 (1990).
11. K. R. Applegate and J. A. Glomset, *J. Lipid Res.*, **32**, 1635 (1991).
12. G. Boswinkel, J. T. P. Derksen, K. Vant Riet, and F. P. Cuperus, *J. Am. Oil. Chem. Soc.*, **73**, 707 (1996).
13. R. H. Barton and C. J. O'Connor, *Aust. J. Chem.*, **50**, 355 (1997).
14. Y. Itabashi and T. Takagi, *Lipids*, **15**, 205 (1980).
15. T. Takagi, *Prog. Lipid Res.*, **29**, 277 (1990).
16. L. Q. Xie, K. E. Markides, and M. L. Lee, *Anal. Biochem.*, **200**, 7 (1992).
17. A. Watanabe, *J. Am. Oil Chem. Soc.*, **74**, 1569 (1997).
18. J. A. W. Engbersen and F. Van Stijn, *Chem. Phys. Lipids*, **16**, 133 (1976).
19. C. M. Lok, *Chem. Phys. Lipids*, **22**, 323 (1978).
20. B. H. Johnston, A. C. Knipe, and W. E. Watts, *Tetrahedron Lett.*, No. 43, 4225 (1979).
21. C. M. Lok, A. P. J. Mank, and J. P. Ward, *Chem. Phys. Lipids*, **36**, 329 (1985).
22. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York (1973).