notes on methodology

Synthesis of racemic 1,2-diolein

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SUMMARY 1,2-Diolein has been synthesized by protecting the 3-position of glycerol during acylation by means of the pyranyl ether linkage. This linkage is easily broken under conditions leading to very limited isomerization of the diglyceride.

KEY WORDS diolein · synthesis · tetrahydropyranyl ether · 1,2-diglycerides · isomerization

No simple, general method is at present available for the synthesis of 1,2-diglycerides (1, 2).

Blocking of one of the primary hydroxyl groups of glycerol by a benzyl, trityl, or pyranyl group followed by esterification involves the later removal of the ether group. If this is accomplished by hydrogenolysis, the method is limited to the preparation of saturated 1,2-diglycerides. Breaking of the ether bond by acid catalysis is generally believed to result in isomerization of the 1,2-diglyceride.

In the present communication we report results indicating that the pyranyl glycerol ether linkage can be split by acid catalysis under conditions which do not effect a significant isomerization of 1,2-diglycerides containing long-chain fatty acids. In this way a generally applicable route is open for the synthesis of 1,2-diglycerides.

PROCEDURE AND RESULTS

1-Tetrahydropyranyl glycerol was synthesized according to Barry and Craig (3) from allyl alcohol.

1,2-Di-(oleoyl-9,10-H^3*) 3-O-tetrahydropyranyl glycerol* was synthesized from 1-*O*-tetrahydropyranyl glycerol and oleoyl-9,10-H^3 chloride (4) according to standard procedures. For convenience "tetrahydropyranyl" is replaced by "pyranyl" in the remainder of this article.

1,2-Di-(oleoyl-9,10-H^3*) 3-O-trityl glycerol* was similarly synthesized by acylation of 1-*O*-trityl glycerol (5).

The last two compounds were purified by preparative thin-layer chromatography (TLC) to a radiopurity of >98%.

The extent of splitting of the ether bond and the formation of 1,2- and 1,3-diglycerides was determined as follows. The reaction products were separated by TLC on Silica Gel G using heptane–ethyl ether–acetic acid 79:20:1 as solvent. With this system a good separation of the 1,2- and 1,3-diglycerides is obtained (6). The spots were detected with iodine vapor and scraped off, and their radioactivity was determined by liquid scintillation counting.

In preliminary experiments the acid-catalyzed reaction rates were compared for the dioleoyl trityl and dioleoyl pyranyl derivatives. Under the conditions specified below, a 10 min reaction time effected a splitting of 94% of the pyranyl ether, but only 7% of the trityl ether linkage. The percentage of 1,3-diglyceride formed constituted only 1.6 and 1.2% respectively of the total diglyceride produced. By contrast, after 2 hours' reaction only 50% of the trityl ether was split but the percentage of 1,3-diglyceride in the diglycerides was found to be about 30.

It therefore appeared that the pyranyl ether linkage was more reactive than the trityl linkage and that a quantitatively satisfactory splitting of the pyranyl ether could be obtained with almost negligible isomerization.

A time study of the acid-catalyzed degradation of the dioleoyl pyranyl glycerol ether is given in Table 1. In this experiment 150 mg of the starting material was dissolved in 3 ml of ethyl ether plus 3 ml of methanol at room temperature. At zero time 3 drops of concentrated hydrochloric acid (Merck, reagent grade 36.4%, density 1.19) were added. At the times given, 1 ml of the reaction mixture was pipetted into a test tube containing 1 ml of heptane plus 1 ml of water. The upper phase was further washed until neutral; 5 µl of it was subjected to TLC as described above. The spots were scraped off and their radioactivity was determined by liquid scintillation counting.

TABLE 1 Percentage Composition of the Reaction Product after Acid Degradation of 1,2-Dioleoyl 3-*O*-Tetrahydropyranyl Glycerol

	Reaction time (min)					
	0	1	5	10	20	22 hr*
Starting compound	98.8	69.7	24.1	6.6	1.2	—
1,2-diolein	<0.1	28.5	73.4	87.2	88.6	—
1,3-diolein	<0.1	0.2	1.6	3.0	6.3	—
More polar compounds	1.0	1.6	0.9	3.2	3.9	—
1,2-diolein in diglyceride	—	99.3	97.9	96.7	93.7	41.5

* Only the percentage composition of the diglyceride is given, as considerable methanolysis had occurred.

In a larger scale experiment, 360 mg of the dioleoyl pyranyl glycerol ether was dissolved in 10 ml of ether plus 10 ml of methanol, and 10 drops of concd HCl were added. After 10 min, 10 ml of water was added and then 25 ml of heptane. Preparative TLC of the extracted lipids yielded 87% of the activity as 1,2-diolein. Other conditions of solvent, acidity, time, and temperature gave no better yield of 1,2-diolein than those used in the experiment summarized in Table 1.

These experiments show that the pyranyl glycerol ether linkage is more acid labile than the trityl glycerol ether linkage and that the pyranyl ether can be split under conditions leading only to a very limited isomerization of a 1,2-diglyceride.

The method has the advantage, in the synthesis of unsaturated 1,2-diglycerides, over that of Baer and Buchnea (7) in not requiring the protection of fatty acid double bonds during hydrogenolysis of the ether linkage. Unlike the latter method it can be used only for the synthesis of racemic diglycerides. The method described is less wasteful than the procedure proposed by Mattson and Volpenhein (2) involving incomplete acylation of the 2-monoglyceride.

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